Univerzita Karlova v Praze

Přírodovědecká fakulta

Studijní program: Antropologie a genetika člověka

Studijní obor: Antropologie a genetika člověka

Katedra antropologie a genetiky člověka



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Příbuzenské vztahy a morfologické znaky lidské kostry u genealogicky dokumentovaných souborů: využití v bioarcheologii a forenzní antropologii.

Kinship relationships and morphological features of the human skeleton in genealogically documented osteological assemblages: use in bioarchaeology and forensic anthropology.

Disertační práce

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Praha, 2021

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Podpis

Poděkování

Na tomto místě bych chtěl poděkovat všem, bez jejichž pomoci by má doktorská práce nikdy nemohla vzniknout.

Předně děkuji mému školiteli Petru Velemínskému za vedení mé práce, a poskytnutí takového zázemí a podpory, jaké si jen může student přát. Stál za mnou již od bakalářské práce v době, kdy jinak asi jen málokdo věřil, že můj zamýšlený projekt může uspět, a to znamená opravdu mnoho. Stejně tak děkuji konzultantovi práce Jaroslavu Brůžkovi, především za jeho rady a pomoc při tvorbě publikací a psaní této práce.

Dále chci poděkovat mým nejbližším, kteří mi pro studium poskytli ty nejlepší možné podmínky a vždy mě podporovali v mých zájmech. Díky nim se mi ve studiu dostalo příležitostí, jako asi málokomu, zvláště v tomto oboru.

Mimo nich se na práci přímo či nepřímo podílelo přes osm desítek mých dalších blízkých či vzdálených členů rodiny, kterým tímto děkuji za jejich ochotu, podporu a pomoc, bez které bych se neobešel: Františku Andresovi st. a jeho rodině; Gustavu Andresovi st. a jeho rodině; Janě Blechové a její rodině; rodinám Antonína Cvrčka, Jaroslava Cvrčka, Karla & Karla Cvrčka, a Luďka Cvrčka; Petru Drbovi a jeho rodině, Petru Eretovi, Heleně Kleczkové a její rodině, Aleně Klímové a její rodině, Františku Koppovi, Otovi Lacinovi a jeho rodině, rodině Menšíkově, Evě Palmové, Petru Paučkovi, Lukáši Rokosovi a jeho rodině, Petru Šorejsovi a jeho rodině, Heleně Tomanové a její rodině, a Janě Tomanové. Děkuji také Pavlu Čaškovi a jeho rodině. In memoriam děkuji mým prarodičům Karlu a Jarmile Cvrčkovým, a dále Zdeňku Rezkovi, neboť tato práce mohla vzniknout také díky jejich odkazu.

V neposlední řadě děkuji svým kolegům Petře Brukner Havelkové, Zdeňku Chundelovi, Marku Jantačovi, a Jitce Vítkové z Antropologického oddělení Národního muzea, kteří mi vždy ochotně pomáhali jak při práci v terénu, tak i v laboratořích. Děkuji též všem kolegům, kteří se podíleli na vlastním výzkumu a přípravě publikací: Miluši Dobisíkové, Sylvě Drtikolové Kaupové, Vítězslavu Kuželkovi, a Lence Půtové (Antropologické oddělení, Národní muzeum), Markétě Čechové, Jánu Dupejovi, Rebece Rmoutilové, Janě Velemínské, a Elišce Zazvonilové (Katedra antropologie a genetiky člověka PřF UK), Františku Fortinovi (Česká genealogická a heraldická společnost v Praze), Ivě Grossové (Vojenský ústav soudního lékařství, ÚVN Praha), Jiřímu Hadravovi a Tomáši

Jorovi (Katedra zoologie PřF UK), Martinu Horákovi (Radiodiagnostické oddělení nemocnice Na Homolce), Viktoru Chrobokovi (Fakultní nemocnice Hradec Králové); Ondřeji Naňkovi (Anatomický ústav 1. LF a VFN), a Luboši Vostrému (Česká zemědělská univerzita v Praze). Alastairu Millarovi děkuji za konzultace anglické gramatiky a pomoc s jazykovou korekturou publikací, Jakubovi Štěpánovi za fotodokumentaci a obětavou pomoc jak v terénu, tak i v laboratoři, a Michalovi Tkočovi (Entomologické oddělení, Národní muzeum) za pomoc s technickou přípravou této práce. Taktéž děkuji za spolupráci Tomáši Jiráskovi, Kateřině Vadinské a ostatním zaměstnancům patologie nemocnice Královské Vinohrady.

Disertační práce byla ve svém průběhu podpořena Grantovou agenturou Univerzity Karlovy (GAUK č. 1276217), Grantovou agenturou České republiky (GAČR 19-13265S), a Interním grantem Národního muzea (Osteobiografie a paleopatologie ostatků hraběcího rodu Swéerts-Sporck v kontextu dobových písemných pramenů, Čechy, 19. století). Po celou dobu studia byla pak také podporována Ministerstvem kultury České republiky (DKRVO, Národní Muzeum, 00023272).

Stále jsou naši mrtví s námi
a nikdy vlastně nejsme sami

A přicházejí jako stíny
ve vlasech popel kusy hlíny

Tváře jakoby vymazané
a přece se jen poznáváme

Po chrpách které kvetly vloni
slabounce jejich ruce voní

Tiše mne zdraví jako svého
hrbáčka času přítomného

Jan Skácel

Abstrakt

Vliv biologických příbuzenských vztahů na morfologii lidské kostry představuje pro bioarcheologii i forenzní výzkum jedno z aktuálních témat. Ať už jde o odhalení příbuzenských vazeb na anonymním pohřebišti nebo otázku individuální identifikace na základě rodinné podobnosti, výzkum je limitován řadou faktorů. Především je to nedostatek osteologických souborů s genealogickou dokumentací. Většina takových souborů také zahrnuje jen malý počet jedinců, a je tedy omezený i počet různých stupňů příbuznosti. S tím souvisí i nedostatek, popřípadě absence metod vhodných jak pro exaktní vyjádření míry morfologické podobnosti mezi jedinci, tak i pro statistické vyhodnocení výsledků. Pro tuto disertační práci se však naskytla příležitost analyzovat několik osteologických souborů s genealogickou dokumentací ze 17. až 20. století, celkově zahrnujících téměř jedno sto jedinců. To představuje jeden z dosud největších takových celků na světě. Jednotlivé soubory zahrnují i několik případů příbuzenských sňatků a po nich následující generace. Cílem první části disertační práce tak bylo navrhnout nové metodické přístupy vyjádření míry podobnosti jedinců na základě různých typů morfologických, resp. patologických znaků lidské kostry, a dále zjistit, zda a do jaké míry morfologické znaky kostry odrážejí stupeň biologické příbuznosti jedinců. Cílem druhé části práce bylo zjistit vliv příbuzenských vztahů, zdravotního stavu a dalších faktorů na stabilní izotopy uhlíku a dusíku v kostním kolagenu. Výsledky prokázaly, že distribuce nemetrických osteologických znaků, morfologických znaků a maximální výšky čelních dutin či vývojové anomálie skeletu statisticky signifikantně korespondují se stupněm biologické příbuznosti jedinců. U nemetrických osteologických znaků biologické příbuzenské vztahy reflektují především znaky spojené s přítomností, absencí nebo způsobem vyústění cévních a nervových drah a charakterem jejich průběhu, spojené s nadměrným růstem kosti. Zejména jde o zadní a postranní můstky na prvním krčním obratli (*ponticulus atlantis posterior et lateralis*), přemostění křídlovitých výběžků kosti klínové a spodiny lební (*ponticulus pretygospinosus et pterygoalaris*), přemostění *sulcus mylohyoideus* na dolní čelisti *(ponticulus mylohyoideus*), a kanál pro některou z větví supraklavikulárního nervu v klíční kosti (*canalis intraclavicularis*). Podobně bylo v rodinách nalezeno několik opakujících se kosterních vývojové anomálií se vzácnější populační frekvencí, jako prodloužený bodcovitý výběžek (*processus styloideus elongatus*), různé formy vrozených srůstů krčních obratlů, nevyvinutí hákovitého výběžku hákovité kosti (*hamulus ossis hamati*), změny spojované s vrozeným srůstem patní a loďkovité kosti, či vzájemné morfologické posuny jednotlivých úseků páteře. V případě čelních dutin se blízce příbuzní

jedinci shodovali v různých morfologických znacích jako jsou různé zářezy či vyklenutí jednotlivých laloků dutin, nebo ve stranově zrcadlové podobnosti. Nicméně pozitivní vztah mezi mírou podobnosti jedinců a mírou jejich biologické příbuznosti byl patrný i u znaků, kde závislost na biologické příbuznosti nebyla statisticky signifikantní, jako je celkový objem, povrch a maximální šířka čelních dutin. Navíc, vliv pohlavní příslušnosti na velikost čelních dutin u biologicky příbuzných jedinců se ukázal oproti náhodně vybraným jedincům z populace jako statisticky nevýznamný. Naproti tomu vliv inbreedingu na distribuci sledovaných znaků se jeví jako nejednoznačný. Také v případě analýzy stabilních izotopů se příbuznost skrze odlišné stravovací návyky a způsob života rodin ukázala hlavním faktorem ovlivňujícím izotopové hodnoty jedinců. Disertační práce se skládá z deseti publikovaných studií, které jsou předloženy po její úvodní a souhrnné části.

Klíčová slova: příbuznost, genealogie, nemetrické morfologické znaky, čelní dutiny, kosterní vývojové anomálie, stabilní izotopy uhlíku a dusíku, dědivost

Abstract

The influence of biological relationships on the morphology of the human skeleton is a current theme in bioarchaeology and forensic research. Whether it is the discovery of kinship relationships in an anonymous cemetery or the question of individual identification based on familial similarity, research is limited by a number of factors. Above all, there is the shortage of osteological assemblages with genealogical documentation. Most such samples also include only a small number of individuals, and thus the number of different degrees of their relatedness is limited. Related to this is the lack or absence of methods suitable for either the exact expression of the degree of morphological similarity between individuals or the statistical evaluation of results. However, for this dissertation an opportunity arose to analyze several osteological assemblages with genealogical documentation from the 17th to 20th centuries, comprising almost one hundred individuals. This represents one of the largest such units in the world to date. The individual samples also include several cases of consanguineous marriages with subsequent generations. The aim of the first part of the dissertation is to propose new methodological approaches to expressing the degree of similarity of individuals based on different types of morphological or pathological features of the human skeleton, and to determine whether and to what extent the morphological features of the skeleton reflect the degree of biological relationship between individuals. The aim of the second part of the work is to determine the influence of kinship relationships, health status and other factors on carbon and nitrogen stable isotopes in bone collagen. The results show that the distribution of non-metric osteological traits, morphological traits and the maximum height of the frontal sinuses or skeletal developmental anomalies statistically significantly correspond to the degree of biological relationship between individuals. In non-metric osteological traits, biological relationships primarily reflect traits associated with the presence, absence, or means of outlet of vascular and neural pathways and the character of their courses, associated with excessive bone growth. These are mainly posterior and lateral bridges on the first cervical vertebra (*ponticulus atlantis posterior et lateralis*), bridging of the pterygoid processes of the sphenoid bone and the cranial base (*ponticulus pretygospinosus et pterygoalaris*), bridging of the mylohyoid sulcus on the lower jaw (*ponticulus mylohyoideus*), and the channel for any of the branches of the supraclavicular nerve in the collarbone (*canalis intraclavicularis*). Similarly, several recurrent skeletal developmental anomalies with a rarer population frequency were found in families, such as an elongated styloid process (*processus styloideus elongatus*), various forms of congenital cervical block vertebrae, hamate hamulus

aplasia (*hamulus ossis hamati*), changes associated with congenital calcaneus and navicular bone fusion, and the mutual morphological shifts of individual sections of the spine. In the case of the frontal sinuses, closely related individuals agreed in different morphological traits such as different notches or arches of individual lobes of the sinuses, or in lateral mirror similarity. However, a positive relationship between the degree of similarity between individuals and the degree of their biological relatedness was also evident in features where the dependence on biological relatedness was not statistically significant, such as the total volume, surface area and maximum width of the frontal sinuses. In addition, the effect of sexual dimorphism on frontal sinus size in biologically related individuals proved to be statistically insignificant compared to randomly selected individuals from the population. In contrast, the influence of inbreeding on the distribution of the observed features appears to be ambiguous. In the case of stable isotope analysis, too, relatedness through the different eating habits and lifestyle of families proved to be a major factor influencing the isotopic values of individuals. This dissertation consists of ten published studies, which are submitted after its introductory and summary parts.

Keywords: kinship, genealogy, non-metric morphological traits, frontal sinuses, skeletal developmental anomalies, carbon and nitrogen stable isotopes, heritability

Obsah

1 Úvod ... 1

2 Příbuznost a osteologické znaky, jejich potenciál a limity ... 3

2.1 Příbuznost a morfologická i metrická podobnost kostry ... 3

2.2 Příbuznost, životní podmínky a zdravotní stav ... 8

3 Cíle práce ... 11

4 Materiál a metody ... 12

4.1 Materiál ... 12

4.2 Metody ... 16

5 Výsledky a diskuse: shrnutí předkládaných publikací ... 18

5.1 Příbuznost a morfologická i metrická podobnost kostry ... 18

5.1.1 Nemetrické osteologické znaky ... 18

5.1.2 Čelní dutiny ... 20

5.1.3 Kosterní vývojové anomálie ... 23

5.2 Příbuznost, životní podmínky a zdravotní stav ... 27

6 Aplikace výsledků v bioarcheologii a forenzní antropologii ... 30

7 Směry budoucího výzkumu ... 33

7.1 Příbuznost a morfologická i metrická podobnost kostry ... 33

7.2 Příbuznost, životní podmínky a zdravotní stav ... 36

8 Závěry ... 37

9 Bibliografie ... 39

10 Přílohy ... 65

10.1 Seznam předkládaných publikací ... 65

10.2 Předkládané publikace ... 67

1 Úvod

Otázka vlivu genetických faktorů na variabilitu lidské kostry je v bioarcheologii i forenzní antropologii již několik desetiletí diskutované téma. Pro bioarcheologii je v tomto směru předmětem zájmu odhalení biologických příbuzenských vztahů pohřbených jedinců (Esparza et al., 2017; Johnson, 2018; Sjögren et al., 2020; Brück 2021; Žegarac et al. 2021). Například mnohočetné pohřby jako dvojhroby či trojhroby bývají na základě nálezové situace již předem považovány za pohřby biologicky příbuzných jedinců (Haak et al., 2008; Esparza et al., 2017). Výsledky osteologických i molekulárně genetických analýz jsou však s tímto předpokladem často v rozporu (Boljunčić, 2007; Meyer et al., 2012; Deguilloux et al., 2014), někdy dokonce i mezi sebou navzájem. Známým příkladem je mladopaleolitický trojhrob z Dolních Věstonic. Zatímco prvotní morfologické analýzy ukazovaly na jejich vzájemnou blízkou příbuznost (Vlček, 1991; Alt et al., 1997), první výsledky analýzy DNA tyto závěry odmítly (Krause & Svoboda, 2011), aby následně další výzkum DNA potvrdil příbuznost jen dvou ze tří jedinců, avšak obecně v mateřské linii (Fu et al., 2013). U třetího z jedinců bylo možno blízký příbuzenský vztah v mateřské linii vyloučit, nebylo však možné vyloučit příbuznost v otcovské linii (Mittnik et al., 2016). Do jaké míry se tedy na kostře odráží genetické faktory a které z nich lze pro detekci biologické afinity jedinců použít? Forenzní antropologie se pak snaží odhalit možný vliv biologických příbuzenských vztahů na proces individuální identifikace jedince (Cameriere et al., 2008). Může shoda či podobnost ve výskytu morfologických znaků kostry podmínit falešně pozitivní identifikaci biologicky příbuzných jedinců, tedy záměnu příbuzných jedinců při ztotožnění? Anebo naopak, lze se o znaky ve snaze o identifikaci jedince opřít?

Odpovědi na tyto otázky lze hledat jedině ve výzkumu souborů s doloženými příbuzenskými vztahy, ať už se jedná o žijící rodiny (Stojanowski et al., 2017), nebo osteologické soubory (Case et al., 2017). Snad kromě výzkumu dentálních nemetrických znaků a rozměrů, kdy se využívají sádrové odlitky (Stojanowski et al., 2017, 2018, atd.), je však studium žijících rodin dlouhodobě na ústupu vzhledem k etickým omezením, především pokud jde o medicínsky neindikované dávky rentgenového záření (Kjaer et al., 2012). Osteologické série s biografickou (a genealogickou) dokumentací se tedy stávají nepostradatelnými (Henderson & Cardoso, 2018; Mann et al., 2021). Možnosti výzkumu však naráží na celosvětově omezenou dostupnost takových souborů (Carson, 2006a; Ricaut et al., 2010; Gavrus-Ion et al., 2017). Tyto soubory se pro výzkum většinou naskytnou jen náhodně,

zejména při renovacích rodinných hrobek či rušení hřbitovů, kdy je přikročeno k odbornému výzkumu pozůstatků (např. Lane 1976; Kelley, 1989; Molleson et al., 1993; Gemmerich-Pfister, 1999; Velemínský & Dobisíková, 2005; Drozdová, 2006, Harding et al., 2020; Alterauge et al., 2021; atd.). V jejich případě je samozřejmě vždy nutné brát v úvahu, že někteří členové rodiny nemusí být ve skutečnosti vůbec biologicky příbuzní tak, jak udávají písemné prameny (např. Gerstenberger et al., 1999). Tato pravděpodobnost je ovšem obecně velmi nízká, přibližně pouhé 1 % (Larmuseau et al., 2016, 2017). Bohužel, v některých případech, když je genealogicky dokumentovaný materiál již k dispozici, zůstává příležitost pro výzkum vlivu příbuzenských vztahů na morfologickou variabilitu kostry ne zcela plně využitá (např. Fornaciari et al., 2007).

Pro předkládanou disertační práci se naskytla příležitost analyzovat několik osteologických souborů s genealogickou dokumentací z období 17. až 20. století z území Čech, celkově zahrnujících bezmála sto jedinců, což dohromady tvoří jeden z dosud největších takových celků na světě. Jednotlivé soubory přitom zahrnují i několik případů příbuzenských sňatků a po nich následující generace. Jestliže počet osteologických souborů s genealogickou dokumentací zůstává doposud celosvětově nízký, pak počet souborů zahrnujících současně i jedince pocházející z pokrevních manželství je ojedinělý. Materiál pro tuto práci z části pochází z muzejních sbírek (Velemínský & Dobisíková, 2005), většinou jej však poskytly potomci zkoumaných jedinců kvůli nutnosti renovace jejich hrobek, případně kvůli přesunu ostatků na nové místo. Disertační práci lze rozdělit do dvou tematických oblastí. První se zabývá vztahem mezi mírou podobnosti jedinců na základě morfologických i metrických znaků kostry a stupněm jejich biologické příbuznosti. Řeší také možný vliv genetických faktorů na pohlavní dimorfismus vybraných částí kostry. Stěžejním tématem této části jsou pak nové metodické přístupy pro hodnocení konkrétních znaků a vyjádření míry podobnosti jedinců pro využití v bioarcheologii a forenzní antropologii. Druhá část pak pojednává o vlivu příbuznosti, životních podmínek a zdravotního stavu na stabilní izotopy kostního kolagenu, opět s přesahem do bioarcheologie.

2 Příbuznost a osteologické znaky, jejich potenciál a limity

2.1 Příbuznost a morfologická i metrická podobnost kostry

Utváření a změn některých partií lidské kostry v souvislosti s rodinnými vztahy si badatelé začali všímat již od konce 19. století (např. Charles 1893, 1894; Shepherd, 1893; Symmers, 1895). Tyto objevy pocházející z klinické praxe byly ve své době prezentovány spíše jen jako kazuistiky, a teprve od konce 20. let 20. století začaly na toto téma vznikat systematické studie, ať už na základě žijících rodin, nebo kosterních pozůstatků (Leicher, 1928; Matiegka, 1934; Torgersen, 1951; Selby et al., 1955). Metodika výzkumů se však začala rozvíjet ještě později, od poloviny 20. století (Ullrich, 1969). Postupně se ukázalo, že biologické příbuzenské vztahy se odrážejí u celé řady znaků lidské kostry, z nichž nejvíce diskutované jsou nemetrické osteologické a dentální morfologické znaky (Alt & Vach, 1995; Carson 2006a; Stojanowski & Schillaci, 2006), paranasální dutiny (Szilvássy, 1986), kosterní vývojové anomálie (Rösing, 1986; Case, 2003), a kraniální rozměry (Sjøvold, 1984; Carson 2006b). K tomuto zjištění se dospělo skrze studium míry podobnosti blízce příbuzných jedinců, zejména dvojčat, přičemž jednovaječná dvojčata vykazovala ve sledovaných znacích větší podobnost, než ta dvojvaječná (např. Biggerstaff, 1973; Szilvássy, 1986; Boraas et al., 1988). Větší míra podobnosti byla dále zjištěna u dalších blízce příbuzných jedinců, jako jsou rodiče a děti nebo sourozenci, než u vzdáleně příbuzných jedinců (Velemínský & Dobisíková, 2005). V několika případech se ovšem našly výjimky, zejména u bratranců a sestřenic (bez ohledu na pohlaví), kteří vykazovali větší míru podobnosti, než zmínění blízce příbuzní jedinci (Gemmerich-Pfister, 1999; Velemínský & Dobisíková, 2005).

Na první pohled by se tato zjištění mohla jevit jako dostatečný argument pro využívání těchto znaků v bioarcheologické i forenzní praxi. Hlubší pohled na tuto problematiku však ukázal tři zcela zásadní úskalí, které je nutné při jejich využití brát v úvahu. Tím prvním je dědivost znaků. Studium zvířecích modelů ukázalo, že mezi geny a morfologickými i metrickými znaky lidské kostry neexistuje jednoduchý příčinný vztah (Tyrrell, 2000). Znaky jsou determinovány více geny, které mají aditivní účinek a k jejichž projevu navenek je nutné dosažení určité prahové hodnoty. Tuto hodnotu pak mohou dále ovlivňovat i vnější negenetické faktory, jako je např. výživa, fyziologický stres apod. (Deol et al., 1957). V případě znaků se tak předpokládá platnost tzv. Falconerova modelu (Falconer, 1965; Hauser & De Stefano, 1989). Kromě toho se ukázalo, že i různé formy stejného znaku, jako je např. vrozený srůst krčních obratlů, mohou mít v závislosti na lokalizaci a rozsahu projevu odlišnou

dědivost (Clarke et al., 1998). Tato zjištění zapříčinily určitou skepsi některých badatelů vůči výpovědní hodnotě a potenciálu osteologických přístupů pro detekci biologické afinity jedinců (např. Brown, 2015).

Výzkum v této oblasti se může opřít o výpočet heritability (dědivosti) u sledovaných znaků, tzv. heritability v užším slova smyslu („narrow-sense heritability"; h^2), který udává podíl aditivní genetické složky na celkové varianci vnějšího projevu znaku (Stojanowski et al., 2019). Významná dědivost byla tímto způsobem prokázána např. právě u nemetrických osteologických a dentálních znaků i kraniálních rozměrů, a to nejen u člověka (Sjøvold, 1984; Carson, 2006a, b; Stojanowski et al., 2017, 2018), ale též u některých zvířecích modelů, jako jsou například kočkodanovité opice makakové – Makak rhesus (*Macaca mullata*) (Cheverud & Buikstra 1981a, b, 1982). Některé z nejnovějších populačních studií dokonce prokázaly, že podíl genetických faktorů na celkové distribuci např. nemetrických osteologických a dentálních znaků je natolik významný, že je lze považovat za spolehlivé zástupce genetických dat (Irish et al., 2020). Lze je použít společně s genomovými markery nebo i místo nich, pokud nejsou k dispozici, a lze je využít i jako měřítko genetické vzdálenosti jedinců i populací (Hubbard et al., 2015; Irish et al., 2020; Meza-Peñaloza, 2021). Přítomnost výraznějšího pohlavního dimorfismu a nejrůznějších stresových faktorů jako je enviromentální nebo socioekonomický stres však může mít u některých znaků za následek zvýšení jejich variability a současně snížení jejich heritability (Stojanowski et al., 2018, 2019). Společně s nimi se na tom může podílet i reprodukční izolace, která směřuje ke zvyšujícímu se stupni příbuzenství uvnitř populace prostřednictvím příbuzenského mísení jedinců (inbreeding).

Právě otázka vlivu inbreedingu na distribuci morfologických i metrických znaků kostry představuje další možné úskalí, které je třeba při detekci příbuzenských vztahů na základě kostry brát v úvahu. Pokud vezmeme v úvahu strukturu společnosti a hustotu osídlení především ve starších prehistorických obdobích, ve kterých sídliště často obývalo jen několik málo rodin (Alt & Vach, 1998; Limburský et al., 2010; Velemínský & Cvrček, 2020), pak pravděpodobnost, že v těchto rodinách docházelo k příbuzenskému křížení, není zrovna nízká (Strouhal & Jungwirth, 1979). Proto se musí tento faktor při identifikaci příbuzenských vztahů na pohřebišti brát v úvahu (Johnson & Lovell, 1994; Sholts et al., 2010). U člověka rozumíme pod pojmem *inbreeding* zvláštní případ příbuzenského vztahu, kdy dochází k manželství biologicky příbuzných osob, příbuzenskému sňatku, jehož cílem je zejména posílení vztahů v rodině či v komunitě, udržení či ještě lépe posílení socioekonomického

postavení rodiny apod. (Hamanová & Hruban, 2000a). Z pohledu klinické genetiky je příbuzenské (konsanguinní) manželství definováno jako svazek dvou jedinců, kteří jsou příbuzní na úrovni bratrance a sestřenice druhého stupně (tzn. sdílejí alespoň jeden pár pra-prarodičů) nebo i vyššího stupně (Bittles, 2001). S přibývajícím stupněm inbreedingu mezi jedinci klesá variabilita jejich genomu, klesá počet heterozygotů a naopak se zvyšuje počet homozygotů (Fisher, 1949). To by pro detekci příbuzenských vztahů na pohřebišti bylo samozřejmě přínosem. Na druhé straně však Lerner (1954) vyslovil hypotézu, že navzdory předpokládané genetické uniformitě mají inbrední jedinci v rámci své linie tendenci být více variabilní než jedinci pocházející z mísení mezi liniemi. A to proto, že inbrední jedinec se může během svého vývoje vyrovnávat s vlivy vnějšího prostředí hůře a podléhat jim více, než jedinci s vyšší variabilitou genomu. Tuto hypotézu pak následně několik studií potvrdilo, nejen u člověka, ale též u modelových organismů (např. Markow & Martin, 1993; Deng, 1997). Je faktem, že inbreeding přináší pro další generace určitá rizika, zejména zvýšení exprese autozomálně recesivních nebo polygenních onemocnění, včetně některých s pozdním nástupem, dále zvýšení počtu mrtvě narozených dětí, úmrtí kojenců, vývojových vad (Bennett et al., 2002; Rudan et al., 2003; Álvarez et al., 2011). Tyto odchylky jsou známkou tzv. inbrední deprese, tedy snížení schopnosti přežití jedinců v důsledku příbuzenského křížení (Charlesworth & Willis, 2009). Naproti tomu nebylo prokázáno, že by pokrevní manželství například významně zvyšovala počet potratů. Počet porodů v takových manželstvích je dokonce statisticky vyšší, než u nepříbuzných manželství (např. Bittles & Black 2010; Hamamy et al. 2011; Tadmouri et al. 2009). Nezvyšuje se také riziko pro výskyt autozomálně dominantních onemocnění, pokud je postižený jeden z rodičů, ani pro recesivní onemocnění vázané na pohlavní chromozom X, pokud jsou oba rodiče zdraví (Hamamy et al. 2007). A stejně jako jsou známy možné negativní dopady inbreedingu na potomky, tak je také známo, že tento proces nepřináší pouze posilování negativních vlastností, ale také vlastností pozitivních. Inbreeding proto nelze v žádném ohledu brát jako čistě nežádoucí jev s negativními důsledky (Kříženecký, 1919; Hamanová & Hruban, 2000b).

Dopady inbreedingu na člověka se věda zabývá již více než jedno století (Kříženecký, 1919; Fisher, 1949), zvláště v poslední desetiletích představuje aktuální téma, a to nejen z hlediska populační genetiky (Pemberton & Rosenberg, 2014), lékařské genetiky, výskytu onemocnění (Bener et al., 2007), či ontogenetického vývoje (Bittles, 2003), ale také ze socioekonomického hlediska (Teeuw et al., 2014). Zájem badatelů směřuje zejména k příslušníkům některých panovnických dynastií, jako je královský portugalský rod Braganzů či

rod Habsburských, resp. dynastie Habsbursko-Lotrinská, u nichž byla frekvence příbuzenských sňatků a míra inbreedingu natolik vysoká, že se o nich dokonce hovoří jako o „laboratoři inbreedingu“ (Álvarez et al., 2009, 2015, 2019; Ceballos & Álvarez, 2013). Předmětem těchto studií však nejsou kosterní pozůstatky, ale historické prameny, zahrnující ikonografii, zaznamenaná onemocnění, rodokmeny a z nich vyplývající proměny míry jejich příbuznosti a inbreedingu (např. Turliuc et al., 2019; Vilas et al., 2019; Sadeghi & Sisti, 2020). Dopady příbuzenských sňatků na variabilitu lidské kostry však byly diskutovány jen v několika málo studiích a přinesly nejasné výsledky (např. Niswander & Chung, 1965).

Například, dentální rozměry vykazovaly u inbredních jedinců oproti jedincům s běžnými příbuzenskými vztahy zvýšenou variabilitu (Niswander & Chung, 1965). V případě tělesné výšky (McQuillan et al., 2012; Habicht et al., 2015) byla variabilita jedinců naopak snížená, a snížená byla také jejich průměrná výška oproti běžné populaci. Socieokonomické postavení jedinců, které se na finální tělesné výšce taktéž podílí mimo převažující genetické faktory (např. Silventoinen et al., 2003), přitom nemělo na výšku jedinců přímý vliv. Obdobně, vlivu inbreedingu byl přisuzován i zvýšený výskyt kosterních vývojových anomálií u archaických a izolovaných populačních skupin (Merbs, 2004; Ríos et al., 2015, 2019; Trinkaus, 2018). Je však nutné brát v úvahu, že prevalence určitých znaků se může mezi populacemi značně lišit (Palancar et al., 2020; Ríos & Cardoso, 2020), ačkoli to stále nevylučuje možnost, že k jejich zvýšené prevalenci vedlo právě příbuzenské mísení. Otázka vlivu inbreedingu lidskou kostru se tedy ve světle dosavadních studií stále jeví jako nedostatečně prostudovaná a diskutabilní, což i některé z nich připouští (Habicht et al., 2015). Především potom zcela chybí studie zabývající se touto problematikou u kosterních pozůstatků s genealogickou dokumentací recentních populací Evropy.

Třetí zásadní úskalí využití osteologických znaků pro detekci příbuzenských vztahů představuje metodika výpočtu heritability znaků, stanovení míry podobnosti mezi jedinci a statistických přístupů pro její vyhodnocení. Zatímco metodika pro zaznamenání nejrůznějších znaků a rozměrů na kostře je dobře ustálená a mění se jen minimálně (např. Finnegan & Faust, 1974; Szilvássy, 1974; Knussmann, 1988; Hauser & de Stefano, 1989; Turner et al., 1991; Barnes, 2012; Nikolova et al., 2018), metodologie statistického zpracování dat naráží na řadu nedostatků, popř. u některých typů znaků je i opomíjená. Výpočet heritability, jenž představuje nástroj pro vyjádření podílu genetických faktorů na celkové varianci vnějšího projevu znaku (Ghosh et al., 2021), naráží na 4 zásadní limity: 1) tento přístup je určen zejména pro kontinuální proměnné, kterým jsou metrické znaky (Sjøvold, 1984; Relethford,

1994; Carson, 2006a; Dudzik & Kolatorowicz, 2016; Gavrus-Ion et al., 2017); 2) hlavním zdrojem dat pro výpočet heritability jsou jednovaječná a dvojvaječná dvojčata (Snustad & Simmons, 2009; Paul et al., 2020), přičemž výzkum žijících lidí je limitován zejména etikou. Výzkum osteologických souborů s genealogickou dokumentací naráží na nedostatek nebo úplnou absenci dvojčat, tudíž musí být založen na jiných příbuzných prvního stupně (Sjøvold, 1984); 3) k úspěšnému výpočtu heritability je potřeba dostatek dat, přičemž většina genealogicky dokumentovaných souborů zahrnuje nedostatečný počet jedinců (Strouhal, 1992; Spence, 1996; Thurzo et al., 2001; Slavec 2004; Velemínský & Dobisíková, 2005, atd.); 4) způsob zaznamenání dat, která vstupují do výpočtu, může výslednou heritabilitu významně ovlivnit (např. Carson, 2006b; Stojanowski et al., 2019; Paul et al., 2020). Kromě toho, heritabilita se u různých typů znaků může značně lišit. Například heritabilita nemetrických dentálních znaků se jeví jako nižší, než heritabilita dentálních rozměrů (Stojanowski et al., 2018).

Většina studií zabývající se distribucí morfologických a metrických znaků kostry v souvislosti s možným vlivem genetických faktorů se tedy ubírala jiným směrem. Porovnávaly podobnost jedinců zejména popisným způsobem, většinou však jen na základě několika málo znaků či rozměrů (např. Spence, 1996; Vlček, 1997; Drozdová, 2006). Pokud se nějaké studie zabývaly statistickým zpracováním dat, šlo zejména o porovnání frekvencí a variability zaznamenaných znaků s referenčními soubory (Case et al., 2017). Matematickém vyjádřením míry podobnosti mezi jedinci na základě rozsáhlejších datových souborů se dosud zabývalo jen několik studií (Wiltschke-Schrotta, 1988; Alt & Vach, 1991; Heinrich & Teschler-Nicola, 1991; Gemmetrich-Pfister, 1999; Velemínský & Dobisíková, 2005; Paul & Stojanowski, 2017). Většina z nich však byla zatížena značnými nedostatky. Jedná se zejména o závislost výsledků na referenčních populačních frekvencích, tedy obtížnou srovnatelnost výsledků napříč časem a prostorem (Velemínský & Dobisíková, 2005), a/nebo je současně výpočet konkrétní hodnoty pouze pro jednoho jedince (Wiltschke-Schrotta, 1988). Dalšími limity jsou citlivost metod na neúplnou zachovalost materiálu, nemožnost odečtení všech znaků u všech porovnávaných jedinců, nízký počet jedinců, či podmínka nezávislosti hodnocených znaků (Alt & Vach, 1991; Heinrich & Teschler-Nicola, 1991; Gemmetrich-Pfister, 1999). Případně byla na zvolenou formu dat aplikována ne zcela vhodná statistická metoda (Paul & Stojanowski, 2017). Naprostá většina těchto metod proto byla použita pouze jedinkrát, s tím, že i někteří z uvedených autorů poznamenávají nezbytnost vytvořit optimálnější statistické přístupy (Alt & Vach, 1991; Gemmetrich-Pfister, 1999). Volba

vhodného přístupu je hraje v problematice příbuznosti a utváření kostry zcela zásadní roli (Velemínský & Dobisíková, 2005).

2.2 Příbuznost, životní podmínky a zdravotní stav

Na jedné straně existují morfologické osteologické znaky, jejichž vnější projev (fenotyp) je významně podmíněn genetickou složkou. Potom také ale existují znaky, jejichž projev je podmíněn zejména negenetickou složkou, na jejichž vnějším projevu se podílí především životní podmínky a zdravotní stav (Saunders, 1978; Capasso et al., 1999). I tyto znaky jsou do ale určité míry geneticky podložené a lze pro detekci příbuzenských vztahů do značné míry využít. Uvažovalo se o tom již před více než půl stoletím, a to včetně familiární paleopatologie (Ullrich, 1969). Na základě výpočtů heritability se ukázalo, že genetické predispozice je možné nalézt i u takových znaků, které jsou spojovány spíše se socioekonomickým postavením jedinců, působením nespecifického stresu, fyzickou zátěží, či různými onemocněními. Je to např. lineární hypoplazie skloviny (Lawrence et al., 2021), kazivost a postup ztráty zubů za života (Boraas et al., 1988), nebo změny svalových a vazivových úponů (Kuželka, 2018; Herrou et al., 2021) apod. Je však nutné brát v úvahu, že tyto znaky se nemusí vyskytovat pouze u biologicky příbuzných jedinců. Příkladem může být situace, kdy jedince spojuje míra fyzické zátěže skrze jejich socioekonomické postavení a/nebo pohlavní příslušnost. To se odráží ve změnách vyvinutí či charakteru míst svalových a vazivových úponů a charakteru degenerativních změn kloubních plošek či v průběhu osifikace, a přitom se může jednat o biologicky příbuzné (Angel et al., 1987; Yammine, 2013; Kuželka, 2018; Cockerill et al., 2021). Nicméně právě proto může analýza znaků významně ovlivněných životními podmínkami zásadním způsobem přispět ke zvýšení pravděpodobnosti určení biologické afinity mezi jedinci (např. Vlček, 1997; Kuželka, 2018). Zvláště je-li současně přihlédnuto k možnému podílu genetických faktorů na jejich utváření, i když ten nemusí být tak významný, jako u skupin znaků uvedených v předcházející podkapitole (Lawrence et al., 2021).

Životní podmínky a zdravotní stav ve vztahu k příbuznosti se však na kostře nemusí odrážet pouze prostřednictvím vnějších morfologických znaků. Další možný způsob, jak analyzovat možný dopad příbuzenských vazeb na lidskou kostru, jak těch biologických, tak i těch sociálních, je analýza chemického složení kostí, konkrétně hodnoty a poměr stabilních izotopů uhlíku a dusíku (Longin, 1971; Bocherens, 1992). Tento přístup představuje nejčastější způsob rekonstrukce stravy minulých populací (Lee-Thorp, 2008). Kromě toho experimentální studie prokázaly, že frakcionaci a distribuci izotopů v těle mohou ovlivňovat

také např. patofyziologické procesy, zejména ty doprovázené kachexií (tzn. vyhubnutím, metabolizováním tělesných proteinů) a tím změněnou dusíkovou bilancí organismu (Welle, 1999; Reitsema, 2013; Salesse et al., 2019). Kachexie je projevem řady chronických onemocnění, jako je TBC, nádorové stavy atd, ale objevuje se i u přestárlých osob (stařecká kachexie) či u jedinců dlouhodobě znehybněných (např. Welle, 1999; Reitsema, 2013). Přitom onemocnění (nejen ta s důsledky pro metabolismus) nemusejí být jen získaná, ale též zděděná (Fornaciari & Giuffra, 2013; Giuffra & Fornaciari, 2013; Whitmore & Buzon, 2019). Oba typy onemocnění se přitom mohou v rodině opakovat, a jejich nástup a závažnost mohou být dokonce ještě dále ovlivněny např. zmíněným příbuzenským mísením (Rudan et al., 2003). O podílu genetických faktorů se uvažuje také u náchylnosti k samotné kachexii (Tan et al., 2008, 2011).

Většina minulých studií, které prokázaly dopad fyziologických faktorů na izotopové hodnoty uhlíku a dusíku, se však opírá o data získaná z tkání s rychlým tempem biosyntézy, jako je moč, krev, nebo vlasy (např. Fuller 2004; Mekota et al. 2006; D'Ortenzio et al. 2015). Naše znalosti o tom, jak výše uvedené procesy ovlivňují izotopové složení lidské kosti (která se obrací v řádu let nebo dokonce desetiletí), jsou stále omezené (Katzenberg & Lovell, 1999; Richards & Montgomery, 2012). Izotopové studie zaměřené na moderní kosterní soubory se známou anamnézou jsou stále velmi vzácné (Katzenberg & Lovell; 1999; Salesse et al., 2019). Omezený je také počet studií zabývajících se analýzou izotopů paleopatologických případů z archeologických nálezů (Olsen et al., 2014; Dent, 2017). Tento alternativní přístup však není pro rozvoj poznání zcela optimální, neboť je omezen pouze na patologie s kostním projevem a paleopatologická diagnóza často zůstává nejasná. Také je zde obtížné stanovit variabilitu hodnot stabilních izotopů uhlíku a dusíku u nepatologických jedinců. Absence patologických změn na kostech totiž nemusí nutně znamenat absenci patologického stavu (Wood et al. 1992; DeWitte & Stojanowski, 2015). Otázkou také je, zda jedinec postižený nemocí přežil dostatečně dlouho na to, aby byl do pomalu remodelované kostní tkáně začleněn změněný izotopový signál. Konečně je nutné brát v úvahu, že pozdější efekty určitých patologických stavů se mohou od jejich časných stádií lišit (Welle, 1999).

Hodnoty stabilních izotopů uhlíku a dusíku potom vedle patofyziologických změn mohou také reflektovat sociální strukturu populace (Kaupová et al., 2018; 2019; Rey et al., 2019; Sjögren et al., 2020). Toho by jako pomocného nástroje bylo možné využít při multidisciplinárním výzkumu pro detekci příbuzenských vazeb, či dokonce pro individuální identifikaci, zejména historických osobností (Minozzi et al., 2020). Do jaké míry jsou ovšem

rozdíly v izotopových hodnotách uhlíku a dusíku ovlivněny biologickou příbuzností jedinců, respektive jejich rodinnou příslušností a sdílením jejich životních podmínek, a do jaké míry převažuje tento faktor nad jinými faktory jako je zdravotní stav jedinců, jejich pohlavní příslušnost, porodnost u žen, či dlouhodobé změny prostředí, to zůstává dosud nezodpovězeno. Tento poznatek může zpětně přispět ke zlepšení výpovědní hodnoty izotopové analýzy v rekonstrukci stravy minulých populací.

3 Cíle práce

Disertační práce měla čtyři hlavní cíle:

1) vzhledem k výše zmíněným nedostatkům dřívějších studií navrhnout nové metodické přístupy pro hodnocení různých morfologických znaků lidské kostry a následné vyjádření míry podobnosti jedinců;

2) analyzovat morfologické a metrické znaky kostry u jedinců s doloženými příbuzenskými vztahy a zjistit, zda míra podobnosti jedinců na základě těchto znaků koresponduje se stupněm jejich biologické příbuznosti;

3) posoudit možné důsledky příbuzenských sňatků (inbreedingu) mezi jedinci a důsledky na distribuci hodnocených znaků;

4) na základě hodnot stabilních izotopů uhlíku a dusíku u jedinců s doloženými příbuzenskými vztahy a biografickou dokumentací, zjistit, do jaké míry odrážejí jejich příbuzenské vazby, životní podmínky a zdravotní stav.

4 Materiál a metody

4.1 Materiál

V rámci disertační práce bylo analyzováno 6 různě rozsáhlých genealogicky dokumentovaných osteologických souborů z období 17. až 20. století z území Čech v rozsahu 2 až 7 generací:

1) Největší ze studovaných souborů zahrnoval celkem 65 jedinců z 19. až 20. století v rozsahu 5 generací (39 mužů a 26 žen; od 1 týdne věku až do 91 let). Kosterní ostatky pocházejí z 13 rodinných hrobek a 4 hrobů z 10 římskokatolických hřbitovů na území Čech. Jedná se o členy různých větví příbuzných jedné širší rodiny (Obr. 1). V souboru se vyskytují tři příbuzenské sňatky a u dvou z nich i po nich následující generace. Většinou se jednalo o příslušníky selského stavu, rolníky a statkáře, a také o rodiny úředníků a řemeslníků.

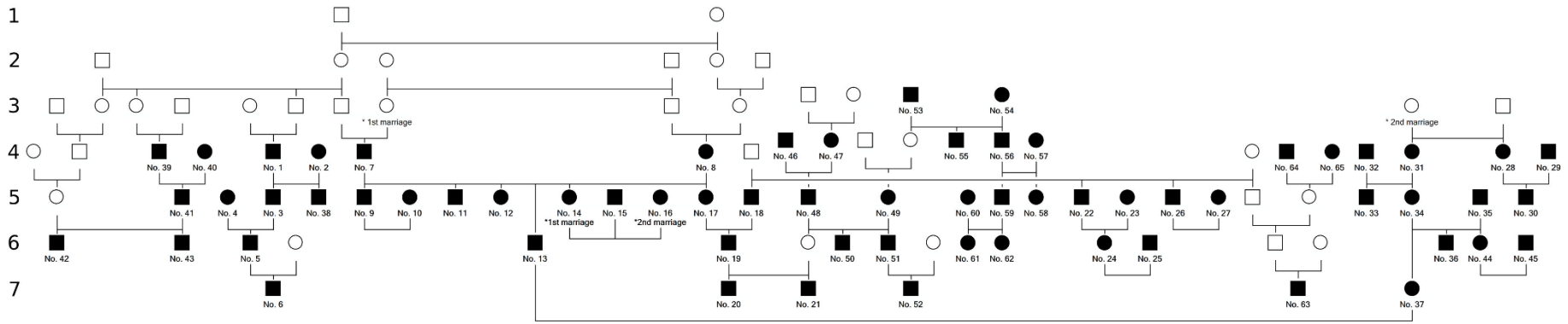
2) Druhý soubor tvořily kosterní pozůstatky 9 členů spojených šlechtických rodů Špork a Swéerts-Sporck, říšských hrabat, z období 17. až 20. století v rozsahu 7 generací (5 mužů a 4 ženy; 7 až 81 let), kteří byli pohřbeni v rodinné hrobce v kostele Nejsvětější Trojice v Kuksu. Ve třetí generaci došlo k příbuzenskému sňatku (Obr. 2).

3) 8 jedinců z 18. až 20. století ve 4 generacích (4 muži a 4 ženy; 10 až 86 let) zahrnoval soubor z rodinné hrobky statkářů z obce Vetlá (okres Litoměřice) (Obr. 3).

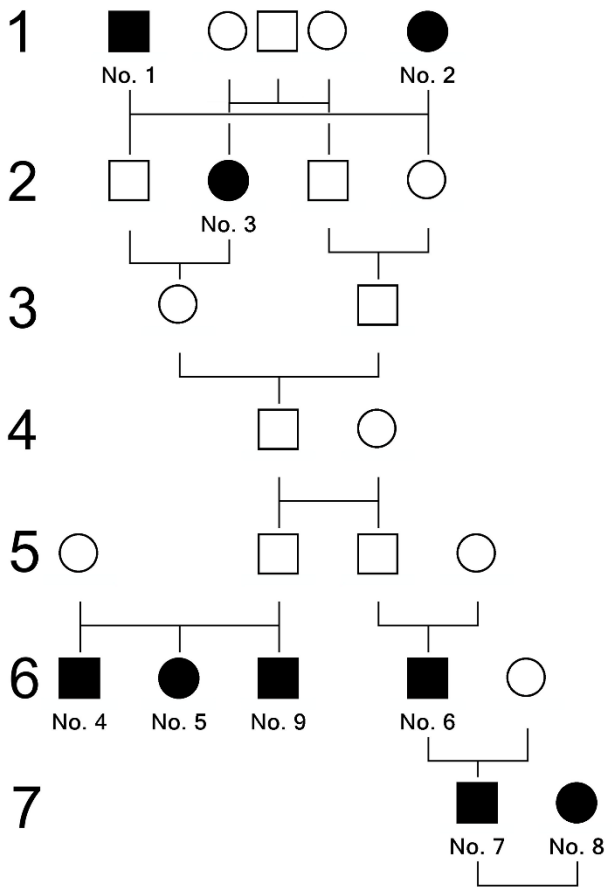
4) 4 jedinci z 19. až 20. století ve 2 generacích (rodiče, jejich dcera a její manžel; 69 až 86 let). Jedná se o rodinu dělníků (Obr. 4).

5) Poslední dva soubory, které byly studovány, pocházely ze sbírek Hrdličkova muzea člověka (Univerzita Karlova, Přírodovědecká fakulta). Zahrnovaly celkem 7 jedinců v rámci 2 rodin (v první 2 muži a 2 ženy, ve věku 36 až 84 let; ve druhé rodiče a syn, ve věku 22 až 57 let) z 19. až 20. století ve 2 generacích (Obr. 5). Ve větším z těchto dvou souborů byly zaznamenány dva příbuzenské sňatky. Jednalo se o rodinu průmyslníků a rodinu řemeslníků.

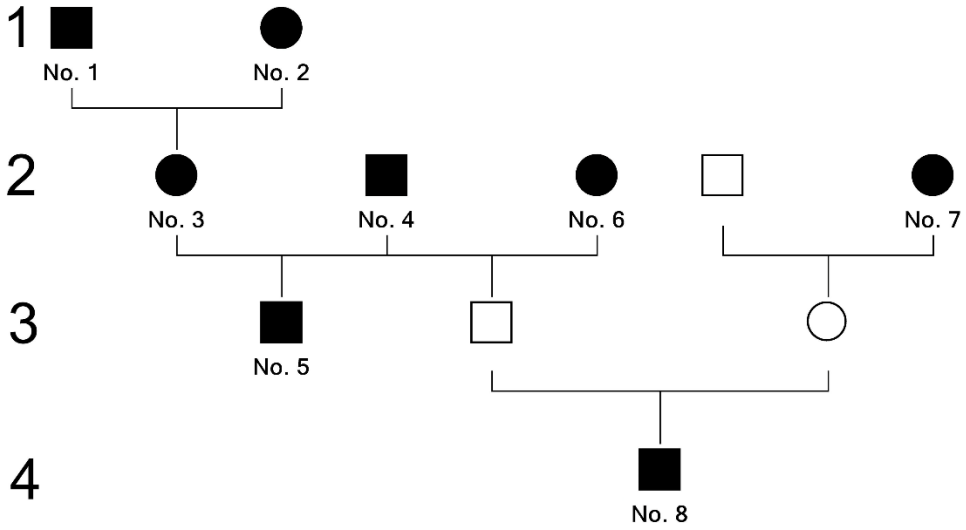
Více detailů o studovaném materiálu je podrobně uvedeno v jednotlivých předkládaných publikacích (Cvrček et al., 2018, 2020, 2021a, b, c, d; Cvrček &Velemínský, 2020, 2021; Drtikolová Kaupová et al., 2020, 2021).



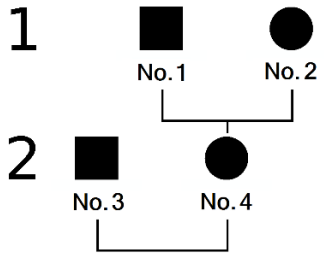
Obr. 1. Rodinné vztahy největšího ze studovaných souborů zahrnující 65 jedinců (černě vyznačeni), 19. až 20. století.



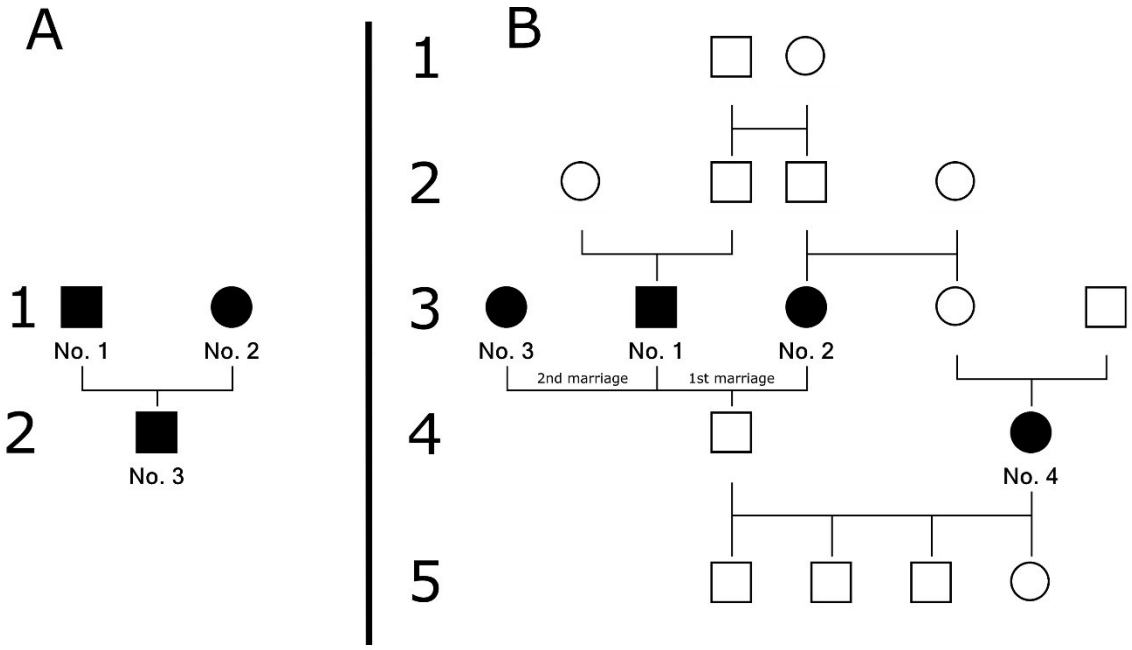
Obr. 2. Rodokmen studovaných jedinců (černě vyznačeni) ze šlechtických rodů Šporck (Nos. 1-3) a Swéerts-Sporck (Nos. 4-9). Kuks, 17. až 20. století.



Obr. 3. Vztahy mezi jedinci z rodinné hrobky sedláků ve Vetlé, 18. až 20. století (černě vyznačeni).



Obr. 4. Vztahy mezi jedinci z rodinné hrobky dělníků, 19. až 20. století.



Obr. 5. Genealogie dvou rodinných souborů (A, B) ze sbírek Hrdličkova muzea člověka, PřF UK, 19. až 20. století (studovaní jedinci černě vyznačeni).

4.2 Metody

Ke splnění cílů práce byly z morfologických a metrických znaků kostry hodnoceny morfologické nemetrické osteologické znaky (Finnegan & Faust, 1974; Hauser & De Stefano, 1989), čelní dutiny (jejich morfologie, základní rozměry) (Szilvássy, 1986; Cameriere et al., 2008), a kosterní vývojové anomálie (Barnes, 2012; Case et al., 2017), jejichž přehled je uveden v jednotlivých předkládaných publikacích k disertaci.

Pro nemetrické osteologické znaky jsme se spoluautory navrhli vlastní metodu hodnocení míry podobnosti jedinců, jejíž výsledkem je tzv. „koeficient podobnosti (similarity coefficient, SC) (Cvrček et al., 2018; Cvrček &Velemínský, 2021). Vyjadřuje míru podobnosti mezi dvěma jedinci, a čím vyšší je jeho hodnota, tím větší je míra jejich podobnosti, a tedy tím větší je pravděpodobnost jejich biologické příbuznosti. „Koeficient podobnosti“ je založen na kvantifikaci shody nebo neshody ve výskytu znaků mezi dvěma jedinci v případech, kdy znak je přítomen (+) alespoň u jednoho z nich. Tento přístup není závislý na průměrných populačních frekvencích hodnocených znaků. Jeho použití je tedy možné v kterékoli populaci a výsledky jsou srovnatelné. Metoda také umožňuje přisuzovat různým variantám vyjádření stranového výskytu bilaterálních znaků při porovnání dvou jedinců různou váhu. Tento přístup byl následně využit také při hodnocení kosterních vývojových anomálií (Cvrček et al., 2021b, c).

Pokud jde o vyjádření míry podobnosti jedinců na základě čelních dutin, neúplná zachovalost orbitální a nasální partie neumožňovala hodnotit dutiny včetně celé jejich báze, aplikovat tzv. supraorbitální linii („supraorbital line“, SOL). Tato linie představuje ohraničení báze dutin pomocí horizontální tečny, která se dotýká horních okrajů stropů očnic (Nikolova et al., 2018). Na základě práce Borovanského (1936) a Nussenové (2007) jsme proto výše popsaný přístup modifikovali a definovali tzv. vnější supraorbitální linii („external supraorbital line“, ESOL). Tato horizontální linie se dotýká horních okrajů supraorbitálních okrajů čelní kosti (margo supraorbitalis ossis frontalis). Při její aplikaci pro oříznutí báze dutiny je vždy nutné držet se frankfurtské horizontály (Nikolova et al., 2018). V našem případě byla stanovena pomocí landmarků u virtuálního 3D modelu lebky. Pro výpočet míry podobnosti jedinců podle základních rozměrů dutin jsme poté použili metodu Szilvássyho (1974), která byla původně vytvořena pro vyjádření míry asymetrie velikosti čelních dutin jedince. Míra podobnosti jedinců podle morfologických znaků dutin byla získána pomocí výpočtu cosinové podobnosti (Singhal, 2000). Data pro lineární model byla potom dále

upravena pomocí arcsinové transformace (Studebaker, 1985). Tento přístup byl při porovnání morfologie čelních dutin použit poprvé.

V případě všech hodnocených znaků byl následně vztah mezi mírou morfologické podobnosti biologicky příbuzných jedinců a stupněm jejich biologické příbuznosti testován zejména lineárního modelu (např. Zou et al., 2003). Tento přístup opět nebyl při řešení této problematicky dříve použit. Stupeň biologické příbuznosti jedinců byl za tímto účelem vyjádřen pomocí logaritmu jejich biologické vzdálenosti vypočítané na základě tabelární metody (Falconer & Mackay, 1996) podle VanRadena (1992), přičemž logaritmus byl použit pro automatickou eliminaci biologicky nepříbuzných jedinců, jejichž biologická vzdálenost je nula, a logaritmus nuly není definován (Cvrček et al., 2018). Míra podobnosti jedinců byla dále graficky zobrazována pomocí shlukové analýzy (Murtagh, 1985), K-means aplikovaným na souřadnice multidimenzionálního škálování (Hartigan & Wong, 1979) a jeho varianty SMACOF: stress majorization algorithm (de Leeuw & Mair, 2009). Pomocí statistických metod, jako je Wilcoxonův test s Bonferroniho korekcí (Siegel, 1956; Bonferroni, 1936) či ANOVA byly potom testovány rozdíly v míře morfologické podobnosti jednotlivých rodin, zejména mezi jedinci s běžným stupněm příbuznosti a jedinci se zvýšeným stupněm příbuznosti v důsledku příbuzenských sňatků. Statistická významnost rozdílů frekvencí sledovaných znaků byla ověřována pomocí Fisherova exaktního testu (Fisher, 1922) s Benjamini–Hochbergovou korekcí typu FDR (false-discovery-rate) (Benjamini & Hochberg, 1995).

Vedle morfologických znaků kostry jsme se zabývali analýzou stabilních izotopů uhlíku a dusíku v kostním kolagenu. Kostní kolagen byl izolován metodou Longina (1971) modifikovanou Bocherensem (1992). Pro statistické zpracování výsledků byly použito několik různých metod (jednorozměrné analýzy, lineární model, smíšené modely) (Drtikolová Kaupová et al., 2020). Detailní popis použitých metod je opět uveden v jednotlivých předkládaných publikacích.

5 Výsledky a diskuse: shrnutí předkládaných publikací

5.1 Příbuznost a morfologická i metrická podobnost kostry

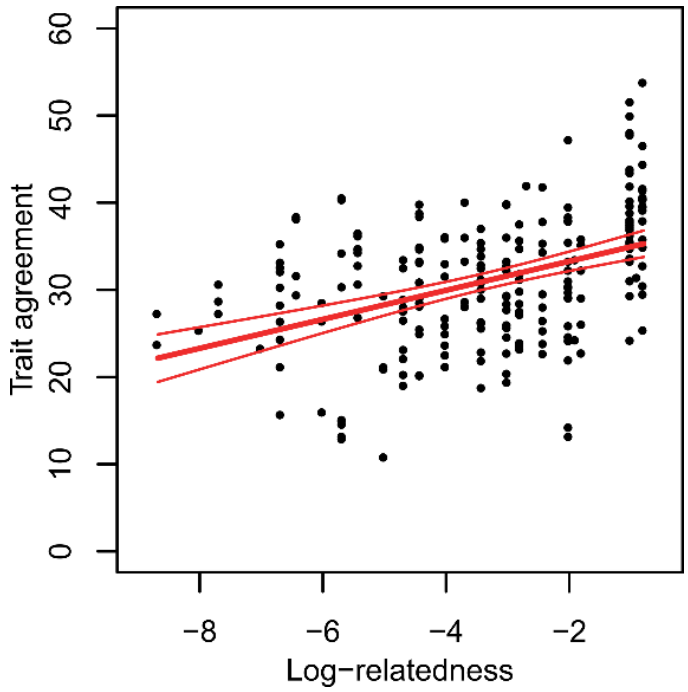
Vztahem mezi morfologickou podobností jedinců a stupněm jejich biologické příbuznosti a možným vlivem příbuzenských sňatků i dalších faktorů na variabilitu sledovaných morfologických znaků jsme se zabývali ve studiích Cvrček et al. (2018, 2020, 2021a, b, c, d), Cvrček & Velemínský (2020, 2021), a Drtikolová Kaupová et al. (2020). Výsledky je možné rozdělit to tří skupin.

5.1.1 Nemetrické osteologické znaky

Nejprve jsme se zaměřili na hodnocení morfologických nemetrických osteologických znaků (Cvrček et al., 2018; Cvrček & Velemínský, 2021), přičemž velký důraz byl kladen na metodiku výpočtu míry morfologické podobnosti mezi jedinci pomocí tzv. koeficientu podobnosti (similarity coefficient, SC).

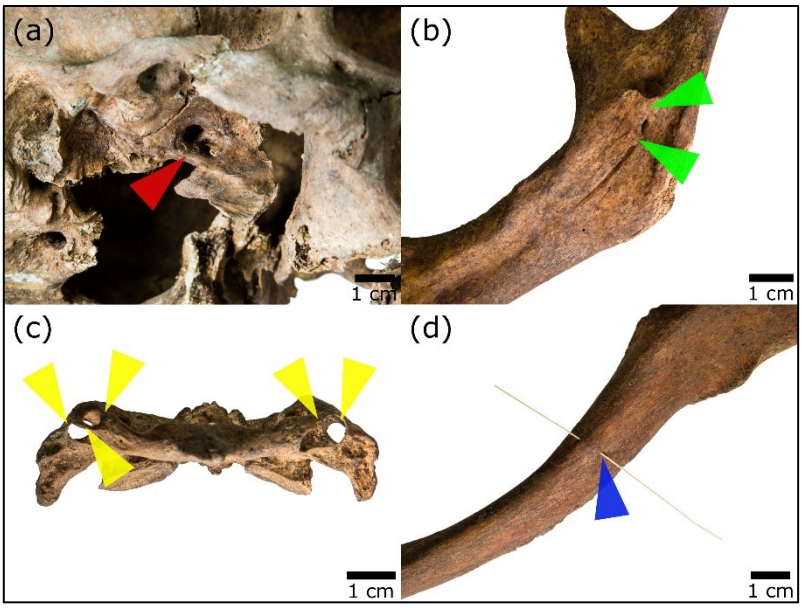
Lineární model, který byl v naší studii poprvé při řešení této problematiky aplikován (Obr. 6) prokázal, že existuje signifikantní vztah mezi mírou morfologické podobnosti jedinců vypočtené na základě SC a mírou jejich biologické příbuznosti. Kromě toho srovnání s dříve použitými metodami vyjádření míry morfologické podobnosti jedinců, jako je Essen-Möllerův vzorec (Velemínský & Dobisíková, 2005) či „faktor podobnosti" (Wiltschke-Schrotta, 1988) vytvořený na základě práce Sjøvolda (1976-77), ukázalo, že SC přináší oproti nim optimálnější výsledky. Ani tak však plně neplatí, že čím větší je stupeň příbuznosti jedinců, tím větší je míra jejich morfologické podobnosti (Cvrček et al., 2018; Cvrček & Velemínský, 2021). V řadě případů bylo možné pozorovat větší míru podobnosti mezi více vzdálenými příbuznými, jako jsou bratranci a sestřenice (bez ohledu na pohlavní příslušnost) a prarodiče a vnoučata, než mezi blízkými příbuznými, jako jsou rodiče a jejich děti, resp. sourozenci. Na tento fakt již dříve upozornily dvě předcházející studie využívající jiné metody výpočtu podobnosti jedinců (Gemmerich-Pfister, 1999; Velemínský & Dobisíková, 2005). Příčina tohoto fenoménu nicméně nebyla dosud spolehlivě vysvětlena. Naproti tomu v našem výzkumu však nebyl prokázán statisticky signifikantní rozdíl mezi jedinci s běžnou mírou biologické příbuznosti a jedinci se zvýšenou mírou biologické příbuznosti v důsledku pokrevního manželství. Byly porovnávány dvě příbuzné rodiny, o stejném počtu jedinců a generací, přičemž v jedné z rodin došlo v první sledované generaci ke dvojnásobnému příbuzenskému sňatku, v dalších generacích docházelo k již běžným manželstvím bez

příbuzenských vazeb (Cvrček et al., 2018). Navzdory předpokladu, že inbreeding obecně vede k redukci variability (např. Habicht et al., 2015) se zdá, že na variabilitu nemetrických osteologických znaků nemá, alespoň u této rodiny, velký dopad.



Obr. 6. Vztah mezi biologickou vzdáleností (osa x) a mírou podobnosti jedinců vypočítané na základě osteologických nemetrických znaků pomocí „similarity coefficient“ (osa y). Převzato ze Cvrček et al. (2018).

Dokumentované biologické příbuzenské vazby jedinců našeho souboru potom na základě srovnání s referenčními soubory podpořil opakovaný výskyt několika populačně vzácnějších znaků. Šlo zejména o znaky, související s přítomností, absencí či způsobem vyústění cévních a nervových drah a charakterem jejich průběhu (Velemínský, 2000; Velemínský et al., 2008). V souladu se závěry předcházejících studií (Ossenberg, 1970; Cheverud & Buikstra, 1981a, b, 1982) jsme zjistili, že příbuznost jedinců odrážely zejména znaky mající hyperostotický charakter, tedy spojené s nadměrným růstem kosti. Šlo o kostní můstky přemosťující průběh cévních a nervových drah (Obr. 7), jako jsou např. zadní a postranní můstky prvního krčního obratle (*ponticulus atlantis posterior et lateralis*), přemostění křídlovitých výběžků kosti klínové a spodiny lební (*ponticulus pretygospinosus et pterygoalaris*), přemostění *sulcus mylohyoideus* dolní čelisti *(ponticulus mylohyoideus*), či u klíční kosti částečné přemostění nebo úplný kanál pro některou z větví supraklavikulárního nervu (*canalis intraclavicularis*) a další (např. Selby et al., 1955; Ossenberg 1974; Saunders & Popovich, 1978; Éry, 1990; Pietrusewsky & Douglas, 1992; Kaur et al., 2012).



Obr. 7. Příklady familárně se vyskytujících hyperostotických nemetrických osteologických znaků (označeny šipkami) mající souvislost s průběhem cévních a nervových drah (Cvrček et al., 2018): *ponticulus pretygospinosus* (a), *ponticulus mylohyoideus* (b), *ponticulus atlantis posterior et lateralis* (c), a *canalis intraclavicularis* (d).

K ověření příbuzenských vztahů a posouzení možného vlivu inbreedingu na distribuci morfologických znaků by bylo ideální hodnotit i nemetrické dentální znaky (Stojanowski et al., 2017, 2018; Irish et al., 2020). U studovaných osteologických souborů je ovšem nebylo možné plně reprezentativně vyhodnotit. Hlavní překážkou byly velmi časté intravitální či postmortální ztráty zubů u většiny jedinců, a v případě zachovalých zubů pak značná abraze okluzních ploch korunek, kazy, či protetické úpravy, zejména korunky a můstky z nejrůznějších materiálů, jako jsou polymerní pryskyřice, porcelán či drahé kovy (Velemínský & Dobisíková, 2005; Cvrček et al., 2018).

5.1.2 Čelní dutiny

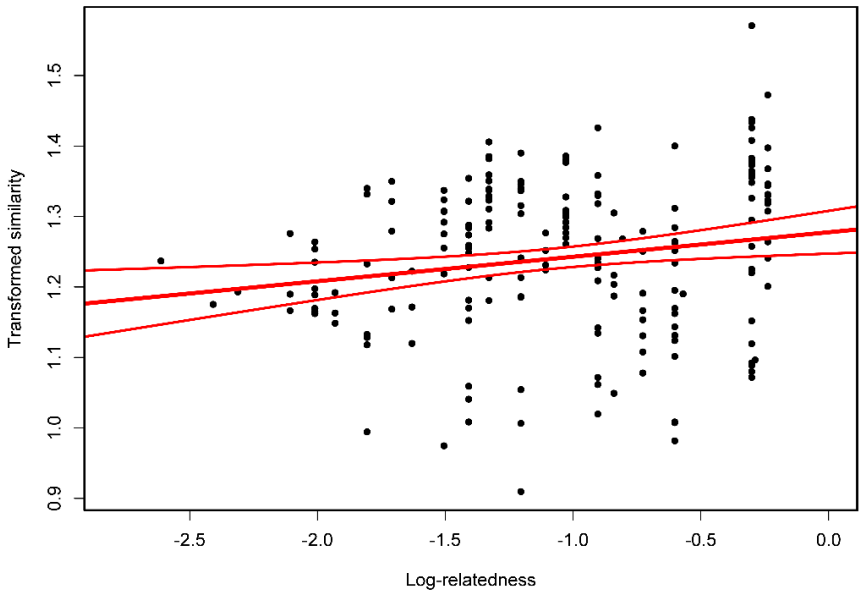
Předmětem druhé oblasti studia vztahu morfologických znaků a biologické příbuznosti bylyzákladní rozměry čelních dutin (*sinus frontalis*) a jejich morfologické znaky (Cvrček et al., 2020,2021d; Cvrček & Velemínský, 2020).

Dvě studie (Cvrček et al., 2020, 2021d) byly založeny na základním měření virtuálních povrchových 3D modelů čelních dutin (celkový povrch a objem, a maximální výška a šířka) získaných pomocí počítačové tomografie (CT) lebek a hodnocení několika morfologických znaků jejich 2D siluet, které byly vybrány na základě již dříve publikovaných studií, jako je např. jejich typ, asymetrie, počet laloků, apod. (Szilvássy, 1986; Yoshino et al.,

1987; Reichs, 1993). Vymezení báze dutin pro zpracování dat bylo založeno na novém přístupu využívajícím „externí supraorbitální linii“ (ESOL). Kromě výpočtu míry podobnosti jedinců na základě jednotlivých metrických a morfologických proměnných a vztahu výsledných hodnot k jejich známému stupni příbuznosti jsme se v první řadě zaměřili na úspěšnost klasifikace pohlaví jedinců na základě hodnocených rozměrů dutin.

Výsledky ukázaly, že mezi muži a ženami nebyl v rozměrech dutin statisticky signifikantní rozdíl a klasifikace pohlavní příslušnosti nebyla úspěšná tak, jako u jiných souborů. I když se může zdát, že toto zjištění je v přímém rozporu s výsledky studií, které napříč různými populacemi prokázaly mezi muži a ženami signifikantní rozdíly (např. Benghiac et al., 2015; Michel et al., 2015; Motawei et al., 2016; Akhlaghi et al., 2016; Choi et al., 2018), ve skutečnosti obě naše studie jejich výsledky pouze zpřesňují. Jako nejpravděpodobnější vysvětlení se totiž jeví skutečnost, že studie zjišťující pohlavní rozdíly napříč populacemi analyzovaly jedince biologicky nepříbuzné, náhodně vybrané z populace. Naproti tomu, náš výzkum byl založen na biologicky příbuzných jedincích, u nichž můžeme předpokládat stírání pohlavních rozdílů právě v důsledku působení genetických faktorů (Cvrček et al., 2020, 2021d). Tento předpoklad podporuje i zjištění, že naměřené rozměry dutin zbavené faktoru velikosti pomocí geometrického průměru (Mosimann, 1970) odrážejí dokumentované příbuzenské vztahy jedinců podstatně méně, než neupravené rozměry (Cvrček et al., 2021a).

U největšího ze studovaných souborů, který zahrnoval 41 jedinců z různých větví příbuzných jedné širší rodiny (Cvrček et al., 2020), bylo zjištěno, že v případě rozměrů příbuznost signifikantně odráží pouze maximální výška dutin. Nicméně, při porovnání jedinců s běžným stupněm příbuznosti a jedinců pocházejících z dvojnásobného příbuzenského sňatku se u všech rozměrů ukázalo, že u jedinců zatížených inbreedingem je variabilita míry jejich podobnosti menší a míra podobnosti naopak větší než u jedinců s běžnými rodinnými vztahy. Statisticky signifikantní rozdíl byl nalezen ale pouze u jejich celkového povrchu a objemu. Pokud jde o morfologické znaky, i v jejich případech byl zaznamenán statisticky signifikantní vliv biologické příbuznosti na míru podobnosti jedinců (Obr. 8), i když v menší míře než u osteologických nemetrických znaků.

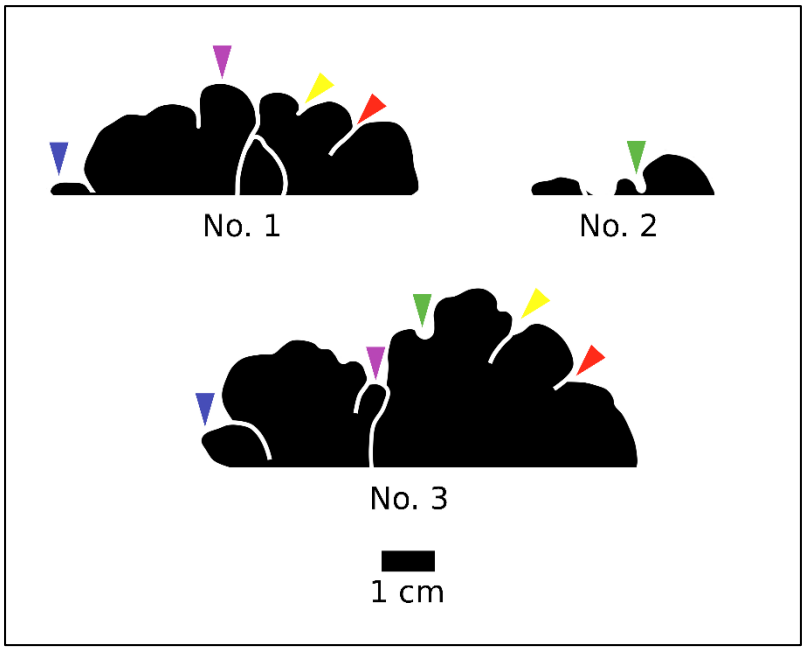


Obr. 8. Vztah mezi biologickou vzdáleností (osa x) a mírou podobnosti jedinců vypočítané na základě morfologických znaků čelních dutin (osa y). Převzato ze Cvrček et al. (2020).

Srovnání míry morfologické podobnosti jedinců s běžnými rodinnými vztahy a jedinců zatížených inbreedingem ovšem neukázalo mezi těmito skupinami statisticky signifikantní rozdíl. Nicméně variabilita míry podobnosti jedinců rodiny s příbuzenským sňatkem byla oproti srovnávací skupině jedinců menší. V neposlední řadě, podobně jako u nemetrických osteologických znaků, bylo na dutinách studovaných jedinců nalezeno několik specifických morfologických odchylek, které ještě více podporovaly nám známé biologické příbuzenské vazby mezi jedinci.

Ve třetí studii zabývající se čelnímu dutinami (Cvrček & Velemínský, 2020) jsme potom měli k dispozici pouze předozadní rentgenové snímky, u nichž bylo navíc díky velmi dobré zachovalosti možné použít již dříve zavedené ohraničení báze dutin pomocí supraorbitální linie (SOL) (Nikolova et al., 2018). V tomto případě, s ohledem na velmi malý počet jedinců (sedm jedinců ze dvou rodin, 3 + 4), jsme se zabývali pouze morfologickými znaky dutin ve vztahu k dokumentovaným rodinným vztahům, avšak za použití stejné metodologie jako v předcházejících dvou studiích (Cvrček et al., 2020, 2021d). Nízký počet jedinců podmínil, že lineární model nebylo možno zde vytvořit. Přesto i výsledky této studie ukázaly, že výskyt individuálních morfologických znaků čelních dutin koresponduje s mírou příbuznosti jedinců. Největší míra podobnosti byla zjištěna mezi otcem a synem, a mezi matkou a synem (Obr. 9). Oproti tomu morfologická podobnost mezi nepříbuznými rodiči

byla nižší. U více vzdálených biologicky příbuzných, jako jsou bratranec a sestřenice či neteř a strýc nebo neteř a teta nebyla shledána žádná zjevná morfologická podobnost.



Obr. 9. 2D siluety čelních dutin některých blízce příbuzných jedinců (No. 1 a 2 manželé a jejich syn No. 3) získaných na základě RTG snímků lebek s vyznačenými podobnostmi v jejich utváření. Převzato ze Cvrček & Velemínský (2020).

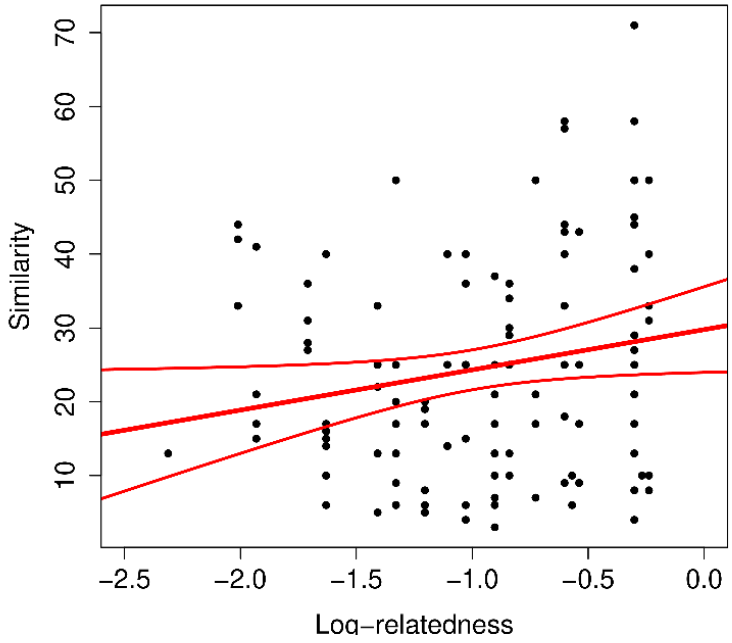
5.1.3 Kosterní vývojové anomálie

Konečně, třetí studovanou skupinu morfologických znaků představují kosterní vývojové anomálie (Cvrček et al., 2021 a, b, c; Drtikolová Kaupová et al., 2020). Na rozdíl od předchozích skupin znaků je tato skupina vysloveně na pomezí klasických morfologických znaků a patologických změn, neboť některé znaky zde mohou mít také klinický význam a negativně ovlivňovat život svého nositele.

Případová studie na kosterních pozůstatcích desetiletého chlapce žijícího a zemřelého v období 1. světové války (1907–1917) ukázala, že na distribuci kosterních vývojových anomálií se kromě možných stresových faktorů, jako je nedostatečná výživa či nespecifický stres v podobě onemocnění, zjevně podílí i genetické faktory (Cvrček et al., 2021a). V rámci doložených vztahů v rodinném souboru se chlapec ve výskytu některých osteologických anomálií shodoval zejména se svým dědem a polorodým strýcem. Jednalo se především o poruchu osifikace obratlových oblouků.

Zjištění předcházející studie potom následně potvrdil rozsáhlejší výzkum na základě vyhodnocení výskytu téměř devíti desítek anomálií, který zahrnoval 34 příbuzných jedinců

v rozsahu čtyř generací (Cvrček et al., 2021b), a výzkum 7 příslušníků šlechtického rodu Swéerts-Sporck v rozsahu čtyř generací (Cvrček et al., 2021c). U těchto souborů byly dříve již vyhodnoceny nemetrické osteologické znaky a čelní dutiny (Velemínský & Dobisíková, 2005; Cvrček et al., 2018, 2020, 2021d). Stanovení míry morfologické podobnosti jedinců bylo v obou případech opět založeno na použití „koeficientu podobnosti (similarity coefficient) (Cvrček et al., 2018). A v obou případech výsledky potvrdily, že míra podobnosti jedinců významně reflektuje jejich biologické příbuzenské vazby (Obr. 10). Do statistického vyhodnocení byly zahrnuty pouze ty biologicky příbuzné dvojice, jejichž míra podobnosti nabývala kladné hodnoty. Tedy jen ty dvojice, kterými má smysl se dále zabývat z hlediska podobnosti či rozdílu ve výskytu anomálií. Důvodem byla neúplná zachovalost kosterních pozůstatků, zejména jejich postkraniálních částí, kde se vyskytuje většina hodnocených anomálií. Kromě toho, většina z hodnocených anomálií má nízkou populační frekvenci.

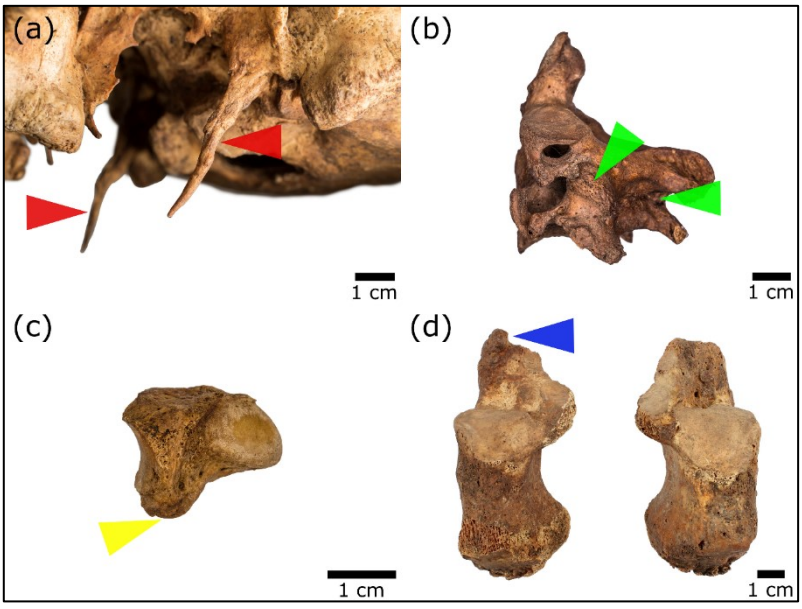


Obr. 10. Vztah mezi biologickou vzdáleností (osa x) a mírou morfologické podobnosti jedinců vypočítané na základě kosterních vývojových anomálií pomocí „similarity coefficient“ (osa y). Převzato ze Cvrček et al. (2021b).

U většího z těchto souborů však nebylo zjištěno, že by existoval statisticky signifikantní rozdíl v míře podobnosti inbredních jedinců a jim paralelně příbuzných jedinců s normální mírou příbuznosti (Cvrček et al., 2021b), podobně jako tomu bylo u nemetrických osteologických znaků (Cvrček et al., 2018). Variabilita míry podobnosti inbredních jedinců ovšem byla větší než u paralelně příbuzných rodin s normálními příbuzenskými vztahy. To by odpovídalo teorii Lernera (1954) o vývojové nestabilitě a větší variabilitě inbredních jedinců.

Tyto výsledky je však nutné považovat za orientační, neboť mohly být negativně ovlivněny neúplnou zachovalostí pozůstatků některých jedinců. Naproti tomu v případě výzkumu kosterních vývojových anomálií rodu Swéerts-Sporck (Cvrček et al., 2021c) nebylo možné případný vliv inbreedingu na morfologii jedinců objektivně posoudit kvůli nízkému počtu studovaných jedinců s výraznějšími generačními rozestupy. Vzhledem ke zvýšené míře morfologické podobnosti některých jedinců s větší mírou biologické vzdálenosti však jeho vliv nelze zcela vyloučit.

Stejně jako u předcházejících znaků byly ve studovaných rodinách nalezeny znaky, které je možné na základě srovnání s jejich výskytem u referenčních populací považovat do jisté míry za rodinné. Jejich analýza přitom také přinesla významné poznatky z hlediska jejich možných klinických projevů. V případě většího ze souborů (Cvrček et al., 2021b; Obr. 11) to byl zejména prodloužený bodcovitý výběžek (*processus styloideus elongatus*), s délkou větší než 3 cm, který může vyvolat tzv. Eaglův syndrom, vyznačující se např. možnou poruchou řeči v důsledku tlaku na měkké tkáně krku (Eagle, 1937). V našem případě však na základě dostupné biografické dokumentace studovaných nositelů této vady nebylo možné potvrdit přítomnost tohoto syndromu.



Obr. 11. Příklady familárně se vyskytujících kosterních vývojových anomálií (označeny šipkami): *processus styloideus elongatus* (a), vrozený srůst druhého a třetího krčního obratle (b), aplázie *hamulus ossis hamati* (c), a „anteater nose sign“ (d). Převzato ze Cvrček et al. (2021b).

Dále byly opakovaně zjištěny různé formy vrozených srůstů krčních obratlů, zejména srůst druhého a třetího krčního obratle. Tato vada je v literatuře považována za dostačující

argument k tomu, aby u jeho nositele byl konstatován tzv. Klippel-Feilův syndrom, v našem případě většinou II. typu (Barnes, 1994). I ten je spojován s dalšími klinickými projevy, jako jsou jiné změny kostry a měkkých orgánů. Ačkoli někteří jedinci s touto anomálií vykazovali více kosterních anomálií, v rámci souboru nebyl nalezen jedinec, u kterého by byly doloženy klinické projevy zmíněného syndromu. Naproti tomu u dalších potenciálních familiárních znaků, jako je nevyvinutí (aplázie) hákovitého výběžku hákovité kosti (*hamulus ossis hamati*), nebo tzv. „anteater nose sign" asociovaný s vrozeným srůstem patní a loďkovité kosti (kalkaneonavikulární koalice), nejsou popisovány významné klinické projevy.

V kontrastu s těmito výsledky, u členů šlechtické rodiny Swéerts-Sporck (Cvrček et al., 2021c) dominovaly jednoznačně vzájemné morfologické posuny jednotlivých částí páteře, a to především mezi krčním a hrudním, a mezi hrudním a bederním úsekem, včetně výskytu krčních žeber nebo naopak neúplného vyvinutí (hypoplazie) dvanáctého páru žeber. Všechny tyto znaky jsou různými projevy poruchy segmentace obratlů (zde zejména tzv. kranializace), který však zpravidla nemá klinický význam (Barnes, 2012). Zdá se tedy, že pro každou rodinu existují různé „vzory" ve výskytu kosterních anomálií. To kosterním anomáliím, trochu paradoxně společně s jejich zpravidla vzácnou populační frekvencí, dává značný význam pro detekci příbuzenských vztahů i proces individuální identifikace.

Na základě všech našich výše zmíněných studií jsme se pokusili odhadnout pravděpodobnost biologické afinity u čtyř jedinců s předčasně rozvinutými a rozsáhlými degenerativně-produktivními změnami kloubů z Prahy z období raného středověku (Drtikolová Kaupová et al., 2020). Neúplná zachovalost kosterních pozůstatků v tomto případě neumožňovala systematické vyhodnocení nemetrických osteologických znaků ani čelních dutin. Nicméně jedinci se shodovali pouze ve výskytu některých populačně běžných nemetrických znaků na lebce. Avšak u tří jedinců bylo nalezeno několik vývojových odchylek skeletu: rozštěp oblouku u šestého krčního obratle, rozštěp oblouků u křížové kosti, spondylolýza. Na první pohled se mohou zdát odlišné a vzniklé na pozadí onemocnění jedinců. Na základě diferenciální diagnostiky jsme dospěli k závěru, že jedinci trpěli mírnější formou epifyzární dysplasie. Tyto anomálie svým charakterem však představují velmi podobné změny, pouze jinak lokalizované, které je možné považovat spíše za odraz biologických vazeb jedinců, než za důsledek patologických stavů (Thurzo & Beňuš, 2003; Cvrček et al., 2021a). Také zjištěné onemocnění u těchto jedinců a jeho výskyt na pohřebišti pouze u těchto jedinců také podporuje pravděpodobnost jejich biologické příbuznosti (Drtikolová Kaupová et al., 2020).

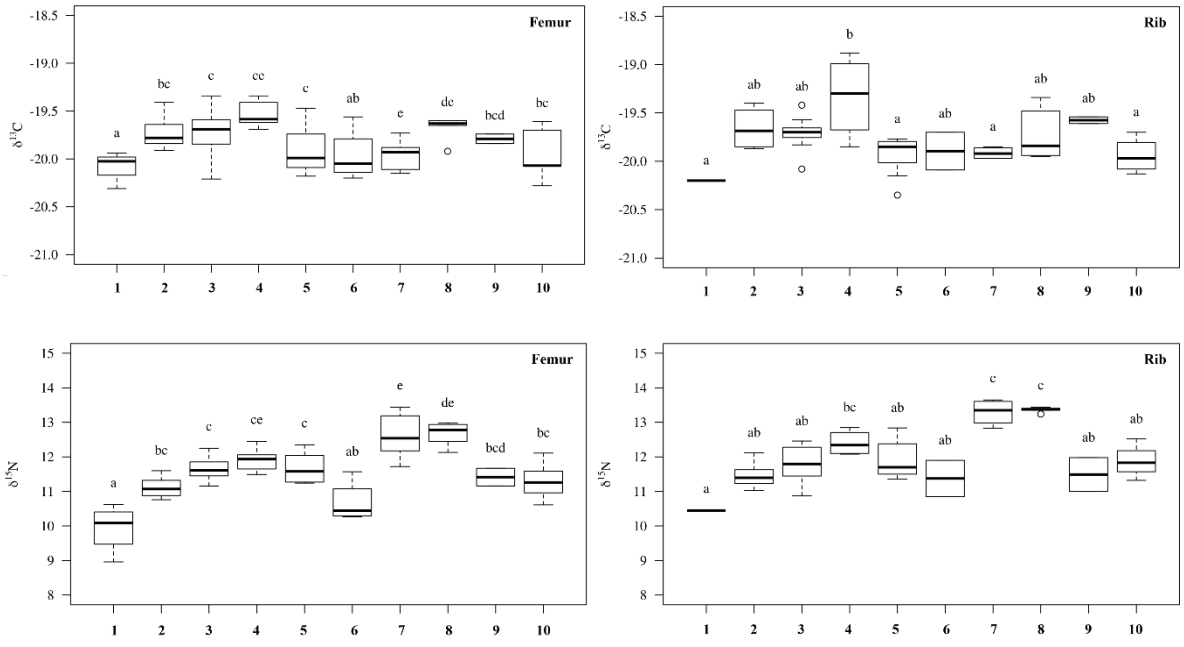
5.2 Příbuznost, životní podmínky a zdravotní stav

Soubor jedinců s genealogickou a biografickou dokumentací nám umožnil také analyzovat, které faktory známé ze života studovaných jedinců mohou významně ovlivňovat hodnoty stabilních izotopů uhlíku a dusíku (Drtikolová Kaupová et al., 2021). Sledoval se jejich vztah k příbuznosti a životním podmínkám podmíněným i socioekonomickým postavením, zdravotním stavem, proměnám prostředí v průběhu času, pohlaví příslušností, počtem porodů apod.

Hodnoty stabilních izotopů uhlíku a dusíku byly měřeny v žebrech a stehenních kostech 65 jedinců pocházejících z 10 rodin různého socioekonomického postavení z 19. až 20. století. Jedinci trpěli širokou škálou chronických onemocnění, které často i vedly k jejich úmrtí, jako např. nádorová onemocnění, tuberkulóza, ledvinová onemocnění apod. Značná část jedinců také zemřela na tzv. sešlost věkem. Na druhou stranu jsme měli k dispozici i případy akutního úmrtí jedince, např. v důsledku mrtvice, či násilného úmrtí. Byl zaznamenán vliv roku úmrtí jedinců na izotopové hodnoty dusíku v žebrech, snad jako důsledek enviromentálních a/nebo společenských změn během studovaného období. Současně jsme zjistili zvýšení izotopových hodnot dusíku u jedinců s výskytem kostních metastáz zhoubných nádorových onemocnění (jak v kostní tkáni z místa metastáz, tak i ve zdravých oblastech kostí postižených metastázami a zdravé tkáně žebra), což koresponduje s nástupem kachexie (vyhubnutí) jedinců a negativní dusíkovou bilancí doprovázejících tento typ onemocnění, kdy vylučování dusíku z organismu převažuje v důsledku katabolismu organismu nad jeho příjmem v potravě formou bílkovin. I přesto však přítomnost chronického onemocnění obecně, jako je karcinom, tuberkulóza či tělesná sešlost v důsledku věku jedince neměla oproti předpokladům na izotopové hodnoty uhlíku a dusíku statisticky významný vliv. Stejně tomu bylo i u dalších sledovaných faktorů. Naopak statisticky významný rozdíl v hodnotách izotopů dusíku i uhlíku mezi rodinami ukázal, že hlavním faktorem ovlivňujícím hodnoty uhlíku a dusíku u jedinců jsou odlišné stravovací návyky v jednotlivých rodinách.

Nejnižší izotopové hodnoty byly zjištěny v rodině, která měla ve svém závěru nejnižší socioekonomický status, zejména dusíku (Obr. 12). Ačkoli původně patřila mezi statkáře, srovnatelné s ostatními studovanými statkářskými rodinami, ve druhé studované generaci došlo nejprve mimo zaběhnuté zvyky k decentralizaci (rozdrobení) rodinného majetku mezi potomky první studované generace. Následně příslušník druhé generace a dědic usedlosti svých rodičů, jehož ostatky jsme měli pro výzkum k dispozici, uzavřel příbuzenský sňatek, který měl socioekonomickou situaci rodiny znovu posílit. Pro děti pocházející z toho

manželství však mělo příbuzenství rodičů fatální následky, což nakonec vedlo k úplnému socioekonomickému propadu rodiny. Nejstarší syn zemřel krátce po narození, druhý syn trpěl velmi vážným duševním onemocněním a zemřel ve věku dvaceti let v ústavu pro choromyslné. Nejmladší syn a jediná dcera se sice dožili dospělosti, zemřeli však bezdětní, a tak tato rodina nakonec vymřela. Nízké hodnoty izotopů byly dále zaznamenány u rodiny námezdních dělníků.



Obr. 12. Boxploty znázorňující izotopové hodnoty uhlíku a dusíku v jednotlivých rodinách (1-10) pro žebra i stehenní kosti. Písmena znázorňují výsledek Tukeyho post hoc testu. Rodiny, které sdílejí stejné písmeno, se navzájem signifikantně neliší. Upraveno podle Drtikolová Kaupová et al. (2021).

Obecně nejvyšší izotopové hodnoty uhlíku a dusíku byly naopak nalezeny u rodin s vyššími socioekonomickými poměry jako jsou statkáři, kteří měli rovněž přístup k vyššímu vzdělání. Kromě toho, v těchto rodinách docházelo v důsledku jejich sňatkové politiky v rámci jejich společenského postavení a nadstandardním vztahům k představitelům katolické církve k opakovaným příbuzenským sňatkům. Ty však na rozdíl o výše zmíněné rodiny neměly negativní dopad na další generace, a naopak znamenaly posílení jejich vlivu v rámci tehdejší společnosti a jejich sociální, společenské vrstvy. Vedle vazeb na katolickou církev měly tyto statkářské rodiny velmi úzké vazby také například na knížecí hospodářské dvory a státní správu. Někteří mužští členové z těchto rodin se proto stávali představiteli obcí, rychtáři a posléze starosty, případně členy nejrůznějších hospodářských a dobročinných organizací. Díky tomu se někteří jejich synové mohli stát knížecími úředníky a správci, zemskými

úředníky, jejich nástupci v regionálních úřadech, případně mohli získat lékařské či teologické vzdělání. Obdobně jejich dcery se vdaly za muže stejného postavení, tedy statkáře a představitele obcí, úředníky, správce apod.

6 Aplikace výsledků v bioarcheologii a forenzní antropologii

Výsledky disertační práce nacházejí uplatnění zejména v oblasti bioarcheologie a forenzní antropologie. Poznatky získané studiem morfologických a metrických znaků kostry u genealogicky dokumentovaného materiálu mohou být přeneseny na anonymní (pre)historické soubory a tak přispět pro detekci biologické afinity anonymních jedinců (Drtikolová-Kaupová et al., 2020) a směřování dalších analýz, jako je například tolik aktuální analýza aDNA (Prüfer et al., 2021). A to zejména tehdy, pokud jsou výsledky osteologického zpracování podpořeny archeologickými poznatky. Biologickou afinitu jedinců mohou naznačovat nejen vícečetné pohřby (např. Alt et al., 1997; Adachi et al., 2006), ale také vzájemná poloha hrobů, velikost a konstrukce pohřebních jam, nebo pohřební výbava, nepřímo tedy socioekonomické postavení (např. Macháček et al., 2016).

Aktuálním příkladem využití morfologických nemetrických osteologických znaků v ověření biologických vazeb je i pohřebiště v Mikulovicích ze starší doby bronzové (Ernée et al., 2020; Velemínský & Cvrček, 2020). Analýza nemetrických osteologických znaků naznačila, že mezi jedinci z vícečetných hrobů existují ve výskytu znaků většinou značné rozdíly. Tudíž pravděpodobnost, že tito jedinci jsou biologicky příbuzní, je nízká. Předběžné, dosud nepublikované nejnovější výsledky dalších analýz, včetně DNA, tento předpoklad potvrdily. Současně se na základě použití lineárního modelu u dospělých jedinců ukázalo, že jejich biologická vzdálenost stanovená na základě genetických dat a míra morfologické podobnosti vypočítaná pomocí koeficientu podobnosti (Cvrček et al., 2018) spolu korespondují stejným způsobem, jako u genealogicky dokumentovaných jedinců (Cvrček et al., 2018; Cvrček & Velemínský, 2021), a to navzdory nepoměrně horší zachovalosti některých částí koster u většiny jedinců. Stejně tak se biologicky příbuzní jedinci shodují ve výskytu některých populačně vzácnějších znaků, jako je *canalis intraclavicularis* a další znaky spojené s vyústěním a průběhem cévních a nervových drah, což podtrhuje jejich již dříve zmíněný význam (např. Ossenberg, 1970; Pietrusewsky & Douglas, 1992; Kaur et al., 2012; Cvrček et al., 2018).

Jiným příkladem využití našich výsledků je výpočet koeficientu podobnosti (Cvrček et al. 2018) na základě nemetrických kraniálních znaků u dospělých jedinců z raně středověkého pohřebiště u druhého kostela na Pohansku (např. Macháček, 2016; Macháček et al., 2016; Sládek & Macháček, 2017). V tomto případě výsledky morfologické analýzy do značné míry korespondují s předpoklady archeologů o možné sociální struktuře populační skupiny

pochované na pohřebišti (Macháček et al., 2016) a společně směřují k vytipování příbuzenských vztahů, které by následně mohla potvrdit analýza aDNA.

Zásadní uplatnění v bioarcheologii se týká také našich výsledků izotopové analýzy. Jak se ukázalo, hodnoty izotopů dusíku a uhlíku reflektují především odlišné stravovací návyky a s tím také související socioekonomický status jedinců, to vše pod vlivem jejich rodinné příslušnosti. Naproti tomu ostatní faktory nehrají tak významnou roli, ačkoli možný vliv některých onemocnění na změnu izotopových hodnot je třeba stále brát v úvahu (Salesse et al., 2019). Tato zjištění podtrhují účinnost a význam izotopové analýzy pro rekonstrukci stravy minulých populací, analýzu jejich sociální struktury, a dokonce také pro individuální identifikaci vytipovaných pozůstatků historických osobností (Kaupová et al., 2019; Drtikolová Kaupová, 2020; Minozzi et al., 2020).

Ve forenzní oblasti se morfologické znaky lidské kostry i její rozměry uplatňují jako nástroj v procesu individuální identifikace jedince. Na jedné straně se anatomické odchylky a rozměry kostry využívají při srovnání zdravotnické dokumentace vzniklé za života se stavem zjištěným posmrtně (např. Macaluso et al., 2014; Verna et al., 2013, 2015; Christensen & Hatch, 2018). I když zůstává faktem, že osteologické znaky neumožňují mezi dvěma jedinci přesně stanovit stupeň jejich příbuznosti a rekonstruovat rodokmeny, je možné je využívat pro stanovení pravděpodobnosti jejich biologické afinity (Thurzo et al., 2002; Cameriere et al., 2008; Ricaut et al., 2010). Na straně druhé jsou sledovány celé anatomické struktury či různé skupiny znaků, a to za účelem stanovení jejich variability v populaci a pravděpodobnosti falešně pozitivní shody mezi dvěma jedinci (Cameriere et al., 2008; Palamenghi et al., 2021). Přestože např. u čelních dutin nebyl doposud prokázán statisticky signifikantní vliv příbuznosti na pravděpodobnost falešně pozitivní identifikace jedince (Cameriere et al., 2008), naše výsledky ukazují, že i tak je u obou směrů nutné možný vliv biologické příbuznosti na proměnlivost jednotlivých znaků či jejich celků (vzorů – „patterns“) brát v úvahu. Ať už jde o možné snížení jejich variability, nebo stírání pohlavního dimorfismu (Cvrček et al., 2020). To zasahuje do dalšího směru forenzní antropologie, a tím je určení pohlavní příslušnosti jako jednoho z nejdůležitějších prvků biologického profilu jedince – a tak i jeho případné individuální identifikace (např. Williams & Rogers, 2006).

Jak pro bioarcheologii, tak i pro i forenzní antropologii přitom platí, že čím širší je škála studovaných znaků, tím větší je šance na úspěšný výpočet míry podobnosti mezi jedinci

i v případě neúplné a/nebo rozdílné zachovalosti jejich pozůstatků (např. Drtikolová-Kaupová et al., 2020; Cockerill et al., 2021).

7 Směry budoucího výzkumu

7.1 Příbuznost a morfologická i metrická podobnost kostry

Potenciál genealogicky dokumentovaných osteologických souborů zahrnující i příbuzenské sňatky a po nich následující generace, které se staly předmětem mé disertační práce, nebyl dosud vyčerpán, a to především v oblasti vztahů mezi morfologií a mírou biologické příbuznosti jedinců. U řady znaků, jenž jsou zvažovány pro další výzkum, se dopad genetických faktorů na jejich variabilitu předpokládal již několik desetiletí. Avšak vzhledem k celosvětové vzácnosti dobře zachovalého genealogicky dokumentovaného osteologického materiálu (Gavrus-Ion et al., 2017) a etickým omezením při studiu žijících rodin pomocí zobrazovacích metod (Kjaer et al., 2012; Cvrček et al., 2020) ovšem bylo dosud téměř nemožné tyto předpoklady v konkrétních případech ověřit. Jedná se např. o celkový tvar hrudní kosti (*sternum*), tvarové varianty různých částí lopatky (*scapula*), či morfologické vzory intrakraniálních znaků a švů lebeční klenby (např. Hauser & de Stefano, 1989; Vlček, 1995; Eisová et al., 2019; Rojas et al., 2021; Cvrček & Velemínský, 2021; atd.). Některé z těchto znaků již byly v závěru disertační práce vyhodnoceny a předběžné výsledky potvrzují vliv biologické příbuznosti na jejich variabilitu alespoň do té míry, že existují statisticky signifikantní rozdíly mezi biologicky příbuznými a nepříbuznými jedinci, s větší mírou podobnosti a menší mírou variability znaků u biologicky příbuzných jedinců.

Familiární dispozice se v poslední době stávají předmětem intenzivního zájmu také u některých stavů, znaků obvykle považovaných za převážně získané, např. v důsledku zátěže (např. Cockerill et al., 2021), a v neposlední řadě také u osteologických znaků, jejichž variabilita je dávána do souvislosti zejména s pohlavním dimorfismem či stárnutím jedince (Vlček, 1997). Již během zpracování materiálu byly v jednotlivých rodinách, často navzdory jejich stejnému socioekonomickému postavení a pracovní zátěži (např. statkáři), zjevné rozdílně se vyskytující změny charakteru míst svalových/vazivových úponů. Současně bylo možné v jednotlivých rodinách pozorovat, že se změny charakteristické pro danou rodinu opakují i po několik generací. Další výzkum by tedy mohl směřovat k jejich exaktnímu vyhodnocení za pomoci nejrůznějších statistických a grafických přístupů. Podobně tomu je i u pohlavně dimorfních znaků, zejména na lebce, u kterých bylo již dříve zjištěno možné stírání signifikantních rozdílů mezi muži a ženami v rámci rodin (Vlček, 1997, 2000; Cvrček et al., 2020, 2021a). Chceme se proto dále zaměřit i na další významné pohlavně diagnostické znaky kostry, jako je např. morfometrie čelní kosti (Čechová et al., 2019, 2021). V tomto případě již

výzkum probíhá a předběžné výsledky naznačují, že signifikantní rozdíly mezi muži a ženami jsou způsobeny rozdíly mezi rodinami, zatímco uvnitř rodin jsou rozdíly jen minimální nebo žádné.

Další výzkum bude směřovat i k posouzení vlivu biologické příbuznosti na znaky vypovídající o stárnutí jedince, jako je např. průběh uzavírání lebečních švů. Přestože je dáván tento proces především do souvislosti s věkem jedince (Nikolova et al., 2019; Fan et al., 2020), na možnou familiární podobnost jedinců v tomto procesu poukázal již Vlček (1997). Od jeho studie se touto problematikou nikdo šířeji nezabýval. Předběžné vyhodnocení dat ukazuje, že věk hraje v postupu uzavírání švů skutečně nejvýznamnější roli, avšak vliv biologické příbuznosti také není zcela zanedbatelný a představuje další významný faktor, který se na tomto procesu podílí. Konečně, zaměříme se také na výše diskutovanou rekonstrukci tělesné výšky jedinců a její změny v souvislosti s jejich způsobem života, zdravotním stavem a stupněm příbuznosti včetně inbreedingu (McQuillan et al., 2012; Habicht et al., 2015).

Kromě osteologických souborů byla během disertační práce vytvořena také kolekce odlitků chrupu 65 žijících potomků jedinců z největšího studovaného souboru (Cvrček et al., 2018, 2020, 2021b). V tomto případě se bude výzkum ubírat dvěma směry. První z nich je morfometrická analýza tvrdého patra ve vztahu k doložené míře biologické příbuznosti jedinců. Tento výzkum již započal v rámci aktuálně probíhající diplomové práce na Katedře antropologie a genetiky člověka Přírodovědecké fakulty Univerzity Karlovy (Eva Vilímková). Podobnost tvaru zubních oblouků a tvrdého patra u blízkých příbuzných demonstroval opět Vlček (1997) u nejstarších generací přemyslovské dynastie. Dalšímu výzkumu v této oblasti za použití moderních výpočetních metod se však dosud nikdo další nevěnoval. Výsledky by mohly znamenat přínos např. pro problematiku predikce vývoje patra u pacientů s orofaciálními rozštěpy (např. Kožejová Jaklová et al., 2021). Druhým směrem, podobně jako v předchozím případě, bude ve vztahu k příbuznosti jedinců analyzována jejich míra podobnosti na základě nemetrických dentálních znaků. Důraz bude kladen na aktuální metodologii hodnocení znaků a různé přístupy statistického vyhodnocení výsledků (např. Stojanowski et al., 2019; Irish et al., 2020; Paul et al., 2020).

Konečně, chceme se také zabývat metrickou podobností jedinců, a to zejména na základě kraniálních rozměrů u největšího ze studovaných souborů. Již nyní jsme se v rámci tématu disertační práce na okraj zabývali problematikou vlivu genetických faktorů a pohlaví

na kraniální rozměry u spojených šlechtických rodů Šporků a Swéerts-Sporcků. Soubor zahrnoval muže i ženy. Minulé studie zjistily napříč různými populacemi signifikantní pohlavní rozdíly ve velikosti lebky u biologicky nepříbuzných jedinců náhodně vybraných ze studovaných populací (Gapert et al., 2009; Toneva et al., 2020). Zjišťovali jsme proto, zda tyto rozdíly jsou přítomny i v tomto případě biologicky příbuzných jedinců. Byly hodnoceny jak přirozeně naměřené rozměry na základě standardní metodologie Martina (Knussmann, 1988), tak i indexy z nich vypočítané a rozměry adjustované pomocí geometrického průměru (Mosimann, 1970; Cvrček et al., 2021d) za účelem vyloučení možného vlivu pohlavního dimorfismu na velikost přirozeně naměřených rozměrů. U všech tří variant dat byl zjištěn pozitivní vztah mezi stupněm příbuznosti a biologické vzdálenosti jedinců, a to i u neupravených naměřených rozměrů, navzdory jejich významnému pohlavnímu dimorfismu. Ve všech třech datových souborech byla navíc vyšší míra podobnosti mezi biologicky příbuznými jedinci oproti nepříbuzným jedincům. To naznačuje, že genetické faktory se mohou v rozměrech lebky odrážet bez ohledu na pohlaví, podobně jako u rozměrů čelních dutin (Cvrček et al., 2021d). Je však nutné připustit existenci dalších faktorů, které mohly výsledky ovlivnit, jako je malá velikost souboru, faktor věku studovaných jedinců, a v neposlední řadě také inbreeding v důsledku přítomnosti příbuzenského sňatku v jedné z generací studované rodiny. Výsledky této práce s názvem „Degree of similarity of cranial dimensions in a documented sample of eight members of the Sporck and Swéerts-Sporck family (Bohemia, 17th–20th centuries)“ se v době odevzdání disertační práce nachází v recenzním řízení v periodiku Slovenská Antropológia.

Výsledky studia dalších morfologických i metrických znaků lidské kostry a jejích patologických změn mohou stejně jako znaky uvedené v předkládaných publikacích disertační práce nalézt uplatnění jak v oblasti bioarcheologie, tak i v různých aspektech forenzní antropologie. Kromě toho se ale v poslední době ukazuje, že výzkum kosterní morfologie u genealogicky dokumentovaných souborů by mohl mít přesah do dalších biologických oborů. Jedním z nich je například mikrobiologie, kde se uvažuje, že střevní mikrobiom představuje sice environmentální, ale současně dědičný faktor, který významně přispívá jak morfologii, tak i hustotě kostí (Hernandez & Moeller, 2021). V neposlední řadě to může být také výzkum epigenetické dědičnosti v souvislosti s příbuzností a změnami životního prostředí v čase a prostoru. Ten je v současnosti omezen na epidemiologické studie a/nebo jen současné populace (Casas & Vavouri, 2020). Genealogicky dokumentované

osteologické soubory přitom mohou nabídnout i několik po sobě jdoucích generací v relativně dlouhém čase a širokém prostoru.

7.2 Příbuznost, životní podmínky a zdravotní stav

V této oblasti bychom se rádi zaměřili zejména na zpřesnění již získaných výsledků izotopové analýzy uhlíku a dusíku, a to 1) analýzou izotopů uhlíku a dusíku ve vlasech zkoumaných jedinců, a 2) analýzou jednotlivých aminokyselin kostního kolagenu jedinců s nádorovým onemocněním. Očekáváme, že výzkum vlasů přinese podrobnější informace o změnách metabolismu jedinců v krátkém období před jejich smrtí, a že výzkum aminokyselin odhalí, zda zjištěné signifikantní zvýšení dusíku u jedinců s metastázami v kostech bylo vyvoláno změnou stravy, či změnou metabolismu v důsledku onemocnění.

Dalším cílem bude potom pokračování výzkumu založeném na analýze stabilních izotopů uhlíku a dusíku (Drtikolová-Kaupová et al., 2021). V době odevzdání disertační práce bylo exhumováno dalších 16 biologicky příbuzných jedinců ve 4 generacích, kteří doplňují již studované jedince (Cvrček et al., 2018, 2020, 2021b; Drtikolová-Kaupová et al., 2021). Soubor je podložen biografickými údaji jedinců. Výzkum bude proto zaměřen zejména na analýzu stabilních izotopů uhlíku a dusíku ve vztahu k výživě a zdravotnímu stavu jedinců, právě s přihlédnutím k překotným změnám způsobu života jednotlivých generací. Nebudou však vynechány ani další osteologické analýzy, které se staly předmětem dosavadních publikací, ani ty, které budou předmětem budoucího výzkumu, jak bylo nastíněno výše.

V neposlední řadě by se další výzkum mohl zabývat vztahy mezi výživou, zdravotním stavem a změnami epigenetické dědičnosti. Podle nedávných studií výživa ovlivňuje změny epigenetické informace, jak na transgenerační i mezigenerační úrovni, a mezi stravou rodičů a metabolickými poruchami potomků existuje významná asociace. Tato zjištění jsou však dosud založena zejména na studiu zvířecích modelů (Roseboom & Watson 2012; Casas & Vavouri, 2020; Guo et al., 2020). U člověka se nabízí studovat tuto problematiku pomocí genealogicky dokumentovaných osteologických souborů.

8 Závěry

V rámci disertační práce jsme navrhli nové metodické přístupy pro hodnocení různých morfologických znaků i rozměrů lidské kostry a následné vyjádření míry podobnosti jedinců. Pro nemetrické osteologické znaky, případně kosterní vývojové anomálie, je to tzv. koeficient podobnosti (similarity coefficient). Hodnocení rozměrů a morfologických znaků čelních dutin pak vycházelo z nového přístupu pro jejich vymezení využívajícího tzv. „vnější supraorbitální linii“ (ESOL), přičemž míra podobnosti jedinců podle rozměrů byla následně vyjádřena převzatou rovnicí dle Szilvássyho (1974) a míra podobnosti podle morfologických znaků byla vyjádřena pomocí cosinové vzdálenosti, v této oblasti dosud nepoužité metody.

Distribuce studovaných morfologických a metrických znaků, až na výjimky jako je celkový povrch, objem a šířka čelních dutin, signifikantně koresponduje se stupněm biologické příbuznosti jedinců. Nicméně tento pozitivní trend byl patrný i u jinak nesignifikantních proměnných. V některých jednotlivých případech však existuje větší míra podobnosti mezi vzdáleně příbuznými jedinci, jako jsou bratranci a sestřenice či prarodiče a vnoučata než mezi blízkými příbuznými, mezi které patří rodiče a děti či sourozenci. Ačkoli není přesně známa příčina tohoto fenoménu, naše zjištění korespondují se závěry některých minulých studií. Kromě toho náš výzkum rozměrů čelních dutin ukázal, že genetické faktory mohou jednak významným způsobem ovlivňovat (snižovat) pohlavní dimorfismus jedinců, a pak že přirozeně naměřené rozměry odrážejí biologické vztahy více než rozměry zbavené faktoru velikosti pro odstranění možného vlivu pohlavního dimorfismu. Tato zjištění mají významný dopad pro bioarcheologii a forenzní antropologii a budou dále rozšiřována výzkumem širší škály znaků.

Kromě pozitivního vztahu mezi mírou morfologické podobnosti jedinců a mírou jejich biologické příbuznosti bylo možné pozorovat zřetelnou podobnost biologicky blízce příbuzných jedinců také ve výskytu jednotlivých konkrétních znaků. U nemetrických osteologických znaků bylo v souladu se závěry minulých studií zjištěno, že biologické příbuzenské vztahy reflektují především hyperostotické znaky související s přítomností, absencí či způsobem vyústění cévních a nervových drah a charakterem jejich průběhu, spojené s nadměrným růstem kosti. Jde o kostní můstky přemosťující průběh cévních a nervových drah, zejména ty s populačně vzácnějším výskytem, jako jsou zadní a postranní můstky na prvním krčním obratli (*ponticulus atlantis posterior et lateralis*), přemostění křídlovitých výběžků kosti klínové a spodiny lební (*ponticulus pretygospinosus et*

pterygoalaris), přemostění *sulcus mylohyoideus* na dolní čelisti *(ponticulus mylohyoideus*), a u klíční kosti částečné přemostění nebo úplný kanál pro některou z větví supraklavikulárního nervu (*canalis intraclavicularis*). Podobně bylo v genealogicky dokumentovaných souborech nalezeno také několik opakujících se populačně vzácnějších kosterních vývojové anomálií, jako prodloužený bodcovitý výběžek (*processus styloideus elongatus*), různé formy vrozených srůstů krčních obratlů, nevyvinutí (aplázie) hákovitého výběžku hákovité kosti (*hamulus ossis hamati*), tzv. „anteater nose sign" asociovaný s vrozeným srůstem patní a loďkovité kosti (kalkaneonavikulární koalice), či vzájemné morfologické posuny jednotlivých částí páteře (tzv. kranializace nebo naopak kaudalizace). V případě čelních dutin se jedinci shodovali v nejrůznějších morfologických útvarech, jako jsou různá zářezy či vyklenutí jednotlivých laloků dutin, nebo dokonce i ve stranově zrcadlové podobnosti.

Vliv inbreedingu na distribuci znaků se však jeví jako nejednoznačný. V případě nemetrických osteologických znaků nebyl v míře podobnosti jedinců pocházejících z příbuzenského sňatku a jedinců s běžným stupněm konsanguinity zaznamenán žádný významný rozdíl. Naproti tomu byla u rozměrů čelních dutin zcela zjevná snížená míra variability a zvýšená míra podobnosti jedinců se zvýšenou konsanguinitou v důsledku inbreedingu, než u kontrolní paralelně příbuzné skupiny, ačkoli signifikantní rozdíl byl prokázán jen u jejich celkového povrchu a objemu. Podobně i v případě morfologických znaků čelních dutin byla variabilita míry podobnosti rodiny s příbuzenským sňatkem byla oproti srovnávací skupině jedinců menší, ale bez signifikantního rozdílu. Větší variabilita jedinců zatížených inbreedingem v důsledku vývojové nestability tedy nebyla zjištěna. Byla však naznačena v případě vývojových kosterních anomálií, ačkoli ani zde nebyly rozdíly mezi jedinci se zvýšenou konsanguinitou a jedinci s běžnými příbuzenskými vztahy signifikantní.

Analýza stabilních izotopů v případě uhlíku a dusíku z kostního kolagenu jedinců s doloženými příbuzenskými vztahy a biografickou dokumentací potom ukázala, že příbuznost, respektive příslušnost k rodině, je hlavním faktorem ovlivňujícím izotopové hodnoty jedinců. Rozdíly mezi rodinami vyplývají z jejich odlišných stravovacích návyků, což úzce souvisí s jejich socioekonomickým pozadím. Významné změny v izotopových hodnotách dusíku byly dále zaznamenány v souvislosti s rokem úmrtí a u jedinců s kostními metastázami nádorů, avšak naproti tomu, vliv chronických onemocnění obecně se ukázal jako nevýznamný, stejně tak i vliv další sledovaných faktorů. I tyto výsledky, které budou dále zpřesňovány, představují významné zjištění pro budoucí bioarcheologický výzkum.

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social status in the early Bronze Age of southeastern Europe. *Scientific Reports*, 11(1), 1–11.

10 Přílohy

10.1 Seznam předkládaných publikací

A. * **Cvrček, J.**, Velemínský, P., Dupej, J., Vostrý, L., & Brůžek, J. (2018). Kinship and morphological similarity in the skeletal remains of individuals with known genealogical data (Bohemia, 19th to 20th centuries): A new methodological approach. *American Journal of Physical Anthropology*, 167(3), 541–556. ´

B. **Cvrček, J.**, & Velemínský, P. (2021). Anthropological research into a family tomb from Vetlá cemetery (Bohemia, 19th–20th centuries), with a focus on the morphological similarity of biologically related individuals. *Journal of the National Museum (Prague), Natural History Series*, 190(1), 45–60. https://doi.org/10.37520/jnmpnhs.2021.003

C. * **Cvrček, J.**, Rmoutilová, R., Čechová, M., Jor, T., Velemínská, J., Brůžek, J., Naňka, O., & Velemínský, P. (2020). Biological relationships and frontal sinus similarity in skeletal remains with known genealogical data. *Journal of Anatomy*, 273(4), 798–809. https://doi.org/10.1111/joa.13246

D. * **Cvrček, J.**, Velemínský, P., Jor, T., Rmoutilová, R., & Brůžek, J. (2021). Frontal sinus anatomy of the noble Swéerts–Sporck family and verification of their biological relationships using similarity analysis. *The Anatomical Record*,304(8), 1717–1731. https://doi.org/10.1002/ar.24564

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F. * **Cvrček, J.**, Drtikolová Kaupová, S., Půtová, L., & Velemínský, P. (2021). A reflection of the unfavorable living conditions caused by the First World War in the diet and health of a ten–year–old boy (1907–1917) from Vetlá cemetery, Bohemia. *International Journal of Osteoarchaeology*. https://doi.org/10.1002/oa.3004

G. * **Cvrček, J.**, Kuželka, V., Jor, T., Dupej, J., Horák, M., Naňka, O., Brůžek, J., & Velemínský, P. (2021). Familial occurrence of skeletal developmental anomalies as a reflection of biological relationships in a genealogically documented Central European sample (19th to 20th centuries). *Journal of Anatomy*. https://doi.org/10.1111/joa.13499

H. ***Cvrček, J.**, Velemínský, P., Dupej, J., Jor, T., & Brůžek, J. (2021). Kinship and the familial occurrence of skeletal developmental anomalies in the noble Swéerts–Sporck family (Bohemia, 17th to 20th centuries). *International Journal of Paleopathology*, 34, 163–167.https://doi.org/10.1016/j.ijpp.2021.07.005

I. * Drtikolová Kaupová, S., Velemínský, P., **Cvrček, J.**, Džupa, V., Kuželka, V., Laboš, M., Němečková, A., Tomková, K., Zazvonilová, E., & Kacki, S. (2020). Multiple occurrence of premature polyarticular osteoarthritis in an early medieval Bohemian cemetery (Prague, Czech Republic). *International Journal of Paleopathology*, 30, 35–46. https://doi.org/10.1016/j.ijpp.2020.04.004

J. * Drtikolová Kaupová, S., **Cvrček, J.**, Grossová, I., Hadrava, J., Půtová, L., & Velemínský, P. (2021). The impact of pathological conditions on carbon and nitrogen isotopic values in the bone collagen of individuals with known biographic data and medical history. *International Journal of Osteoarchaeology*. https://doi.org/10.1002/oa.3022

* impaktované publikace

A

Received: 14 February 2018 | Revised: 20 June 2018 | Accepted: 26 June 2018

DOI: 10.1002/ajpa.23683

WILEY American Journal of PHYSICAL ANTHROPOLOGY

RESEARCH ARTICLE

Kinship and morphological similarity in the skeletal remains of individuals with known genealogical data (Bohemia, 19th to 20th centuries): A new methodological approach

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Funding information
Ministry of Culture of the Czech Republic, Grant/Award Number: DKRVO 2018/17, National Museum; Grant Agency of Charles University, Grant/Award Number: GAUK 1276217; The National Agency for the Agricultural Research, Grant/Award Number: NAZV QJ1510141

Abstract

Objectives: This article proposes a new approach, called the "similarity coefficient" (SC) for verifying family relationships from skeletal remains using nonmetric traits. Based on this method and further analyses, the authors aim to show the degree of similarity between individuals with varying degrees of kinship, including inbred individuals.

Materials and Methods: Our sample includes the skeletal remains of 34 individuals with known genealogical data (four generations, 19th to 20th centuries). A total of 243 skeletal nonmetric traits were evaluated with respect to their anatomical characteristics. The SC was calculated by quantifying the agreement of trait occurrence between individuals. We also identified the traits that support the biological relationships of particular individuals by accounting for their population frequencies.

Results: There was a positive correlation between the morphological similarity of biologically related individuals and their biological distance. In some cases, we found greater degree of morphological similarity between first cousins than among other close relatives such as parents and children. At the same time, there was no statistically significant difference in the degree of similarity between inbred individuals and common relatives. Proven family relationships were best reflected by cranial traits, especially bone bridges associated with the courses of blood vessels and nerves.

Conclusions: The use of skeletal nonmetric traits for the detection of relatives is possible. There is a relationship between biological distance and the degree of morphological similarity in related individuals. It also appears that inbreeding, despite previous assumptions, does not lead to a significant reduction in morphological variation.

KEYWORDS

genealogical documented sample, inbreeding, kinship analysis, morphological nonmetric traits, similarity coefficient

1 | INTRODUCTION

The identification of family relationships based on the skeleton is one of the most difficult goals to achieve in bioarcheological or forensic research (Esparza et al., 2017). Earlier, the term "kinship analysis" was frequently used in connection with this objective. However, in bioarcheology, the use of this term has been criticized (Johnson & Paul, 2015; Ensor et al., 2017). We can discuss the patterns of phenotypic similarity and the possibilities of their use for kinship analysis (Stojanowski & Hubbard, 2017). There are two approaches to achieve this aim: DNA analysis (Ricaut et al., 2010; Deguilloux et al., 2014) and morphology. In this case, we use (a) osteological and dental nonmetric traits (NMTs) (Stojanowski & Schillaci, 2006; Paul & Stojanowski, 2015; Stojanowski & Hubbard, 2017), (b) selected bone dimensions (Carson, 2006a; Martínez-Abadías et al., 2009), and (c) genetically conditioned anomalies manifested in the skeleton (Anderson, 1968; Berry, 1975; Case et al., 2016). According to previous studies (Rösing, 1986, 1990; Ritschmeier et al., 1984; Ricaut et al., 2010), the greatest importance is attributed to nonmetric (NM) phenotypic traits. Dental NMTs are preferred (Turner et al., 1991; Scott & Turner, 1997; Scott & Irish,

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2017), as they are expected to have a clearer genetic basis and minimal environmental influence (Berry, 1976; Biggerstaff 1979; Nichol, 1989; Townsend et al., 1992; Alt & Vach, 1995). According to Johnson & Lovell (1994) and Prowse & Lovell (1996), dental and osteological NMTs reflect biological affinity to a similar extent. In addition, the evaluation of dental traits is significantly limited by abrasion and intravital and postmortem tooth loss (Alt & Vach, 1998; Leroux, 2012).

Researchers were already aware of the possible familial occurrence of anatomical variants by the end of the 19th century (Shepherd 1893; Symmers, 1895). Matiegka (1934) suggested the potential use of anatomical variances for the detection of relatives in the case of the Gravettian skeletons of Předmostí near Přerov. Nevertheless, research aimed directly at the verification of biological affinity in past populations did not appear until the late 1960s (Ullrich, 1969). The use of NMTs for verifying biological relationships is of utmost importance in numerous smaller groups (Velemínský & Dobisíková, 2005; Case et al., 2016), where there may be some indications (e.g., archeological) of biological relationships (Adachi et al. 2006; Česnys & Tutkuviene, 2007; Meyer et al., 2012; Brown, 2015). In some cases, NMTs were used as a supporting argument for identifying the remains of historical personalities and their family members (Molleson, 1987; Thurzo et al., 2001; Slavec, 2004).

The use of NMTs for the detection of family relationships is based on the assumption that these traits show a genetic basis (Alt & Vach, 1998). Genetic studies of animal models (Grüneberg, 1952; Deol, 1955) have shown that Falconer's model of inheritance applies to NMT (Falconer, 1965; Hauser & De Stefano, 1989).

In the case of humans, two approaches can be used to study the heritability of NMTs: X-rays of living humans (Torgersen, 1951; Selby et al., 1955, Saunders & Popovich, 1978) and genealogically documented osteology series (Sjøvold, 1984; Carson, 2006b). Both approaches have shown that many NMTs have an inherited basis. These studies have mainly evaluated features related to cranial sutures and vascular and neural pathways. Studies using documented osteological samples have also shown that the degree of morphological similarity of individuals depends on the degree of biological kinship (Lane, 1976; Rösing, 1986, 1990; Strouhal, 1992; Pietrusewsky & Douglas, 1992; Spence, 1996). These studies, however, lack a statistical representation of the degree of similarity between individuals at a particular degree of kinship. Spence (1996) and Velemínský & Dobisíková (2005) show that the greatest similarity exists between father and son. They also found a clear degree of similarity among first cousins of the same or opposite sex. On the other hand, morphological similarity among siblings was not so obvious. The greatest difference was observed among biologically unrelated family members, that is, the females who married into the family (Velemínský & Dobisíková, 2005). In some cases, there may exist a similar degree of similarity among first cousins as in the case of other closely related individuals (Gemmerich-Pfister, 1999). However, for whatever reason there may be more similarity among cousins than among closely related individuals, which has not been explained. All the above studies evaluated predominantly cranial NMTs (Berry & Berry, 1967; Howells, 1973; Molto, 1983; Hauser & De Stefano, 1989). Some authors, however, are skeptical about the use of osteological methods for the detection of family relationships (Brown, 2015), because there seems to be no simple correlation between genes and NMTs (Tyrrell, 2000). On the other hand, according present authors NMTs may be used for the detection of familial relationships (Velemínský & Dobisíková, 2005; Ricaut et al., 2010; Case et al., 2016).

Osteological samples with preserved documentation are rare on a global scale (Carson, 2006b; Ricaut et al., 2010; Gavrus-Ion et al., 2017). These samples are mostly found in family tombs (Velemínský & Dobisíková, 2005; Drozdová, 2006), church crypts (Berry, 1975; Molleson et al., 1993), or documented cemeteries (Lane 1976; Kelley, 1989; Gemmerich-Pfister, 1999). The possibility of studying trait heritability and the morphological similarity of biologically related individuals is therefore still very limited. For research purposes, we have obtained an extensive osteological sample (compared with other samples, e.g., Spence, 1996; Slavec, 2004; Velemínský & Dobisíková, 2005; Drozdová, 2006) with genealogical documentation. This sample made it possible to verify whether NM osteological traits can be used for the detection of family relationships. The aim of this work was to show the degree of morphological similarity between individuals with known genealogical relationships and to answer the following questions:

1. Do NMTs similarity patterns scale with documented genetic relationships among individuals? Can they be used for kinship analysis?
2. Is there a positive correlation between morphological similarity and the biological distance between individuals?

2 | MATERIALS AND METHODS

2.1 | Materials

The genealogically documented sample consisted of the skeletal remains of 44 adult individuals (27 males and 17 females) aged 20–90 years, and one a neonate male. They are members of five different branches of one family over four generations in the 19th–20th centuries. They came from nine family tombs in the country of Bohemia, belonging to members of one family who gave us permission to use these remains for research purposes. Exhumations were conducted under the condition of repairing the family tombs. All biographical data were known for each individual (age, sex, relationships of individuals, state of health, pictures during life, etc.); these data came from the family, regional registry offices, hospitals, and some institutions, mainly State Regional Archives in Prague, Třeboň, and Litoměřice, Prague City Archive, Archive of the Prague Archbishopric, and Vatican Secret Archives. Identification of the individuals was based on documents from the family, documents from cemeteries, and on the position of the bodies in the tomb. For each tomb, there was a list of buried individuals with complete information. The 34 best-preserved adult individuals (21 males and 13 females, generations No. 4–7, family tree: Supporting Information, Figure S1) were selected for studying biological relationships. Although the possibility exists (approximately 1 %) that some family members may not be biologically related (Larmuseau et al., 2016, 2017), the relationships and

other biographical data (sex, age, state of health, etc.) of these individuals are considered to be highly reliable; doubts do not exist. After termination of the study, the skeletal remains were reburied in their tombs. All data were stored in the archive of the Department of Anthropology of the National Museum in Prague.

Three consanguineous marriages are documented in the sample. The first instance comprises doubly biologically related spouses Nos. 7 and 8 (first cousins and second cousins, generation No. 4). The second case is the son of these spouses, No. 13, and his biologically related wife, No. 37 (generation No. 5/6). The third case of inbreeding is male No. 41 and his sons Nos. 42 and 43 (the mother of these sons is the second cousin of their father, generation No. 5–6). From the first mentioned marriage, five offspring (generation No. 5) could be evaluated: sons Nos. 9, 11, and 13 and daughters Nos. 14 and 17; and then in the next two generations Nos. 6 and 7 (males Nos. 19, 20, and 21) from daughter No. 17 were evaluated. The remains of their next daughter, No. 12 (generation No. 5), could not be included in the study due to very poor preservation. Spouses Nos. 13 and 37 had only one son, but his remains could not be examined (he was cremated). The remains of a third inbred family, which included No. 43, who was is a neonate, could also not be included in this study due to poor preservation.

We used two different skeletal samples to determine reference data for our study. These samples were mainly chosen for their chronological and geographical similarity to our genealogically documented collection. There is also partial overlap in socio-economic status.

The first sample is the recent Pachner collection (Pachner, 1937; Kujanová et al., 2008; Bigoni et al., 2010). This sample contains adult individuals who lived in the second half of the 19th century and the first third of the 20th century. The collection comprises 143 skulls (78 males and 65 females; aged 20–88 years) and a larger number of postcranial skeletons (485 individuals). Regarding the number of skulls, an adequate number of postcranial bones were evaluated. However, some parts of the postcranial skeleton were missing in the sample (ribs, patella, talus, or calcaneus), and in these cases, the remains from the second sample were evaluated.

The second sample is from the 18th century from the crypt of the Church of Our Lady Victorious in Prague (Stloukal, 2013). We evaluated 107 adult individuals (49 males, 53 females, and 5 undetermined; aged 20–60 years with 24 adults of undetermined age) and salvages of solitary bones.

2.2 | Methods

2.2.1 | Data recording methods

Patterns of phenotypic similarity were identified with NMTs used in previous studies (Sjøvold 1984; Gemmerich-Pfister, 1999; Velemínský & Dobisíková, 2005) that were assumed to have a genetic basis (243 traits: 133 on the skull, bilateral; 110 on the postcranial skeleton, 92 of which were bilateral); in some cases, their incomplete forms were also used (Hauser & De Stefano, 1989). NMTs can be divided in different ways (Ossenberg, 1970, 2013; Hanihara & Ishida, 2001a, b, c, d). In this study, we divided NMTs into seven categories based on their characteristics (Velemínský et al., 2008):

- Category A—traits related to cranial sutures (epigenetic variants): 47 traits.
- Category B—traits related to the presence, absence or nature of foramina: outlet of vascular or nerve routes or characteristics of its course ("bridging"): 86 traits.
- Category C—traits related to the presence, absence, or characteristics of joint facets and their changes: 40 traits.
- Category D—traits related to disorders of ossification, nonjunction of ossification centers or their absence: 33 traits.
- Category E—traits related to hyperostotic activity with the presence osseous tori, tubercles, spines, or bridges: 21 traits.
- Category Fa—traits related to changes in the area of muscle/ligament attachments: loss of bone tissue: 9 traits.
- Category Fb—traits related to changes in the area of muscle/ligament attachments: fibrous ossification resulting in the genesis of osseous prominences: 7 traits.

A list of all NMTs is provided in Supporting Information, file No. 2 (Tables S1–S7).

The presence (+) or absence (−) of each trait was evaluated. If a bone was damaged or missing at the site, the trait could not be evaluated (0).

Owing to frequent intravital loss of teeth and artificial tooth replacements, pronounced abrasion, and the frequent occurrence of dental caries (occasionally with fillings), we could not evaluate dental traits.

2.2.2 | Analytical methods

We have developed our own approach to express the degree of morphological similarity between individuals. It is based on a quantification of the agreement or disagreement in the occurrence of NMTs between two individuals in cases in which the trait is present (+) in at least one of the individuals. For bilateral traits, which represent the majority of traits, the traits must be present on at least one side of one of the individuals in the comparison pair.

We created a numerical expression of the degree of agreement or disagreement in the occurrence of the trait (Tables 1 and 2). For bilateral traits, each side was considered separately.

Based on the sum of agreement (A_i) and disagreement (B_i) in the occurence of all evaluated traits No. 1–n between two individuals, we calculate their "similarity coefficient" (SC) according to the following formula:

TABLE 1 Numerical expression of agreement and disagreement between two individuals for unpaired traits

	Situation	Degree of agreement: A_i	Degree of disagreement: B_i
1	Agreement of both individuals in the trait	4	0
2	Disagreement of both individuals in the trait	0	4
3	At least one individual can not be evaluated in the trait	0	0

$$SC = 100 \times \frac{A_1 + A_2 + A_3 + \ldots + A_n}{(A_1 + A_2 + A_3 + \ldots + A_n) + (B_1 + B_2 + B_3 + \ldots + B_n)},$$

where A_1, A_2, … A_n represent the degree of agreement between two individuals for trait No. 1–*n*, and B_1, B_2, … B_n represent the degree of disagreement between two individuals for trait No. 1–*n*. If two individuals do not have any currently rated traits, then SC is 0.

SC was calculated between all evaluated individuals according to the numerical expression of agreement (or disagreement) in traits occurrence, representing the basic data for further analysis:

1. We compared the degree of similarity between inbred family members and parallel-related family members with common relationships (level of significance α = 0.05) using the Wilcoxon signed-rank test (Siegel, 1956).
2. We calculated a coefficient of relationship for each possible pair in the sample (578 combinations). The coefficient was estimated using a tabular method (Falconer & Mackay 1996) based on the method of VanRaden (1992). Unlike the SC for each pair, the logarithm of their coefficient of relationship (logarithm of biological distance) was plotted. This step eliminates entirely unrelated individuals because their coefficient of relationship is always zero, so the logarithm is not defined. Thus, it is possible to focus on only the degree of kinship between biologically related individuals. We used analysis of variance to test the relationship between SC and the logarithm of biological distance for all pairs in the sample (level of significance α = 0.05).
3. The resulting degree of similarity of individuals was ordinated in two dimensions using multidimensional scaling (Gower, 1966). This approach based on distance (100 minus the SC for individuals) reconstructs the coordinates in a parametric space. The K-means method (Hartigan & Wong, 1979) was used at the coordinates to find the positions of known clusters. Every individual then belonged to the closest cluster.
4. We also used cluster analysis (Murtagh, 1985), which was performed by the complete linkage hierarchical method, where the distance between two clusters was determined as the distance between their most distant individuals. The SC of each pair was converted into a distance such that the greatest similarity indicated the smallest distance and vice versa.

Based on the work of Velemínský & Dobisíková (2005), we calculated the Essen-Möller formula (Ferák & Sršeň, 1981; Zvára, 1999) to compare our results from paragraphs Nos. 2 and 3 above. We performed this calculation in two ways: (1) based on the data used for our calculations (+, −, 0); and (2) based on traits that are completely preserved and can be evaluated in all individuals (+, −).

Independent of the previous approach, the selection of traits with a population frequency of <10% (Gemmerich-Pfister, 1999; Case, 2003) allowed us to directly capture the traits that supported the biological kinship of particular individuals within specific families and could therefore be considered to be familial. We chose possible family traits based on the frequencies (incidence on individual) of the two aforementioned reference samples from the 18th and 20th centuries. For the chosen traits, the difference in the occurrence of these traits between the genealogically documented sample and the reference samples was tested using Fisher's exact test (Fisher, 1922) with a significance level of α = 0.05. For multiple tests, *p* values were adjusted according to the Benjamini–Hochberg false discovery rate method (BH-fdr) (Benjamini & Hochberg, 1995).

All these calculations were performed in the R program.

3 | RESULTS

3.1 | Statistical results

We obtained SC between all individuals based on all traits according a numerical expression of agreement and disagreement (Tables 1 and 2 and Figure 1). We observed a relatively high degree of similarity among closely related individuals, such as mother No. 4 and son No. 5, father No. 1 and sons Nos. 3 and 38, brothers Nos. 3 and 38, mother No. 2 and son No. 3, father No. 18 and son No. 19, father No. 19 and son No. 21, brothers Nos. 20 and 21, brothers Nos. 18 and 22, mother No. 23 and daughter No. 24, and mother No. 34 and daughter No. 37. The doubly related spouses Nos. 7 and 8 (first cousins and second cousins) and their children Nos. 9, 11, 13, 14, and 17 had a special status in the family tree. The SC between

TABLE 2 Numerical expression of agreement and disagreement between two individuals for bilateral traits

	Situation	Degree of agreement: A_i	Degree of disagreement: B_i
1	Agreement of both individuals in the presence of a trait on both sides	4	0
2	Agreement of both individuals in the presence of a trait only on one side	4	0
3	Agreement of both individuals on their first side, disagreement on their second side	3	1
4	Disagreement of both individuals on both two sides, but their cross-similarity	2	2
5	Disagreement of both individuals in the presence of a trait on one side of one individual	1	3
6	Disgareement of both individuals in the absence of a trait on both sides	0	4
7	One side of both individuals cannot be evaluated, agreement on their second side	2	0
8	One side of both individuals cannot be evaluated, trait absence on their second side	0	0
9	One side of both individuals cannot be evaluated, disagreement on their second side	0	2
10	Both sides of both individuals cannot be compared or evaluated.	0	0

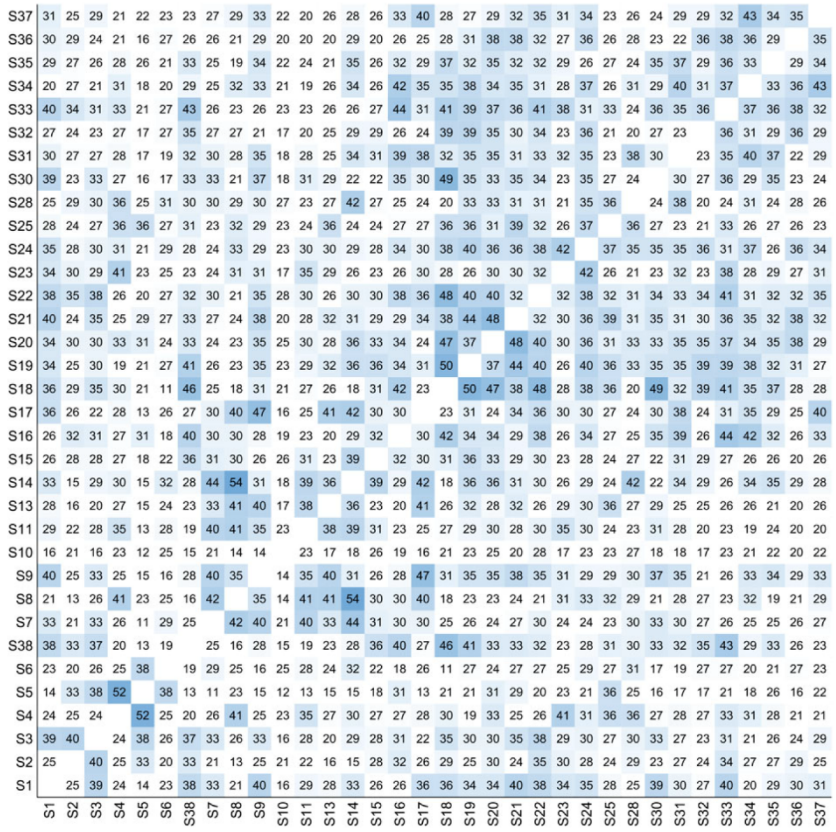


FIGURE 1 The values of similarity coefficient (SC) of all individuals (S1–S38) by all traits. The darker the color, the greater the values of SC [Color figure can be viewed at wileyonlinelibrary.com]

the two biologically related spouses male No. 7 and female No. 8 (first cousins and second cousins) was approximately the same (SC = 42) as that found in other closely related individuals with common family relationships (see Figure 1, e.g., female No. 2 with son No. 3: SC = 40; and brothers Nos. 18 and 22: SC = 48). Statistically, there was no significant difference between members of the inbred family (four generations Nos. 4–7, individuals Nos. 7, 8, 9, 17, 18, 19, and 20) and those of parallel-related family with common relationships (four generations Nos. 4–7, individuals Nos. 1, 2, 3, 4, 5, 6, and 38) using the Wilcoxon signed-rank test with p value = .26.

In some cases, greater similarity was found between the grandparent and the grandchild than between the parent and the offspring, for example, male No. 20 was more like his grandfather (No. 18) than his father (No. 19), and male No. 36 was more like his grandfather (No. 32) than his father (No. 35).

Furthermore, in some cases, there was a greater degree of similarity between first cousins than between these individuals and their other close biological relatives (siblings or parents-offspring). There was a greater degree of similarity between the doubly related spouses Nos. 7 and 8 (first cousins and second cousins) than between father No. 7 and his daughter No. 17, as an example. Furthermore, male No. 19 was more like his first cousin (No. 24) than his mother (No. 17) or his son (No. 20). Female No. 24 was then slightly more similar to her first cousin No. 19 than to her father (No. 22). Additionally, male No. 7 was more like his first cousin No. 1 than his daughter No. 17 or his grandson No. 19. Female No. 31, however, was more like her sister No. 28 and granddaughter No. 34 than her first cousin No. 8, but at the same time, she was more similar to her cousin No. 8 than to her grandson No. 36.

For comparison, the resulting values of the Essen-Möller calculation according variant A did not reflect proven family relationships. The resulting values were unrealistically high (Supporting Information, file No. 3, Figure S2). More realistic values were found for variant B (Supporting Information, file No. 3, Figure S3), but these values also did not reflect many of the family relationships.

The following is a division of all observed traits into cranial (Supporting Information, file No. 3, Figure S4) and postcranial traits (Supporting Information, file No. 3, Figure S5), (Tables 1 and 2). Cranial traits reflect proven family relationships better than postcranial traits. The degree of morphological similarity between individuals based on cranial traits was similar to the degree of similarity according to all traits (Figure 1). In terms of the division of all studied traits into categories according to their characteristics (categories A to Fb: Supporting Information, file No. 3, Figures S6-S12), the traits in category B (Supporting Information, Figure S7) had the highest noticeable values, which were the ones associated with vascular and neural routes and the characteristics of their courses ("bridging"). The degree of morphological similarity between individuals according to this trait category was similar to the degree of similarity of cranial traits (Supporting Information, Figure S4) and likewise to the degree of their similarity according to all studied traits (Figure 1). Traits in categories A

(associated with cranial sutures), C (associated with joint changes), D (ossification disorders) and E (hyperostotic traits) reflected the documented relationships considerably less than the traits in other categories. In these categories, false positives were also present to a large extent and were prevalent in some places. Significantly unsatisfactory results were obtained for categories Fa and Fb (changes in the area of muscle/ligament attachments). For the most part, the resulting values in these two categories did not correspond with the documented relationships, especially the values in category Fb.

If we relate the SC between two biologically related individuals on the basis of all traits (Figure 1) to the logarithm of their biological distances (i.e., the coefficient of relationship), an analysis of variance on that model indicated a strong dependence of these two variables (p value <.0001; Figure 2). As a comparison, there was a much smaller dependence of these two variables (p value = .04) using resulting values of the Essen-Möller formula according to variant A (Supporting Information, file No. 3, Figure S13). However, because of the degree of similarity between all individuals based on this approach and the large number of outlying values, we did not consider this result to be relevant. According to variant B, the dependence of these two variables was not statistically significant (p value = .8), (Supporting Information, file No. 3; Figure S14).

In the resulting graph of multidimensional scaling (Figure 3), the two-parent relatives of Nos. 7 and 8 and their children Nos. 9, 11, 13, 14, and 17 (marked in green) form the most distinct group. In addition, members of the single family Nos. 31, 32, 33, 34, 36, and 37 (mother and father, son and daughter, grandson, and granddaughter, respectively) are close relatives (red). The other groups are mother No. 2 and son No. 3 (blue), grandmother No. 4 and grandchild No. 6 (marked in gray), mother No. 23 and daughter No. 24 (blue), and finally, male relatives (red) Nos. 19, 20, 21, and 22 (father, sons and uncle, respectively), where male No. 18 is also a relatively close member (father of No. 19 and brother of No. 22).

A high degree of similarity among some closely related individuals was also confirmed by cluster analysis (Figure 4). In the first cluster, we again observed the close position of the children (Nos. 11 and 14) and

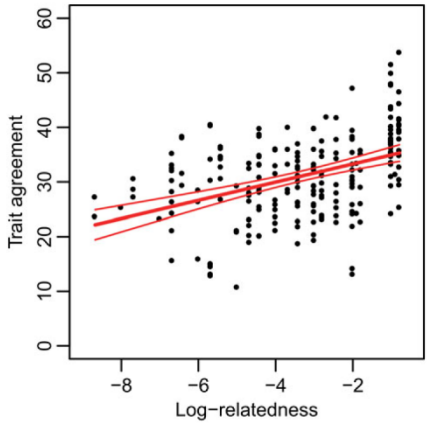


FIGURE 2 Linear model of the similarity coefficient (SC) (y-axis) based on all traits (Figure 1) by log-relatedness of individuals (x-axis). Note: log-relatedness disregards unrelated individuals [Color figure can be viewed at wileyonlinelibrary.com]

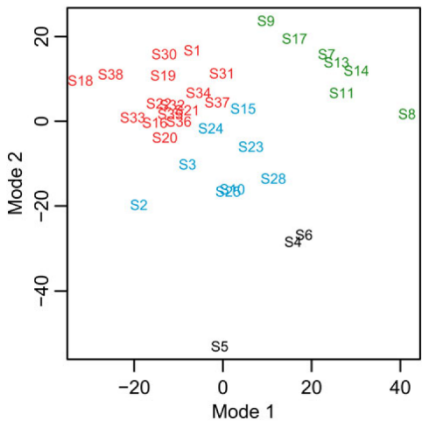


FIGURE 3 A scatter plot displaying coordinates in a parametric space assigned to each individual using multidimensional scaling. Colors indicate cluster assignment obtained by K-means applied to multidimensional scaling coordinates [Color figure can be viewed at wileyonlinelibrary.com]

siblings (Nos. 9, 17, and 13) of the doubly biologically related spouses Nos. 7 and 8. In the second cluster, the greatest similarities were between female No. 23 and her daughter No. 24, female No. 4 and her son No. 5, male No. 18 and his son No. 19, female No. 2 and her son No. 3, sisters Nos. 28 and 31, brothers Nos. 20 and 21, and female No. 37, and her parents Nos. 34 and 35. For ease of orientation in the graphs of these two analyses, we present a family tree that clearly shows the relationships between the evaluated individuals.

3.2 | Possible familial traits

In the genealogically documented sample and accounting for the reference population frequencies of the traced traits, five possible familiar traits were found (or 12, including complete and incomplete forms, Table 3). Four of the five traits were related to the routes of blood vessels and nerves (category B). In the case of the *ponticulus atlantis posterior*, an unusual double bridge was even found in male No. 7 and his son No. 9, which are both cases on the left. The fifth trait, the *facies condylaris partita*, is one of the features associated with changes in articulated surfaces (category C). The occurrences between biologically related individuals, along with their blood relations, are shown in Table 4 and Figure 6. According to Fisher's exact test, there was a statistically significant difference in the occurrence of four traits between the genealogically documented sample and the reference samples: the *ponticulus mylohyoideus* (complete and incomplete), the *ponticulus atlantis lateralis* (complete and incomplete), the *canalis intraclavicularis*, and the *facies condylaris partita* (Table 3).

In addition to these familial traits, 15 other rare or less common NMTs existed in the sample but were shared by only two to three individuals within just two generations. These NMTs cannot therefore be said to be familial at this time, yet they are well documented by the morphological similarity of closely related individuals (Table 5). Again, five of these traits were associated with the routes of blood vessels and nerves (category B), three were associated with cranial sutures (category A), four were associated with joint changes (category C), and three were hyperostotic (category E).

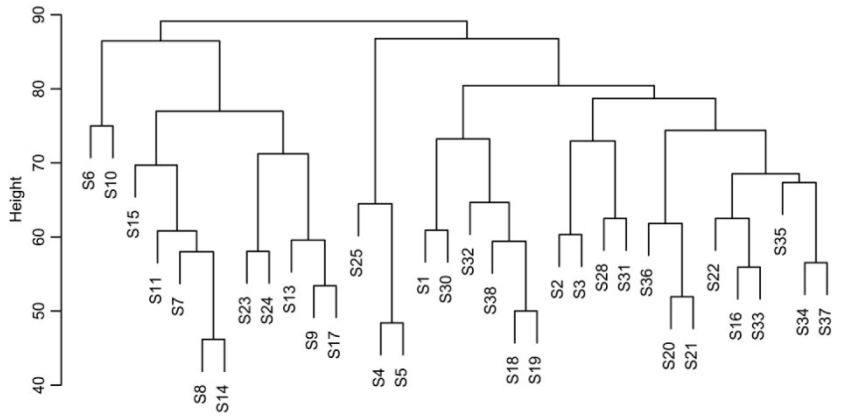


FIGURE 4 Agglomerative clustering of all evaluated individuals (no. 1–38) by similarity coefficient (SC) (all traits; Figure 1)

4 | DISCUSSION

4.1 | Statistical methods for evaluation of the morphological similarity of individuals

We have proposed a new procedure to assess the degree of morphological similarity between biologically related individuals—the SC. In the past, several statistical procedures have been proposed for verifying biological relationships (Ullrich, 1969; Wiltschke-Schrotta, 1988 based on Sjøvold, 1976–77; Heinrich & Teschler-Nicola 1991; Alt 1991; Alt & Vach, 1991, 1992, 1995, 1998). However, these methods have some limitations (Velemínský & Dobisíková, 2005), and the works of Alt (1991) or Alt & Vach (1995, 1998) were often focused on multistage dental traits. Based on the previous work of Velemínský & Dobisíková (2005), we calculated the Essen-Möller formula and tried to compare these calculations with the results of our new method. The "similarity factor" from Wiltschke-Schrotta (1988), which was based on Sjøvold (1976-77) and was also used by Velemínský &

TABLE 3 Possible familial traits found in the genealogically documented sample and their incidence compared to the incidence of these traits in the reference sample (reference frequency < 10%), with a statistical comparison of trait frequencies in the samples using Fisher's exact test

			Genealogical documented sample						Reference sample (Pachner collection)						Fisher's exact test	
			Incidence on individual			Incidence on side			Incidence on individual			Incidence on side			Incidence on individual	Incidence on side
No.	Trait:	Cat.	*N*	Inc.	%	*N*	Inc.	%	*N*	Inc.	%	*N*	Inc.	%	*p* value	*p* value
	Ponticuli basales ossis sphenoidalis:															
97	Pterygospinosus: complete	B	25	2	8.0	56	3	5.4	140	4	2.9	274	4	1.5	.313	.171
98	Pterygospinosus: incomplete	B	22	5	22.7	48	8	16.7	128	13	10.2	259	18	7.0	.227	.086
99	Pterygoalaris: complete	B	26	3	11.5	56	3	5.4	140	4	2.9	280	5	1.8	.156	.206
100	Pterygoalaris: incomplete	B	24	0	0.0	52	0	0.0	134	9	6.7	266	11	4.1	.416	.283
	Ponticulus mylohyoideus:															
110	Complete	B	27	10	37.0	58	13	22.4	54	3	5.6	108	5	4.6	.009**	.007**
111	Incomplete	B	27	12	44.4	58	15	25.9	54	10	18.5	108	11	10.2	.042*	.036*
	Ponticulus atlantis:															
112	Posterior: complete	B	20	3	15	43	6	14.0	85	12	14.1	172	19	11.1	~1.000	.604
–	*Posterior: double complete*	*B*	*20*	*2*	*10*	*43*	*2*	*4.7*	*85*	*1*	*1.2*	*172*	*2*	*1.2*	*.161*	*.251*
113	Posterior: incomplete	B	18	3	16.7	40	5	12.5	80	10	12.5	187	13	7.0	.756	.383
114	Lateralis: complete	B	19	4	21.1	41	6	14.6	77	2	2.6	160	3	1.9	.037*	.010**
115	Lateralis: incomplete	B	18	4	22.2	40	4	10	76	1	1.3	157	2	1.3	.020*	.038*
133	Canalis intraclavicularis	B	27	7	25.9	59	11	18.6	155	8	5.2	310	10	3.2	.015*	.001***
134	Facies condylaris partita	C	25	8	32.0	53	10	18.9	132	13	9.9	268	15	5.6	.024*	.010**

p values were adjusted according to the Benjamini–Hochberg false discovery rate method (BH-fdr). *N* = number of evaluated individuals/sides, Inc. = incidence, % = frequency.

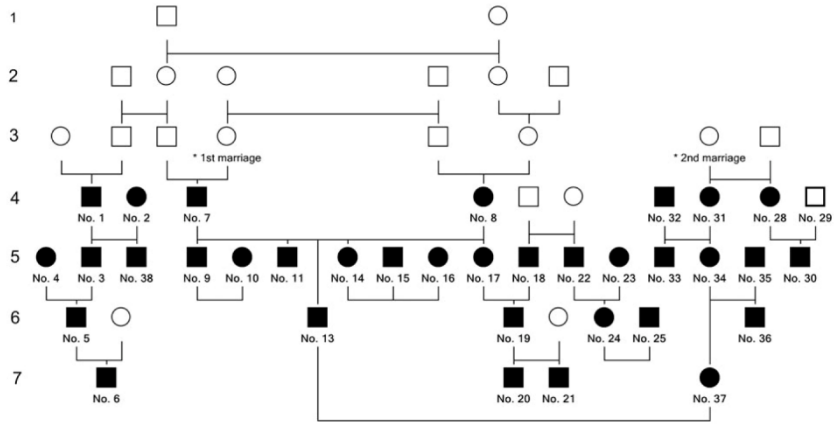


FIGURE 5 Family tree (black marks) of evaluated individuals (no. 1–38)

Dobisíková (2005), was unusable for our purposes. The resulting value is valid for only one individual. It is not an expression of the degree of similarity between two individuals. For this reason, we cannot apply the same statistical approaches used for our method to the similarity factor.

The Essen-Möller formula is derived from the Bayesian formula for calculating the likelihood of occurrence of the relevant phenomenon (Ferák & Sršeň, 1981; Zvára, 1999). This method expresses the probability of the relationship between two individuals, which is similar to our method, based on coincidence in the presence of the traits. However, we encounter some problems with this method. (1) This approach also does not reflect side differences in the occurrence of traits. (2) Knowledge of the frequencies of all observed traits in the population that the studied individuals are derived from is necessary. We performed the calculation with two variants of data: (A) based on the same data as those used in our calculations (+, −, 0) and (B) based on traits that are completely preserved and can be evaluated among all individuals (+, −). Unfortunately, only 16 traits (6.6%) were completely preserved among all individuals, and the dispersion of the resulting values was relatively narrow. Neither variant is a good reflection of the proven family relationships. Compared to the results of our method, the correlation between morphological similarity and biological distance was weak (in variant A) or not statistically significant (in variant B).

Unlike the Essen-Möller formula (and also e.g., approach by Wiltschke-Schrotta, 1988 based on Sjøvold, 1976–77), our method offers the possibility that the side occurrence of assessed traits has different weights in verifying biological relationships. The calculation is also not dependent on the population frequencies of the monitored traits.

The preservation of skeletal remains, which can result in missing data, is the basic limitation of statistical evaluation. A high degree of similarity between two individuals can also be attributed to a large number of identical traits (i.e., positively evaluated), whereas a small number of co-occurring (i.e., co-evaluated) traits can be present in both individuals. As we can see, ideally, individuals should be compared based on the same set of traits. However, observance of this condition usually leads to a reduction in the number of compared individuals and traits, which underlies the noticeably low value of the results. However, the poor conservation of the remains is less of a problem when we use our SC approach. This method can also evaluate partially incomplete data. In our sample, there is the example of the very poorly preserved remains of male No. 5. We could assume a high degree of similarity between this individual and all other

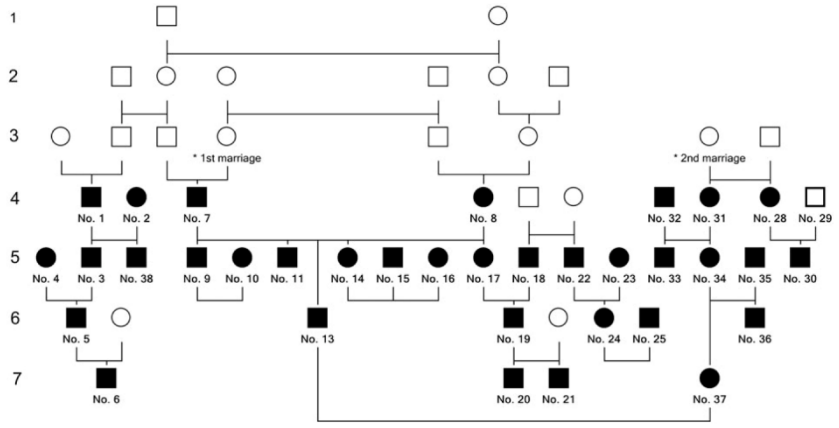


FIGURE 6 Blood relations of individuals (no. 1–38) with possible family traits (Table 3)

TABLE 4 Occurrence of possible familial traits in the genealogically documented sample (reference frequency < 10%) between biologically related individuals (light gray) and their family tree (black marks; Figure 6)

		Trait											
		97 Ponticuli basales ossis sphenoidalis	98	99	100	110 Pont. mylohyoideus	111	112 Ponticuli atlantis	113	114	115	133	134
		Pterygospin. completus	Pterygospin. incompletus	Pterygoalar. completus	Pterygoalar. incompletus	Completus	Incompletus	Posterior completus	Posterior incompletus	Lateralis completus	Lateralis incompletus	Canalis intraclav.	Fac. cond. partita
		B	B	B	B	B	B	B	B	B	B	B	C
Individuals	1	– –	– –	– –	– –	– –	– –	– –	– –	+ –	– –	– –	– +
	2	0 0	0 0	0 0	0 0	– –	– –	0 0	0 0	0 0	0 0	– 0	0 0
	3	– –	– –	– –	– –	– +	– –	– –	– –	– 0	– 0	– –	– –
	4	– –	0 0	– –	0 0	– 0	– 0	0 0	0 0	0 0	0 0	– –	0 0
	5	– 0	– 0	– 0	– 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	6	– –	– –	– –	– –	– –	– –	0 0	0 0	0 0	0 0	0 –	– –
	38	– –	– 0	– –	– 0	– –	– –	– –	0 –	– –	0 –	+ +	+ +
	7	– –	– –	– –	– –	+ –	– –	+ +	– –	+ +	– –	0 –	– –
	8	0 0	0 0	0 0	0 0	+ +	+ –	0 0	0 0	0 0	0 0	– +	0 0
	9	– –	+ –	– –	– –	– +	+ –	+ +	– –	– –	– –	+ +	– –
	10	– –	– –	– –	– –	+ –	– –	– –	– –	– –	– –	– –	– –
	11	0 –	0 –	0 –	0 –	– –	– –	– –	– +	0 0	0 0	– –	0 –
	13	– –	– –	– –	– –	– –	+ +	0 0	0 0	– –	– –	– –	+ –
	14	0 0	0 0	0 0	0 0	– +	– –	– –	– –	+ 0	– 0	– 0	– +
	15	– –	+ –	– –	– –	– –	– –	– –	0 –	– –	– –	– –	– 0
	16	– –	0 0	– –	0 0	0 –	0 –	0 0	0 0	0 0	0 0	– –	– 0
	17	– –	– –	– –	– –	– –	+ –	0 0	0 0	0 0	0 0	+ +	+ +
	18	– –	0 0	– –	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	19	– –	+ +	– –	– –	– –	– +	– –	– –	– –	– –	– –	– –
	20	– –	+ +	– –	– –	– –	– –	– –	– +	+ –	– +	– –	– –
	21	– –	– –	– –	– –	– –	– –	– –	– –	+ –	– +	+ –	– –
	22	– –	– –	– –	– –	– –	– +	– –	– –	– –	– –	– –	– –
	23	0 0	0 0	0 0	0 0	– –	– –	– 0	– 0	0 0	0 0	– –	– +
	24	– –	– –	– –	– –	– –	– +	– 0	+ 0	– –	– +	– –	– –
	25	– –	– –	+ –	– –	0 –	0 –	0 –	0 0	0 –	0 –	– –	– –
	28	0 –	0 0	0 –	0 0	+ +	– –	0 0	0 0	0 0	0 0	0 –	– –
	30	– –	– –	– –	– –	– –	+ +	– –	– –	– –	– –	– –	– –
	31	– –	– –	– –	– –	0 0	0 0	– –	+ +	– –	+ –	– +	– –
	32	+ 0	– 0	– 0	– 0	+ –	– +	0 0	0 0	0 0	0 0	+ +	0 0
	33	– +	– –	+ –	– –	+ 0	– 0	– –	– –	– –	– –	– –	+ –

(*Continues*)

TABLE 4 (Continued)

	Trait																			
	97 Ponticuli basales ossis sphenoidalis		98	99		100		110 Pont. mylohyoideus		111		112 Ponticuli atlantis		113		114		115		133	134
	Pterygospin. completus		Pterygospin. incompletus	Pterygoalar. completus		Pterygoalar. incompletus		Completus		Incompletus		Posterior completus		Posterior incompletus		Lateralis completus		Lateralis incompletus		Canalis intraclav.	Fac. cond. partita
	B		B	B		B		B		B		B		B		B		B		B	C
34	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
35	–	–	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	+
36	–	+	–	–	+	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
37	–	–	+	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–

+ = the presence of the trait; – = the absence of the trait; 0 = the trait could not be evaluated.

individuals (a small number of simultaneously evaluated traits and a high number of identical traits present between two individuals). Despite this assumption, however, the results reflect documented family relationships. Male No. 5 was the most similar to his son (No. 6), mother (No. 4), father (No. 3), and grandmother (No. 2). The remains of female No. 14 were also very poorly preserved. Nevertheless, she was, in line with the documented relations, most similar to her mother (No. 8), her father (No. 7), her sister (No. 17), and her aunt (No. 28).

During our study, we also tried different variants of the numerical expression of agreement and disagreement between two individuals, which are proposed in Tables 8 and 9. While the specific values of the SC were always slightly different, the degree of similarity between individuals remained approximately the same as that shown in Figure 2.

4.2 | The rate of morphological similarity between cousins

In view of the results of Gemmerich-Pfister (1999) and Velemínský & Dobisíková (2005), the degree of similarity between first cousins was assessed. According to the SC, in many cases, there was a greater degree of similarity between first cousins than between individuals and their parents, descendants, or siblings (see Figure 1, e.g., male No. 19 with his first cousin No. 24: SC = 40, and male No. 19 with his mother No. 17: SC = 31). Very similar conclusions have been drawn by studies on the remains of eight members of the noble Swéerts-Sporck family (Velemínský & Dobisíková, 2005) despite their use of other statistical methods, such as the percentage expression of agreement or disagreement in the occurrence of traits, or Essen-Möller formula (Zvára, 1999). There were two couples in this sample, and their similarity was lower than the one between father and son but higher than the similarity among siblings (Velemínský & Dobisíková, 2005). Similarity among first cousins is also given by Gemmerich-Pfister (1999), a study which used different statistical methods (multivariate and bivariate analysis) than those used here. We cannot explain the cause of this phenomenon reliably.

In some cases, there may also be greater similarity among grandparents and grandchildren than between parents and children, as some of our results show (see Figure 1, e.g., male No. 18 with grandson No. 20: SC = 47; vs male No. 19 with son No. 20: SC = 37). Even in this case, questions arise that are similar to those in the case of first cousins, as the closest shared relatives of first cousins are their grandparents. The following questions are offered for further investigation: Is this phenomenon random? Does it have a constant population occurrence or does it only occur in some families?

4.3 | The predictive value of individual type of traits

4.3.1 | Cranial versus postcranial traits

Cranial traits are more appropriate than postcranial traits for evaluation by the SC, as observed in previous studies (Berry & Berry, 1967; Hauser & De Stefano, 1989). This finding has been proven by prior studies (Sjøvold, 1984; Rösing, 1990; Spence, 1996; Velemínský & Dobisíková, 2005; Carson, 2006b; Movsesian, 2013). It may be caused

TABLE 5 Rare traits in the population (reference frequency < 10%) shared by closely related individuals

			Genealogical documented sample		Reference samples (Pachner collection; Church of our Lady Victorious*)					
					Incidence on individual			Incidence on side side		
No.	Trait:	Cat.	Individuals	Side	*N*	Inc.	%	*N*	Inc.	%
65	Ponticulus palatinus	B	No. 19, 20 No. 32, 33	Both sides, left side right side, left side	136	8	5.9	273	8	2.9
85	Canalis basilaris medianus	B	No. 38, 6	–	140	2	1.4	–		
86	Canalis craniopharyngeus	B	No. 7, 8, 14	–	138	8	5.8	–		
109	For. mentale accessorium	B	No. 22, 24	Both left side	52	2	3.8	104	2	1.9
131	For. suprascapulare	B	No. 20, 22	Both sides, left side	155	15	9.7	313	17	5.4
9	Oss. internasale	A	No. 9, 17	–	138	8	5.8	–		
16	Sutura incisiva	A	No. 5, 6	Both sides	139	1	0.7	278	2	0.7
29	Oss. suturae sagittalis	A	No. 18, 20	–	112	4	3.6	–		
150	Walmsley's ("riding") facet	C	No. 1, 3, 38 No. 33, 37	Both sides	150	2	1.3	302	2	0.7
165	Fac. art. tali acc. lateralis*	C	No. 30, 36	Both sides	49	2	4.0	153	6	4.0
169	Fac. art. calc. ant. Absens*	C	No. 2, 3, 38	Both sides	47	2	4.3	151	3	2.0
172	Divided proximal art. facet of first metatars*	C	No. 4, 5	Both sides	42	4	9.5	129	9	7.0
217	Tuberculum praecondylare	D	No. 2, 38	Both sides	139	6	4.3	264	8	3.0
222	Proc. styloideus elongatus	D	No. 1, 3 No. 19, 21/22, 24 No. 36, 34	Both sides left side/both sides both sides	28	1	3.6	85	3	3.5
227	Trochanter tertius	D	No. 34, 37	Both sides	148	5	3.4	296	7	2.4

N = number of evaluated individuals/sides, Inc. = incidence, % = frequency.

by several factors. More attention has been paid to cranial traits than to postcranial traits (Berry & Berry, 1967; Reinhard & Rösing, 1985; Hauser & de Stefano, 1989; Gemmerich-Pfister, 1999; Hanihara et al., 2003; Kaur et al., 2012a; Mann et al., 2016; etc.). Additionally, in cases of selective choice of remains, the greatest attention is paid to skulls (Vlček, 2000; Slavec, 2004). Many nongenetic factors, such as soft tissue and the extent of physical activity, also contribute significantly to the appearance of many traits of the postcranial skeleton, mainly changes in the area of muscle/ligament attachments or some articular facets, or some traits are on the borderline of disease and developmental defects, such as spondylolysis (Kennedy, 1989; Capasso et al., 1999). Nevertheless, Finnegan (1978) considers postcranial traits to be more appropriate than cranial traits. As an argument, he states his own experience with better resistance and preservation of the postcranial skeleton in archeological material. However, the preservation of our material and material from other studies (Spence, 1996; Slavec, 2004) does not match this argument.

4.3.2 | Traits according to anatomical character

Category B (traits associated with the course and outlet of vascular and neural routes) best reflects genealogical relationships. On the other hand, according to Ossenberg (1970) and Cheverud & Buikstra (1981a, b, 1982), these traits (foramina) have lower heritability than hypoostotic and hyperostotic traits. However, our results overlap; from an anatomical point of view, we have combined two basic groups of traits in this category in our study: (1) foramina and (2) osseous bridges through or over vascular and neural foramina or routes, which these authors classified as hyperostotic traits. Furthermore, in some past studies, the similarity of individuals was observed on the basis of the hyperostotic traits we rank in this category, for example, the partition of foramina (Strouhal, 1992; Vlček, 2000) and bridges (Pietrusewsky & Douglas, 1992; Velemínský & Dobisíková, 2005).

The other categories (A, C, D, E, Fa, and Fb) generally do not reflect the documented relationships. The largest limitation in category A (traits associated with cranial sutures) is the obliteration of sutures (Gualdi-Russo et al., 1999). The traits that belong to category C (traits associated with changes in articulated surfaces) may particularly reflect the traits associated with their direct division or connection, for example, contact surfaces between the talus and calcaneus, according to the results of some studies (Spence, 1996; Ricaut et al., 2010). This assumption is also supported by the results of our work (Table 5). Traits such as facets around the femoral head, squatting facets (the tibia/talus), and accessory sacroiliac facets are associated especially with physical activity (Kostick, 1963; Capasso, 1999; Radi

et al., 2013). At the end of the 19th century, Charles (1893, 1894) and later Wood-Jones (1944) did not exclude the inheritance of acquired traits associated with changes in articulated surfaces.

There are several cases of a high degree of morphological similarity between closely related individuals in categories D (hypoostotic traits) and E (hyperostotic traits), but these traits do not reflect the relationships to the extent of the traits classified in category B. For these traits, it is not impossible that in addition to genetic components (Saunders & Popovich, 1978; Hauser & de Stefano, 1989), external factors—such as stress, nutrition, physical activity, and illness—can significantly contribute to their origin (Roeder, 1953; Capasso, 1999; Case et al., 2006; Léonard et al., 2013).

Traits in categories Fa and Fb (traits related to changes in the area of muscle/ligament attachments) positively correlate with sex and age (Rogers et al., 1997; Kalichman et al., 2007), and their development is associated with physical activities (Capasso et al., 1999) or diseases (Resnick et al., 1975). However, they cannot be completely ignored, as genetic components explain 20% of their development variation (Kalichman et al., 2007). On the other hand, additive genetic factors were not found (Kalichman et al., 2007). The presence of a genetic component indicates individuals referred to as "bone formers" (Rogers et al., 1997). In our sample, on the basis of similarity in the occurrence and development of traits associated with changes in the area of muscle insertions, we could refer thus to the closely related males Nos. 18, 19, 20, and 21 (father, son, and two grandsons).

4.4 | Possible familial traits

There are different criteria for selecting rare, possibly familial traits (Case, 2003). Our work draws on that of Gemmerich-Pfister (1999). Traits with a population frequency of 1%–10% are considered rare (traits with frequency ≤1% are considered very rare). This frequency is a relatively broad interval. However, the detection of familiar characteristics using lower frequencies has not been successful (Gemmerich-Pfister, 1999).

Within our sample, we recorded five traits whose population frequency is <10% that could be considered familial based on their increased occurrence among biologically related individuals: the *ponticuli basales ossis sphenoidalis* (*pterygospinosus completeus/incompletus*, *pterygoalaris completus*), the *ponticuli atlantis* (*posterior*, *lateralis*), the *ponticulus mylohyoideus*, the *facies condylaris partita*, and the *canalis intraclavicularis*. Four traits relate to the course of vascular and neural routes (category B). Three of these traits have a hyperostotic characteristic (Ossenberg, 1970): they are bone bridges across the blood vessels with nerves running along the surface of the bone (Hauser & De Stefano, 1989). According to Ossenberg (1970), traits of this characteristic have greater heritability than vascular and nerve outlets (foramina). This finding was confirmed by studies that verified their familial occurrence in living people (Selby et al., 1955; Romanus & Tovi, 1964; Ossenberg 1974; Saunders & Popovich, 1978). Some studies of genealogically documented remains have confirmed the importance of these traits for the detection of family relationships (Pietrusewsky & Douglas, 1992: the *ponticulus mylohyoideus*; Velemínský & Dobisíková, 2005: the *ponticuli sellae* and *ponticuli basales ossis sphenoidalis*). For most traits, a statistically significant dependence on age and sex has not been proven, and occurrence on a particular side is not preferred (Dodo, 1986; Schilling et al., 2010; Kaur et al., 2012b; Kawakubo et al.; 2013). According to Kaur et al. (2012b), these traits are under genetic control. Their occurrence has often been detected prenatally (Hayek, 1929; Hochsteter, 1940; James et al., 1980; Zivanovic, 1980) and in children (Dodo, 1980; Česnys, 1985; Mann et al., 2016).

We found a statistically significant difference in the occurrence of four possible familial traits between the genealogically documented sample and reference samples: the *ponticulus mylohyoideus* (complete and incomplete), the *ponticulus atlantis lateralis* (complete and incomplete), the *canalis intraclavicularis*, and the *facies condylaris partita*. We could not, however, verify the heritability of individual traits (narrow-sense heritability) for the following reasons: (1) a small number of individuals compared to previous studies (Sjøvold, 1984; Carson, 2006b) and (2) missing data. Only Sjøvold (1984) and Carson (2006b) have addressed the heritability of morphological traits, as these studies are mainly limited by a lack of skeletal material with known genealogical data (Carson, 2006b; Ricaut et al., 2010). In addition, both authors rated the same traits (Berry & Berry, 1967) in a genealogically documented skull collection from Hallstatt and produced different results: Carson (2006b) found lower heritability than Sjøvold (1984) for most traits. This difference can be explained by the use of different statistical methods, the inclusion of broader family relationships and differences in trait evaluation (Carson, 2006b). Due to the use of different methodological approaches, comparing the results of the two works is problematic (Ricaut et al., 2010).

Based on our knowledge of heritability and the conditions of its calculation, it is our opinion that the calculation of heritability in NM osteological traits in historical populations is highly controversial. The reasons are as follows:

1. Heritability is mainly found in metric traits, both in humans (Sjøvold, 1984, 1995; Relethford, 1994; Doi, 2000; Carson, 2006a; Martínez-Abadías et al., 2009; Dudzik & Kolatorowicz, 2016; Gavrus-Ion et al., 2017) and in animal models (Formoso-Rafferty et al., 2017; Stojanowski et al., 2017).
2. The primary source of knowledge regarding trait heritability is the study of monozygotic or dizygotic twins (Snustad & Simmons, 2009; Biggerstaff, 1973; Townsend et al., 1992). However, this approach has significant limitations as it applies to the study of past populations. Studies on living people using imaging methods are no longer conducted (Torgersen, 1951; Selby et al., 1955; Saunders & Popovich, 1978) as this approach encounters a number of ethical issues. Genealogically documented osteological samples remain as the only possible source for studying the heritability of NMTs. Such samples are very rare (Carson, 2006b; Ricaut et al., 2010), twins are missing, or the sample is negligible or cannot be evaluated. Therefore, at minimum, other first-degree relatives (parents/children, siblings) must be considered (Sjøvold, 1984).
3. Heritability calculations are usually successful with a large amount of data in humans (Gavrus-Ion et al., 2017) or even in animal models (Wood et al., 1991; Formoso-Rafferty et al., 2017). On the other hand, genealogically documented osteological samples

usually include only a few individuals (Strouhal, 1992; Spence, 1996; Thurzo et al., 2001; Slavec 2004; Velemínský & Dobisíková, 2005).

4. There is not always a consensus on the methodology used to rate traits. The method of trait evaluation can significantly affect apparent heritability (Carson, 2006b).

4.5 | Conclusions

The results of our study confirm that patterns of NMT similarity scales match with documented genetic relationships among individuals and that they can be used for kinship analysis. There is a positive correlation between the morphological similarity among biologically related individuals and their biological distance. In some cases, we may find a greater degree of morphological similarity among first cousins (e.g., Nos. 19 and 24) or between grandparents and grandchildren (e.g., Nos. 18 and 20) than with closely related individuals, such as parents and children (e.g., Nos. 19 and 20), siblings with a common relationship (e.g., Nos. 36 and 37), or inbred siblings (e.g., Nos. 9 and 14).

While we might expect that inbreeding leads to a reduction in morphological variation, the degree of morphological similarity in inbred individuals as assessed by our SC does not significantly exceed the degree of similarity among other closely related individuals with normal family relationships. Inbreeding will probably not have a major impact on NMT distributions.

Proven family relationships are best reflected by cranial traits, especially those traits associated with the course and outlet of blood vessels and nerves (category B). Based on population frequencies, we found five traits that we consider to be familial in view of their increased incidence in our sample: the *ponticuli basales ossis sphenoidalis*, the *ponticuli atlantis*, the *ponticulus mylohyoideus*, the *canalis intraclavicularis* (all category B), and the *facies condylaris partita* (category C). For the *ponticulus atlantis posterior* (complete and incomplete), the *ponticulus mylohyoideus* (complete and incomplete), the *canalis intraclavicularis*, and the *facies condylaris partita*, there is a statistically significant difference in their occurrence between the genealogically documented sample and the reference samples.

ACKNOWLEDGMENTS

The authors greatly thank O. Naňka (Institute of Anatomy, 1st Faculty of Medicine, Charles University, Prague) and J. Velemínská (Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague) for access to Pachner's collection. Thanks to T. Jor (Department of Zoology, Faculty of Science, Charles University, Prague) and A. Černíková (Institute of Applied Mathematics and Information Technologies, Faculty of Science, Charles University, Prague) for their help with statistical analysis. They would also like to thank V. Kuželka, M. Dobisíková, and J. Vítková (Department of Anthropology, National Museum, Prague), P. Daňková (Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague), and I. Grossová (Military Institute of Forensic Medicine, The Military University Hospital, Prague) for their help, advice, and useful comments. Thanks to E. A. Carson (Bureau of Justice Statistics U. S., Department of Justice, Washington) for consultations on the issue of traits heritability and to A. Millar and A. Vacl for correcting the text. They would like to thank two reviewers for their useful comments that have contributed to improving the text.

The authors thank the families who gave their consent to study the remains of their ancestors, namely, F. Andres Sr. and his family, P. Drba and his family, E. Palmová, P. Pauček, H. Kleczková, and her family, F. Kopp, P. Eret, M. Cvrček, K. Cvrček, and A. Cvrček. In memoriam, they thank spouses K. Cvrček and J. Cvrčková because this work was also created with their support.

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How to cite this article: Cvrček J, Velemínský P, Dupej J, Vostrý L, Brůžek J. Kinship and morphological similarity in the skeletal remains of individuals with known genealogical data (Bohemia, 19th to 20th centuries): A new methodological approach. *Am J Phys Anthropol*. 2018;167:541–556. https://doi.org/10.1002/ajpa.23683

NÁRODNÍ MUZEUM

Journal of the National Museum (Prague), Natural History Series
Vol. 190 (2021), ISSN 1802-6842 (print), 1802-6850 (electronic)

PŮVODNÍ PRÁCE / ORIGINAL PAPER

DOI: 10.37520/jnmpnhs.2021.003

Anthropological research into a family tomb from Vetlá cemetery (Bohemia, 19th–20th centuries), with a focus on the morphological similarity of biologically related individuals.

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Cvrček J. & Velemínský P., 2021: Anthropological research into a family tomb from Vetlá cemetery (Bohemia, 19th–20th centuries), with a focus on the morphological similarity of biologically related individuals. – Journal of the National Museum (Prague), Natural History Series 190: 45–60.

Abstract: In common osteological practice, there are few opportunities to verify the relationship between morphological similarity and the biological relatedness of individuals. This contribution expands knowledge in this area based on research into a family tomb from Vetlá (Bohemia, 19th–20th centuries), where 131 non-metric cranial traits were monitored in 7 skulls and the degree of similarity between individuals was calculated using a similarity coefficient. Trait frequencies were evaluated within the family and compared to a reference sample. Due to the small number of individuals statistical methods were not applied, and the authors focused on graphical representation of the results. It was confirmed that a positive relationship between the degree of similarity of individuals and their degree of relatedness is visible. At the same time, in the group of biologically related individuals a greater similarity and a lower degree of its variability was apparent than in unrelated individuals. The average frequency of all the evaluated traits together also appears to be higher in the family sample than in the reference sample. In addition, several individual traits were found to support the documented kinship of individuals, not only on the skulls but also on the scapula.

Keywords: non-metric traits, skull, kinship analysis, similarity coefficient, genealogical documented sample.

Received: 8 April 2021 | Accepted: 18 July 2021 | Published on-line: 25 August 2021

Introduction

Given the rarity of genealogically documented osteological samples worldwide (Gavrus-Ion et al. 2017), each additional family grave or tomb that can be studied represents a unique opportunity to expand our knowledge of the influence of biological relationships on the morphological features of the human skeleton and dentition. Studies to date have shown that there is a positive relationship between the degree of biodistance and the degree of similarity of individuals, and that there is conspicuous agreement in closely related individuals in the occurrence of specific traits (e.g. Case et al. 2017, Cvrček et al. 2018, 2020). This relationship

can be used especially in the effort to detect individuals with biological affinity in anonymous (pre)historical burial grounds (e.g. Pietrusewsky & Douglas 1992, Case 2003, Ricaut et al. 2010), or in the identification of family members of historically known personalities whose identity has been lost over time (Horáčková & Vargová 1997, Thurzo & Beňuš 2003).

The degree of similarity between individuals can be assessed using different approaches, from teeth through frontal sinuses to anatomical variants and developmental anomalies of the whole skeleton, etc. (Stojanowski & Schillaci 2006). Non-metric dental traits seem to be the most ideal (e.g. Paul & Stojanowski 2015, 2017; Irish et al. 2020), but are subject to a number of limitations, such as abrasion, intravital and postmortem losses, caries, or fillings (Alt & Vach 1998). The analysis of non-metric osteological traits is a suitable alternative (Johnson & Lovell 1994, Prowse & Lovell 1996); cranial traits are most often used due to several factors, such as the higher number of traits and, as a rule, their better preservation compared to the postcranial parts of the skeleton (Spence 1996, Cvrček et al. 2018), and the fact that in the secondary placement of the remains, the assignment of postcranial bones to the skull may not always be entirely certain (Slavec 2004).

However, the number of studies on this topic using a documented sample remains low. Some authors are even skeptical about the reflection of biological relatedness in the occurrence of non-metric traits, because although they are qualitative traits, they do not show simple monogenic inheritance, but manifest themselves as quantitative traits, i.e. polygenically conditioned with the possible participation of the external environment (Tyrrell 2000, Brown 2015). In the Spring of 2017, the authors were asked to help with the retrieval of skeletal remains during the reconstruction of a 19th–20th century family tomb in Vetlá (part of Vrbice village, Litoměřice District, Bohemia) which belonged to one of the most important local landowner families. This was an opportunity for them to be researched, and above all to answer these questions: 1) Does the degree of morphological similarity among the individuals correspond to their documented relationships? 2) Is it possible to find specific traits that support their biological relationships?

Material and methods

Material

Based on a genealogical search including death records (State Regional Archives in Litoměřice, Prague City Archive), and documents and portraits from the private family archive of the vault's owners, it was possible to identify the remains of 8 individuals with known family relationships from among the remains stored in the tomb (Figs. 1, 2); 7 of these were adults (3 males and 4 females, 33–86 years old), and one was a 10-year-old boy. The last 3 burials (Nos. 5, 7, 8) were in coffins (2 metal and 1 wooden); the remains of the other individuals were laid in one corner of the tomb in the form of a reduction of older burials, but for individuals Nos. 1 and 2 only the skulls could be individually identified with certainty. Due to the diametrically different preservation of the material (adults vs. the juvenile, complete skeletons vs. distinctly incomplete skeletons, or skulls alone), only the skulls were selected to evaluate the degree of similarity of the individuals in this study. The skull of individual No. 1, however, eventually had to be excluded from the evaluation due to very significant post-mortem damage (the maxilla, base and occipital parts of the skull were missing), meaning that only the skulls of 6 adults and 1 juvenile were evaluated (Nos. 2–8). Despite the fact that individual No. 8 was not an adult and the ontogenetic development of his skeleton was not complete, he was left in the evaluated sample because 1) his family affiliation and relationships to the adults are unquestionable; 2) the occurrence of most of the evaluated traits is documented prenatally or in childhood (e.g. Hauser & De Stefano 1989, Mann et al. 2016), albeit that there are statistically significant differences between non-adults and adults in the occurrence of some (Česnys 1985); and 3) his documented chronological age reached the



Fig. 1. Vetlá cemetery, a view inside the family tomb during its opening before exhumation. Photo: J. Štěpán.

upper limit of the interval 0–10 years, within which it is not appropriate to evaluate cranial traits for comparison purposes (Česnys 1985).

Next, the 140 skulls of the Pachner skeletal collection (Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague) were used to determine reference data. This sample consists of the skeletal remains of people from second half of the 19th and the first third of the 20th centuries (Cvrček et al. 2018), and it was chosen for its chronological and geographical similarity to the genealogically documented sample, and the almost certain unrelatedness of individuals according to written records (information on the bones, vital records from Prague City Archive).

Methods

To determine the degree of similarity of individuals, 131 cranial non-metric traits were evaluated (Movsesjan et al. 1975, Hauser & De Stefano, 1989), of which 28 were unilateral and 103 bilateral (see Supplementary file, Tab. S1). The degree of similarity between individuals was calculated using a similarity coefficient (SC) (Cvrček et al. 2018). The relationship between the resulting similarity coefficient of biologically related pairs and the degree of their relationships was graphically ex-

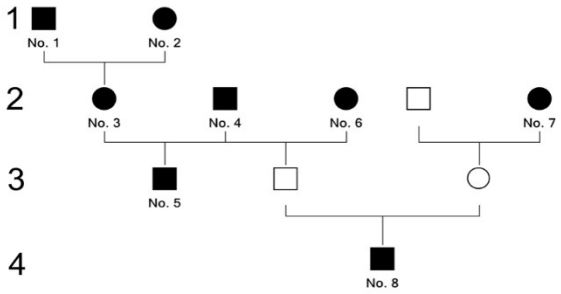


Fig. 2. Family tree of the genealogically documented sample (generations Nos. 1–4). All individuals in the sample have a number, and individuals included in this study (Nos. 1–8) are shown in black.

	2	3	4	5	6	7	8
2	–	40	45	33	30	36	31
3		–	28	28	26	31	30
4			–	38	28	42	31
5				–	29	44	26
6					–	20	33
7						–	28
8							–

Table 1. Similarity coefficient (SC) values for individuals Nos. 2-8 by cranial non-metric traits.

pressed by using the logarithm of their coefficient of relationship (r), which was estimated using a tabular method (Falconer & Mackay 1996) based on the method of VanRaden (1992). However, the low number of individuals explains why statistical processing was not performed in this study (Cvrček et al. 2021).

For all of the evaluated cranial non-metric traits together, the difference in their occurrence between the genealogically documented sample and the reference samples was expressed. At the same time, specific traits which support documented relationships were monitored; their selection was conditioned by a population frequency of less than 10 % (Gemmerich-Pfister 1999, Cvrček et al. 2018), either incidence on individual or incidence on side, and their occurrence in at least two biologically related individuals (Cvrček et al. 2018).

Results

The greatest degree of similarity between biologically related individuals based on cranial non-metric traits exists between mother No. 2 and daughter No. 3, and father No. 4 and son No. 5 (r = 0.5) (Tab. 1). The similarity between boy No. 8 and his grandparents Nos. 4 and 6 (r = 0.25) is greater than the similarity between boy No. 8 and his half-uncle No. 5 (r = 0.125). On the other hand, there is greater similarity between male No. 5 and his grandmother No. 2, between which there is a greater biological distance (r = 0.25), than between male No. 5 and his mother No. 3, whose biological distance, on the other hand, is smaller (r = 0.5). Overall, the degree of similarity of biologically related individuals corresponds to their biological distance (Fig. 3).

If the degree of similarity between biologically related and unrelated individuals within a family is compared (Fig. 4), then biologically related pairs (N = 8) graphically show less variability and a higher median than biologically unrelated pairs (N = 13).

On the other hand, there are only three traits with a population frequency of less than 10 % between close relatives that support their known relationship (see

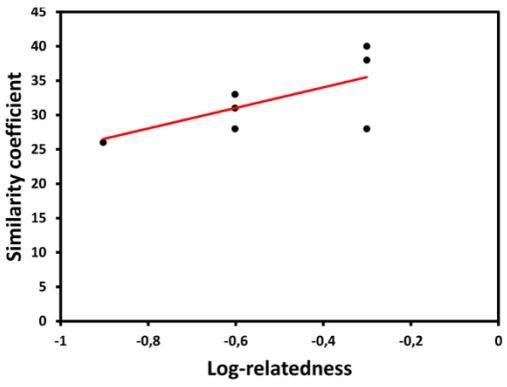


Fig. 3. Relationship between the similarity coefficient (SC) of biologically related individuals Nos. 2–8 (y-axis) and the log-relatedness of individuals (x-axis). The red line represents the regression line. Note: log-relatedness disregards unrelated individuals.

Supplementary, Tab. S1): *foramen zygomaticofaciale partitum* (grandmother No. 6 and grandson No. 8), and *foramen spinosum incompletum* and *ponticulus jugularis internus incompletus* (mother No. 2 and daughter No. 3). However, another five traits were recorded the reference frequency of which is higher than 10 %, but which also occur between close relatives: *ossiculum epiptericum* (mother No. 3 and son No. 5), *sulcus frontalis* (father No. 4 and son No. 5), *incisura frontalis absens* (grandmother No. 6 and grandson No. 8), *foramen palatinum minus accessorium absens* (father No. 4 and son No. 5), *foramen hypoglossale cum spina* (male No. 5 and half-nephew No. 8). Last but not least, the occurrence was noted of two population-rare traits, which, although unique, are similar in their character: female No. 2 has *ossiculum lambdae* and her daughter No. 3 has *ossiculum suturae saggitalis*. Overall, however, a higher average frequency of evaluated cranial non-metric traits is graphically visible in the genealogically documented sample compared to the reference Pachner collection, both on the individual and on the side (Fig. 5).

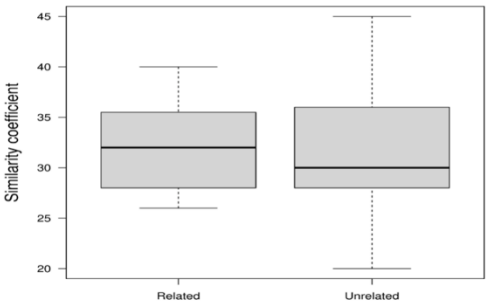


Fig. 4. Variability in the degree of similarity between biologically related (N = 8 pairs) and unrelated (N = 13 pairs) individuals based on the similarity coefficient.

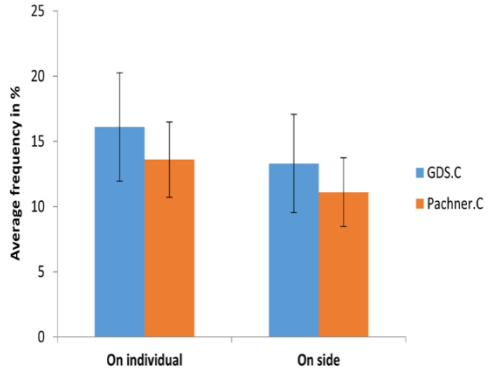


Fig. 5. Average frequency of all evaluated cranial non-metric traits (n = 131) in the genealogically documented sample with comparison of their incidence in the reference Pachner collection both on the individual and on the side. GDS.C = genealogically documented sample: cranial traits, Pachner. C = Pachner colletion: cranial traits.

In addition to the similarities in cranial non-metric traits, the overall examination of the skeletal remains of the family also evinced a striking agreement in the morphology of the suprascapular notch between father No. 4 and son No. 5. The typical sharply formed notch is missing on both sides; conversely, it is wide open and flows smoothly in the superior border of the scapula. This is the so-called type V of the suprascapular notch (Polguj et al., 2011; Fig. 6). The frequency of this anatomical variant in Central European recent populations ranges from about 4.5 to 12.9 % (Polguj et al. 2013, Al-Redouan et al. 2021).

Discussion

This research, in line with previous studies (e.g. Spence 1996, Velemínský & Dobisíková 2005, Case et al. 2017, Cvrček et al. 2018) confirmed that there is a positive relationship between the degree of similarity and the degree of relatedness of individuals. Thus, the smaller the biodistance, the greater the similarity. However, even in this family there was a case where there was a greater similarity between the grandparent and the grandchild than between the parent and the child, as previously shown in other samples (Spence 1996, Drozdová 2006, Cvrček et al. 2018).



Fig. 6. Agreement between father No. 4 (left) and son No. 5 (right) in the morphology of suprascapular notch: type V (arrows).

The small number of individuals and the biological relationships between them were probably reflected in the low number of population-rare cranial traits with a frequency below 10%. However, the graphically apparent increase in the average frequency of all evaluated cranial traits in the family sample compared to the reference sample suggests that even for commonly occurring traits with a population frequency above 10%, their frequency increased due to genetic factors (Hauser & De Stefano 1989, Carson 2006). Therefore, when assessing the degree of similarity between individuals, it is appropriate to also take into account those traits the limit of which either exceeds or meets the 10% population occurrence threshold, but which in comparison to the reference sample are not (statistically) significant. Even these traits can serve as a good indicator of biological affinity (Case et al. 2017, Cvrček et al. 2018), although it is important to consider them in context or on the basis of comparisons with reference data. At the same time, however, it cannot be ruled out that the increase in the average frequency of evaluated traits found is conditioned by the small number of individuals.

The presence of *ossiculum lambdae* in female No. 2 and at the same time *ossiculum suturae saggitalis* in her daughter No. 3 supports the assumption that traits can be found among close relatives which, although at first sight different, are essentially the same (Thurzo & Beňuš 2003). In this case, they are intrasutural ossicles which arise in ontogenesis as a result of the creation of an additional ossification center within the suture during its formation (Hauser & De Stefano 1989); predisposition to their creation can be hereditary (e.g. Molleson 1987, Molleson et al. 1993, Vlček 1997).

Likewise, the postcranial finding of identical suprascapular notch formation between father No. 4 and son No. 5 supports the findings of some previous studies where the authors have observed that the scapula may bear a number of traits, the frequencies of which are not high, that also can serve as a suitable indicator of biological affinity between individuals (Angel et al. 1987, Vlček 1995, Yammine 2013, Cvrček et al. 2018).

Each piece of such research is accompanied by an effort to process the results as objectively as possible using statistical approaches (e.g. Ullrich 1969, Wiltschke-Schrotta 1988, Heinrich & Teschler-Nicola 1991, Alt & Vach 1995, 1998). Unfortunately, this is not always possible (Cvrček & Velemínský 2020). Most genealogically documented samples include only a few individuals, usually no more than ten (e.g. Molleson et al. 1993, Spence 1996, Horáčková & Vargová 1997, Vlček 2000, Thurzo & Beňuš 2003, Velemínský & Dobisíková 2005, Drozdová 2001, Cvrček & Velemínský 2020). In addition, biological relationships need not exist between all the individuals in a sample due to the presence of biologically unrelated individuals, which is also this case. Other limits for evaluating the similarity of individuals within a sample may be the poor preservation of their remains or their too young age, when the skeleton is not yet as fully developed as in adults (Spence 1996). In such circumstances, graphical and descriptive outputs therefore represent a possible alternative to mathematical calculations and statistical tests, the power of which would be low (Ellis 2010). Although this approach may be considered subjective (Asherson 1963), it has been shown that it can be used at least in parallel with statistical evaluation (e.g. Cvrček et al. 2018) – at least because mathematical expressions of similarity can determine the same degree of similarity in individuals with otherwise marked morphological differences, and together with statistical approaches, do not allow the capture of these individual deviations or, conversely, the capture of individual similarities (Szilvássy et al. 1987, Cvrček et al. 2020). However, the character of the relationship between the degree of relatedness and the degree of similarity of individuals, as well as the difference between the degree of similarity of related and unrelated individuals or between the frequency of traits in the family and the reference sample, can also be stated on the basis of scopical and graphical assessment, regardless of statistical conclusions (e.g. Vlček 1997, 2000; Carson 2006, Drozdová 2006, Cvrček et al. 2021). Such an approach is particularly important for cases where a statistical evaluation does not give a significant result despite graphically obvious relationships or differences, which can happen particularly with numerically small samples.

Acknowledgements

The authors greatly thank J. Dupej (Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague) for help with the calculations of degrees of similarity, T. Jor (Department of Zoology, Faculty of Science, Charles University, Prague) for help with the graphical representation of the results, J. Štěpán for creating the field photographic documentation, F. Fortin (Czech Genealogy and Heraldry Society in Prague) for his help with the genealogical research, and A. Millar for linguistic review, and to two reviewers for their comments that have contributed to improving the text. We also greatly thank P. Čašek and his family for supporting this research.

The research was supported by Grant Agency of the Charles University (Grant Number: GAUK 1276217) and Ministry of Culture of the Czech Republic (DKRVO 2019–2023/7.I.c, 00023272).

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Supplementum

		Family sample						Reference sample					
Trait		Incidence on individual			Incidence on side			Incidence on individual			Incidence on side		
		N	Inc.	%	N	Inc.	%	N	Inc.	%	N	Inc.	%
1	*Sutura metopica*	7	0	0	-	-	-	140	9	6.4	-	-	-
2	*Sutura metopica partialis*	7	0	0	-	-	-	140	0	0	-	-	-
3	*Sutura supranasalis absens*	7	4	57.1	-	-	-	140	29	20.7	-	-	-
4	*Fissura metopica*	6	0	0	-	-	-	140	2	1.4	-	-	-
5	*Ossiculum metopicum*	6	0	0	-	-	-	140	0	0	-	-	-
6	*Lophus frontalis*	6	0	0	-	-	-	140	16	11.4	-	-	-
7	*Sutura parametopica*	7	0	0	14	0	0	140	0	0	280	0	0
8	*Ossiculum suturae coronalis*	6	0	0	13	0	0	128	2	1.6	258	2	0.8
9	*Ossiculum internasale*	6	0	0	-	-	-	138	8	5.8	-	-	-
10	*Ossiculum praefrontale*	6	0	0	13	0	0	140	2	1.4	280	3	1.1
11	*Os zygomaticum partitum*	6	0	0	13	0	0	140	0	0	280	0	0
12	*Fissura zygomatica transverza*	6	0	0	13	0	0	140	5	3.6	280	6	2.1
13	*Sutura infraorbitalis*	6	5	83.3	12	9	75.0	140	70	50	280	108	38.6
14	*Sutura incisiva*	7	1	14.3	14	2	14.3	139	1	0.7	278	2	0.7
15	*Sutura incisiva partialis*	7	5	71.4	14	10	71.4	139	35	25.2	278	64	23.0
16	*Ossiculum medianum palatinum anterior*	7	0	0	-	-	-	138	0	0	-	-	-
17	*Ossiculum medianum palatinum posterior*	5	0	0	-	-	-	138	0	0	-	-	-
18	*Ossiculum epiptericum*	7	3	42.9	14	4	28.6	126	23	18.3	260	33	12.7
19	*Stenokrotaphia*	7	0	0	14	0	0	131	1	0.8	267	2	0.7
20	*Sutura frontotemporalis*	7	0	0	14	0	0	128	1	0.8	265	1	0.4
21	*Processus frontalis squamae temporalis completus*	7	0	0	14	0	0	133	8	6.0	272	10	3.7

22	*Processus frontalis squamae temporalis incompletus*	7	0	0	14	0	0	127	8	6.3	262	11	4.2
23	*Processus temporalis squamae frontalis completus*	7	0	0	14	0	0	119	0	0	248	0	0
24	*Processus temporalis squamae frontalis incompletus*	7	0	0	14	0	0	115	1	0.9	244	1	0.4
25	*Processus parietalis ossis sphenoidalis*	7	2	28.6	14	4	28.6	131	19	12.8	269	32	11.9
26	*Ossiculum bregmaticum*	6	0	0	-	-	-	124	1	0.8	-	-	-
27	*Ossiculum suturae sagittalis*	5	1	20	-	-	-	112	4	3.6	-	-	-
28	*Ossiculum incisurae parietalis*	7	1	14.3	14	1	7.1	132	24	18.2	268	30	11.2
29	*Os parietale partitum*	7	0	0	14	0	0	140	0	0	280	0	0
30	*Ossiculum suturae squamosae*	7	0	0	14	0	0	128	3	2.3	266	4	1.5
31	*Squama temporalis partita*	7	0	0	14	0	0	138	0	0	278	0	0
32	*Sutura squamomastoidea*	5	1	20	11	1	9.1	139	56	40.3	279	89	31.9
33	*Processus mastoideus bipartitus*	6	0	0	12	0	0	137	4	2.9	277	4	1.4
34	*Ossiculum lambdae*	7	1	14.3	-	-	-	127	12	9.4	-	-	-
35	*Ossiculum suturae lamboidaea*	7	3	42.9	14	5	35.7	117	51	43.6	239	79	33.1
36	*Os Incae completum*	7	0	0	-	-	-	140	0	0	-	-	-
37	*Os Incae bipartitum*	7	0	0	-	-	-	140	0	0	-	-	-
38	*Os Incae tripartitum*	7	0	0	-	-	-	140	0	0	-	-	-
39	*Os Incae multipartitum*	7	0	0	-	-	-	140	0	0	-	-	-
40	*Os Incae incompletum*	7	0	0	-	-	-	140	3	2.1	-	-	-
41	*Processus interparietalis*	7	0	0	-	-	-	139	6	4.3	-	-	-
42	*Sutura mendosa*	7	1	14.3	14	2	14.3	139	22	15.8	279	36	12.9
43	*Ossiculum asterii*	7	1	14.3	14	1	7.1	132	23	17.4	270	31	11.5
44	*Ossiculum suturae occipitomastoidae*	6	0	0	12	0	0	114	7	6.1	241	7	2.9
45	*Sulcus frontalis*	6	3	50	13	5	38.5	140	45	32.1	280	71	25.4
46	*Incisura trochlearis*	7	2	28.6	14	2	14.6	140	21	15.0	280	23	8.2
47	*Foramen trochleare*	7	1	14.3	14	1	7.1	140	3	2.1	280	3	1.1

48	*Incisura frontalis absens*	7	5	71.4	14	8	57.1	140	54	38.6	280	70	25.0
49	*Foramen frontale*	7	6	85.7	14	10	71.4	140	47	33.6	280	67	23.9
50	*Incisura supraorbitalis*	7	0	0	14	0	0	140	2	1.4	280	2	0.7
51	*Foramen supraorbitale*	7	1	14.3	14	2	14.3	140	22	15.7	280	26	9.3
52	*Foramen nasale absens*	6	2	33.3	12	2	16.7	137	25	18.2	274	30	10.9
53	*Foramen zygomaticofaciale absens*	6	2	33.3	13	2	15.4	140	35	25.0	280	52	18.6
54	*Foramen zygomaticofaciale partitum*	6	3	50	13	3	23.1	140	14	10	280	15	5.4
55	*Foramen ethmoidale anterius absens*	5	0	0	12	0	0	133	0	0	269	0	0
56	*Foramen ethmoidale posterius absens*	5	0	0	11	0	0	136	2	1.5	273	2	0.7
57	*Canalis opticus partitus*	6	0	0	12	0	0	115	10	8.7	248	15	6.0
58	*Foramen infraorbitale absens*	6	0	0	12	0	0	140	0	0	280	0	0
59	*Foramen infraorbitale partitum*	6	1	16.7	12	1	8.3	140	6	4.3	280	6	2.1
60	*Foramen infraorbitale accessorium*	6	1	16.7	12	2	16.7	140	27	19.3	280	30	10.7
61	*Foramen palatinum minus absens*	5	0	0	11	0	0	139	1	0.7	278	1	0.4
62	*Foramen palatinum minus accessorium absens*	5	2	40	11	2	18.2	137	33	24.1	276	51	18.5
63	*Ponticulus palatinus*	7	0	0	14	0	0	136	8	5.9	273	8	2.9
64	*Ponticulus palatinus incompletus*	7	1	14.3	14	1	7.1	136	27	19.9	273	37	13.6
65	*Foramen parietale absens*	7	5	71.4	14	8	57.1	140	85	60.7	280	127	45.4
66	*Foramen parietale inferior*	7	0	0	14	0	0	140	0	0	280	0	0
67	*Foramen squamosum superius*	7	0	0	14	0	0	133	1	0.8	271	1	0.4
68	*Processus parietalis squamae temporalis*	7	0	0	14	0	0	134	2	1.5	273	3	1.1
69	*Foramen mastoideum absens*	6	2	33.3	13	2	15.4	140	32	22.9	280	38	13.6

70	*Foramen mastoideum intrasuturam*	6	3	50	13	5	38.5	118	79	66.9	257	124	48.2
71	*Foramen mastoideum extrasuturam temporale*	6	5	83.3	13	7	53.8	118	71	60.2	259	103	39.8
72	*Foramen mastoideum extrasuturam occipitale*	6	0	0	13	1	7.7	119	14	11.8	256	18	7.0
73	*Foramen mastoideum accessorium*	6	3	50	12	5	41.7	140	32	22.9	280	36	12.9
74	*Foramen tympanicum*	6	0	0	12	0	0	135	20	14.8	271	31	11.4
75	*Foramen marginale*	6	0	0	12	0	0	139	7	5.0	274	10	3.6
76	*Foramen occipitale*	7	2	28.6	-	-	-	140	19	13.6	-	-	-
77	*Foramen condylaris absens*	6	4	66.7	12	4	33.3	140	46	32.9	280	57	20.4
78	*Canalis condylaris absens*	6	4	66.7	12	4	33.3	139	55	39.6	279	74	26.5
79	*Canalis condylaris intermedius*	4	1	25.0	10	3	30	133	53	39.8	270	72	26.7
80	*Canalis condylaris intermedius incompletus*	5	0	0	11	0	0	132	40	30.3	270	48	17.8
81	*Foramen hypoglossale partitum*	3	6	50	12	3	25.0	138	49	35.5	276	61	22.1
82	*Foramen hypoglossale partitum cum spina*	6	2	33.3	12	2	16.7	138	37	26.8	276	47	17.0
83	*Canalis basilaris medianus*	6	0	0	-	-	-	140	2	1.4	-	-	-
84	*Canalis craniopharyngeus*	6	0	0	-	-	-	138	8	5.8	-	-	-
85	*Foramen spinosum incompletum*	6	2	33.3	12	2	16.7	136	14	10.3	275	20	7.3
86	*Foramen spinosum incompletum partialis*	6	5	83.3	12	5	41.7	136	52	38.2	275	74	26.9
87	*Foramen ovale partitum*	6	0	0	12	0	0	137	1	0.7	276	1	0.4
88	*Foramen ovale incompletum*	6	1	16.7	12	1	8.3	134	5	3.7	272	8	2.9
89	*Foramen ovale et spinosum confluens*	6	0	0	12	0	0	138	7	5.1	277	9	3.2

90	*Foramen Vesalii*	6	4	66.7	12	5	41.7	138	86	62.3	277	132	47.7
91	*Ponticulus jugularis externus*	5	0	0	11	0	0	138	18	13.0	277	18	6.5
92	*Ponticulus jugularis externus incompletus*	5	1	20	11	1	9.1	137	33	24.1	276	41	14.9
93	*Ponticulus jugularis internus*	5	0	0	11	0	0	138	1	0.7	277	1	0.4
94	*Ponticulus jugularis internus incompletus*	5	2	40	11	2	18.2	138	6	4.3	277	7	2.5
95	*Ponticulus pterygospinosus*	5	1	20	11	2	18.2	140	4	2.9	274	4	1.5
96	*Ponticulus pterygospinosus incompletus*	5	0	0	11	0	0	128	13	10.2	259	18	7.0
97	*Ponticulus pterygoalaris*	5	0	0	11	0	0	140	4	2.9	280	5	1.8
98	*Ponticulus pterygoalaris incompletus*	5	0	0	11	0	0	134	9	6.7	266	11	4.1
99	*Processus clinoideus medius*	6	1	16.7	12	1	8.3	130	88	67.7	267	146	54.7
100	*Ponticulus carotico-clinoideus*	6	0	0	12	0	0	130	23	17.7	264	36	13.6
101	*Ponticulus carotico-clinoideus incompletus*	4	0	0	9	0	0	102	23	22.5	226	27	11.9
102	*Ponticulus interclinoideus*	4	0	0	10	0	0	94	7	7.4	209	10	4.8
103	*Ponticulus interclinoideus incompletus*	3	0	0	6	0	0	20	2	10	49	4	8.2
104	*Taenia interclinoidea*	6	0	0	12	0	0	126	6	4.8	258	8	3.1
105	*Foramen mentale absens*	5	0	0	10	0	0	52	0	0	104	0	0
106	*Foramen mentale partitum*	5	0	0	10	0	0	52	0	0	104	0	0
107	*Foramen mentale accessorium*	5	0	0	10	0	0	52	2	3.8	104	2	1.9
108	*Ponticulus mylohyoideus*	5	0	0	10	0	0	54	3	5.6	108	5	4.6
109	*Ponticulus mylohyoideus incompletus*	5	0	0	10	0	0	54	10	18.5	108	11	10.2
110	*Facies condylaris bipartita*	5	1	20	11	2	18.2	132	13	9.9	268	15	5.6
111	*Assimilatio atlantis*	6	0	0	-	-	-	140	0	0	-	-	-
112	*Depressio biparietalis circumscripta*	7	0	0	14	0	0	140	3	2.1	280	6	2.1

113	*Depressio interparietalis*	7	0	0	-	-	-	140	23	16.4	-	-	-
114	*Spina trochlearis*	7	1	14.3	14	2	14.3	138	22	15.9	279	31	11.1
115	*Tuberculum marginale absens*	6	3	50	13	4	30.8	140	67	47.9	280	101	36.1
116	*Tuberculum zygomaxillare*	6	2	33.3	13	3	23.1	140	23	16.4	280	34	12.1
117	*Torus palatinus*	7	2	11.8	-	-	-	138	66	47.8	-	-	-
118	*Torus maxillaris*	7	1	14.3	14	2	14.3	134	3	2.2	268	4	1.5
119	*Spina suprameatica*	6	0	0	12	0	0	138	60	43.5	278	102	36.7
120	*Depressio suprameatica*	6	5	83.3	12	9	75.0	135	34	25.2	278	64	23.0
121	*Torus acusticus*	6	0	0	12	0	0	139	1	0.7	275	2	0.7
122	*Tuberculum pharyngeum absens*	6	0	0	-	-	-	139	8	5.8	-	-	-
123	*Condylus tertius*	6	0	0	-	-	-	140	1	0.7	-	-	-
124	*Tuberculum praecondylare*	6	0	0	12	0	0	139	6	4.3	264	8	3.0
125	*Processus paracondylaris*	5	0	0	10	0	0	130	4	3.1	265	7	2.6
126	*Processus retromastoideus*	6	0	0	12	0	0	140	0	0	280	0	0
127	*Linea nuchae suprema*	7	4	57.1	14	8	57.1	140	81	57.9	280	159	56.8
128	*Torus occipitalis*	7	0	0	-	-	-	140	7	5.0	-	-	-
129	*Processus styloideus elongatus*	4	1	25.0	8	2	25.0	28	1	3.6	85	3	3.5
130	*Torus mandibularis*	5	0	0	10	0	0	52	0	0	104	0	0
131	*Fossa pharyngea*	6	2	33.3	-	-	-	138	22	15.9	-	-	-

Table S1: Evaluated non-metric cranial traits (Nos. 1–131) and their frequencies in the genealogically documented assemblage in comparison with the reference frequencies from the Pachner collection (Cvrček et al. 2018).

Received: 24 January 2020 | Revised: 15 May 2020 | Accepted: 15 May 2020

DOI: 10.1111/joa.13246

METHODS

ANATOMICAL SOCIETY Journal of Anatomy WILEY

Biological relationships and frontal sinus similarity in skeletal remains with known genealogical data

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Funding information
Charles University Grant Agency (Grant Numbers: GAUK 1276217, GAUK 1590218); Ministry of Culture of the Czech Republic (DKRVO 2019-2023/7.I.b,00023272).

Abstract

Frontal sinus analysis has potential utility for detecting biologically related individuals. However, the methodological approach to its evaluation, as well as its informative value, have been questioned. The aim of this work is to introduce a new approach to evaluating the frontal sinus using the 'external supraorbital line' (ESOL) and to determine whether there are sex differences within families in frontal sinus measurements and whether frontal sinus similarity reflects known genetic relationships in both measurements and morphology. We examined the skeletal remains of 41 adult individuals (25 males, 16 females), all members of one family over four generations (19th to 20th centuries), including individuals with very close consanguinity. CT images of skulls were acquired, and both the dimensions and morphology of the frontal sinuses were analyzed using their portions above the ESOL. No significant sex differences were found within families based on frontal sinus dimensions. Significant relationships were found between biological distance and the maximum height and morphology of the frontal sinuses. The greatest degree of similarity was found among closely related individuals. Additionally, in several cases, there was a greater degree of similarity between first cousins or grandparents and their grandchildren than among siblings or parents and their children. Total surface, volume and width are not significant indicators of relatedness. Known genetic relationships are also supported by individual morphological features. Variability within families with very close consanguineous relationships was lower than within families with common degrees of consanguinity, although differences are significant only for some variables.

KEYWORDS

dimensions, frontal sinus anatomy, genealogical documented sample, inbreeding, morphology

1 | INTRODUCTION

The formation and variability of craniofacial pneumatization is a relevant subject in research, particularly in terms of its evolutionary aspects, function, development issues, medical relevance and use in forensic practice (Butaric and Maddux, 2016; Choi *et al.*, 2018; Godinho and O'Higgins, 2018; Nikolova *et al.*, 2018). In research on pneumatization, increasing attention is being paid to the frontal sinuses. The variability of their patterns (i.e. configuration, shape, anatomical variations, dimensions) is wide (Szilvássy, 1982; Guerram *et al.*, 2014; Lund *et al.*, 2014; Choby *et al.*, 2018; Štoković *et al.*, 2018). Because these sinuses are encased in the solid frontal bone, they are usually preserved in archaeological contexts and we can evaluate them even if the overall preservation of skeletal remains is

poor (Cameriere *et al*., 2008; Goyal *et al*., 2013). For these reasons, they are of interest to bioarchaeology, forensic anthropology and medicine. There are three main areas of potential application of the frontal sinus in forensic anthropology and bioarchaeology: sex estimation, population affinity estimation and individual identification (Christensen and Hatch, 2018).

Currently, dental and osteological non-metric traits are preferred for the detection of family relationships in skeletal remains, apart from DNA analysis (Stojanowski and Schillaci, 2006). However, the possible use of frontal sinuses for the detection of biologically related individuals has also been discussed (Leicher, 1928; Asherson, 1963). Accordingly, the genetic component of frontal sinus formation is also a subject of interest. This issue has most often been studied in living families (father, mother, children) and dizygotic and monozygotic twins. Frontal sinus similarities have been found in morphology and dimensions between biologically related individuals (Szilvássy, 1986; Kjaer *et al*., 2012). It is believed that the degree of paranasal sinus pneumatization is possibly more influenced by genetic factors more than by the development of anatomical variations of some other paranasal structures (Chaiyasate *et al*., 2007). These types of studies, however, face ethical restrictions in terms of research on living people (Kjaer *et al*., 2012), especially in terms of doses of X-rays without medical indication. The findings were then verified on osteological samples, for which these similarities were also found (Szilvássy *et al*., 1987; Alt *et al*., 1997; Slavec, 2004). It is possible to identify biological affinity even among skeletons in poor condition, although we cannot use frontal sinuses to identify the exact relationship of the individuals or their exact position in the family tree (Cameriere *et al*., 2008).

Until the beginning of the 21st century, osteological studies focused mainly on comparing individuals using simple visual description of sinus morphology based on anteroposterior radiographs (Szilvássy *et al*., 1987; Slavec, 2004). However, this approach could be considered subjective (Asherson, 1963) due to its lack of clear interobserver replicability. Furthermore, dimensions in combination with morphological characteristics have been rarely used (Slavec, 2005). Statistical evaluations as well as medical imaging methods, such as CT and MRI, began only relatively recently to be used in frontal sinus analysis, especially on living people. Each of the recent studies focused on different variables: size based on development categories (Chaiyasate *et al*., 2007), the morphology scoring system used (Cameriere *et al*., 2008) and exact dimensions expressed in units (Kjaer *et al*., 2012). Among osteological samples, the possibilities for study are extremely limited by the availability of genealogically documented material (Carson, 2006; Ricaut *et al*., 2010; Gavrus-Ion *et al*., 2017), its state of preservation and, consequently, the evaluation methodologies available (CT, MRI, statistics).

The question of the influence of frontal sinus pattern inheritance, its informative value, and methodology is still under discussion (Latiff *et al*., 2009; Ito *et al*., 2015). All of the above-mentioned studies dealing with frontal sinus and relatedness assume that genetics affects frontal sinus dimensions and morphology. However, each of them evaluates only a small number of variables; none deals with the issue comprehensively, and they apply different methods. This variability can pose problems for comparisons and transfer of knowledge to other studies (Kjaer *et al*., 2012). Finally, the application of modern imaging methods to enable a much more exact analysis is still in the early stages (Chaiyasate *et al*., 2007). With regard to these facts, the aim of this work is to show the relationship between the individual basic variables (i.e. total surface area and volume, maximum height and width, and morphology) of the frontal sinus and biological kinship.

Using a comprehensive approach based on CT images respecting previously used approaches, we aim to answer the following questions in this order:

First, are there any significant differences between males and females within families in frontal sinus measurements? Whereas significant sex differences in frontal sinus size across different populations have been demonstrated (Akhlaghi *et al*., 2016; Choi *et al*., 2018; Čechová *et al*., 2019), the unresolved question remains as to whether there are significant differences within families. Therefore, we seek to verify that we do not have to take the sex of individuals into account when detecting family relationships because sexual differences are not significant among biologically related individuals.

Secondly, are there positive relationships between the degree of frontal sinus morphological and dimensional similarity and the biological distance of biologically related individuals? Can these variables be used for kinship analysis?

Finally, are there any significant differences in the degree of frontal sinus morphological and dimensional similarity between members of families with very close consanguineous relationships and members of parallel-related families with common degrees of consanguinity?

2 | MATERIALS AND METHODS

2.1 | Materials

The skulls of 41 adult individuals (25 males and 16 females) aged 20–90 years with known genealogical data were available (Cvrček *et al*., 2018). They were members of five branches of one family over four generations in the 19th–20th centuries from Bohemia, Czech Republic (family tree: Supporting Information No. 1, Figure S1, generation Nos 4–7). The skeletal remains researched were provided to us based on written permission from direct descendants of the studied individuals. Research on the remains was made possible by the repair of family tombs and coffins and relocation of the remains. After the research concluded, the skeletal remains were re-buried.

There were two consanguineous marriages and their descendants in the sample. The first instance involved doubly biologically related spouses Nos 7 and 8 (first cousins and second cousins, generation No. 4). From them, six offspring could be evaluated: sons Nos 9, 11 and 13, and daughters Nos 12, 14 and 17 (generation No. 5). The next two generations from daughter No. 17 were also available: male No. 19 (generation No. 6) and his sons, Nos 20 and 21

(generation No. 7). Son No. 13 also had a biologically related wife, No. 37 (generation No. 6).

The second case of individuals with very close consanguinity is represented by male No. 41 and his son, No. 42. The mother of the son is the second cousin of his father (generation Nos 5–6), but her remains were not available. Their second son, No. 43, was a neonate; therefore, he was not included in this study.

Skulls were scanned at the Radiology Department of the Hospital Na Homolce in Prague by computed tomography (CT) using a Somatom Sensation 16 scanner (Siemens, Erlangen, Germany). Acquisition parameters were optimally set with a voxel size 0.49 mm, a slice thickness 0.6 mm and a slice increment 0.3 mm.

2.2 | Methods

Surface models of frontal sinuses were segmented from the CT scans in Morphome3cs software (www.morphome3cs.com) (Musilová *et al*., 2016; Čechová *et al*., 2019) and were further processed and cleaned in Meshlab (Visual Computing Lab, Italian National Research Council), Avizo 7.1 (Visualization Sciences Group, SAS) and Geomagic Studio 2012 (Raindrop Geomagic GmbH, 3D Systems).

Of the total number of 41 individuals, 10 have large postmortem defects: mainly, the bottom of the sinuses is missing or the orbital roofs are damaged (Nos 5, 11, 12, 14, 19, 23, 28, 32, 39, 40). In eight other individuals, the frontal bone was cut during autopsy (a common means of controlling sinus pathological changes in that period, Nos 6, 9, 19, 21, 24, 38, 43, 44); several cases involved chopping directly into the lower parts of the frontal sinuses and roofs of the orbits (Figure 1). Therefore, whole sinuses could not be evaluated, nor could the supraorbital line (SOL), most often used to evaluate radiographs, be employed (Cameriere *et al*., 2008; Nikam *et al*., 2015; Nikolova *et al*., 2018). With regard to these restrictions of our osteological material, we have modified the approach and used the external supraorbital line ('ESOL') instead. All of the meshes were cut along a plane that passes through the upper orbital margins and is parallel to the Frankfurt plane (Borovanský, 1936; Nussen, 2007). A portion superior to this plane was targeted for examination; this part was completely preserved in all individuals. Finally, pictures of frontal sinuses were acquired in the frontal view so that the cutting plane was perpendicular to the computer screen.

2.2.1 | Sex differences

Sex classification was performed in Morphome3cs software using fitting support vector machines (SVM) on the combinations of total surface area and volume and maximum height and width of the frontal sinuses. Equations created for the contemporary Czech population were used on this sample (Čechová *et al*., 2019). The sample on which the equations were based included 45 females and 58 males, aged 21–84 years. SVM carries out classification by finding the hyperplane that maximizes the boundary between the two groups. A radial kernel was employed and the parameters were set depending on the variables to obtain the best results. The SVM algorithm was trained and tested on the whole sample. For more objective results, leave-one-out cross-validation was performed to detect overfitting.

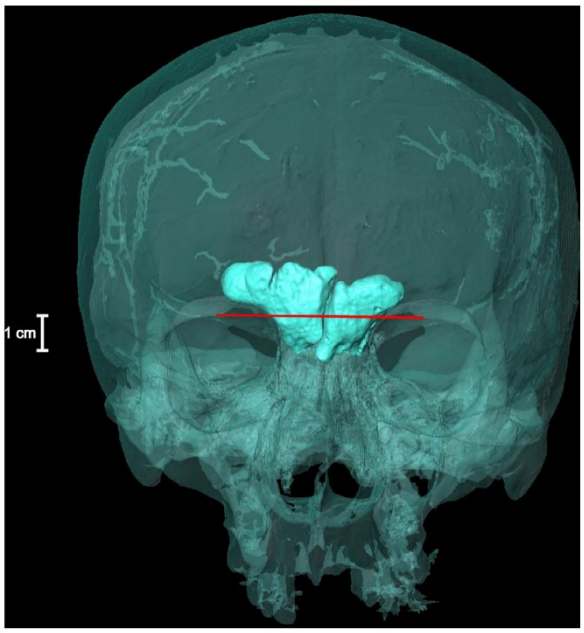


FIGURE 1 CT scan of a skull with a highlighted frontal sinus and 'external supraorbital line' (ESOL, red line), individual No. 17 [Colour figure can be viewed at wileyonlinelibrary.com]

2.2.2 | Evaluation of dimensions

For each individual, four basic dimensions were found: total surface area and volume using Meshlab, and maximum height and width using Avizo 7.1. For each variable, the degree of dimensional similarity ('DS') between two individuals was calculated based on the formula originally designed by Szilvássy (1974) for expression of frontal sinus side asymmetry:

$$DS = 100 \times \frac{A}{B}$$

where A is an individual with a smaller value and B is an individual with a larger value of the given variable. The higher the value of DS, the greater the degree of similarity between individuals. The resulting DS values of each dimension represent the basic data for further analysis:

- We calculated the logarithm of the coefficient of the relationship (logarithm of biological distance) for each possible pair in the sample (820 combinations). The coefficient (r) was calculated using a tabular approach (Falconer and Mackay, 1996) based on the method of VanRaden (1992). Use of a logarithm (logR) eliminates entirely unrelated individuals (their coefficient of relationship is

always zero; thus, the logarithm is not defined) and allows graphical expression of the relationship between the degree of similarity and the biological distance only for biologically related individuals (Cvrček *et al*., 2018). We used a linear model to test the relationship between the DS of each dimension and the logarithm of the biological distance for all related pairs in the sample (level of significance $\alpha = 0.05$). For the graphical representation using a linear model, we performed arcsine transformation of the data on the Y-axis (DS).

- We also used cluster analysis (Murtagh, 1985), which was performed by the complete linkage hierarchical method, where the distance between two clusters was determined as the distance between their most distant individuals. The DS of each pair was converted into a distance such that the greatest similarity indicated the smallest distance and vice versa.
- We compared the degree of similarity between family with common degrees of consanguineous relationships (group A, individuals Nos 1, 2, 3, 4, 5, 6, 38) and parallel-related family with very close consanguinity (group B, individuals Nos 7, 8, 9, 17, 18, 19, 20). Differences were tested using the Wilcoxon signed-rank test (level of significance $\alpha = 0.05$) (Siegel, 1956). The resulting *p*-values were adjusted by Bonferroni correction (Bonferroni, 1936). The variation in each group was also compared using box plots.

2.2.3 | Morphological evaluation

Visual characters were evaluated from pictures of 3D models in frontal view. Nine features were evaluated based on previous publications (Szilvássy, 1986; Yoshino *et al*., 1987; Reichs, 1993) (Table 1).

The degree of morphological similarity ('MS') between biologically related pairs of individuals based on class numbers of the above-described categories was calculated using cosine similarity (Singhal, 2000). Based on these data, we could use a statistical evaluation similar to that used for the metric dimensions (points 1–3).

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions. The data that supports the findings of this study are available in the supplementary materials for this article.

3 | RESULTS

3.1 | Sex differences

Neither combination of variables (total surface area and volume, maximum height and width) provides a clear distinction between males and females. No pattern is observed in the data; thus, there is no reason to separate males and females (Figure 2). Males and females overlap, and some males have much smaller sinus parameters than females (e.g. males Nos 3, 18, 43 vs. females Nos 2, 10, 44, etc.). At the same time, our attempt at classification by sex based on the combination of total surface area and volume showed considerable error. The results after cross-validation suggested a success rate of only 64.1%, and no females were correctly classified (SVM posterior success rate of the male group = 100.0%, SVM posterior success rate of the female group = 0.0%). A combination of maximum

TABLE 1 Visual features and their classifications for expression of frontal sinus morphology

Category	Classification	Class number
Total size, Yoshino *et al*. (1987)	Small (0–6)	1
	Middle (6–12)	2
	Large (12–18)	3
	Very large (> 18)	4
Bilateral asymmetry, Yoshino *et al*. (1987)	Symmetry (100–80)	1
	Slight asymmetry (80–60)	2
	Moderate asymmetry (60–40)	3
	Strong asymmetry (40–20)	4
	Extreme asymmetry (< 20)	5
Superiority, Reichs (1993)	Absent	0
	Left equal to right	1
	Left greater than right	2
	Right greater than left	3
Outline of upper border for left side and right side separately, Yoshino *et al*. (1987)	Absent	0
	Smooth	1
	Scalloped with 2 arcades	2
	Scalloped with 3 arcades	3
	Scalloped with 4 arcades	4
	Scalloped with > 5 arcades	5
Partial septa, Yoshino *et al*. (1987)	Absent	0
	Present in the left side	1
	Present in the right side	2
	Present in the both sides	3
Supraorbital cells, Yoshino *et al*. (1987)	Absent	0
	Present in the left side	1
	Present in the right side	2
	Present in the both sides	3
Shape for left side and right side separately, Szilvássy (1986)	Bean shape	1
	Leaf shape	2
	Fan shape	3
	Pyramid shape	4
Lateral range for left side and right side separately (own category)	Absent	0
	< 1/3 border of orbit	1
	< 1/2 border of orbit	2
	> 1/2 border of orbit	3
	> lateral border of orbit	4

height and width provided a similar result—56.4% of the success rate after cross-validation—but the SVM posterior success rate of the male group was 100.0%, whereas that of the female group was only 14.3% (n = 2; Figure 2). This result very strongly suggests no discernible sex differences.

Individuals Nos 12 and 40 were excluded from the assessment due to their incomplete preservation; only total width could be evaluated.

3.2 | Dimensional similarity

The resulting values of the degree of similarity between individuals based on frontal sinus total surface area, volume, and maximum height and width are shown in Supporting Information No. 2 (Table S1–S4). Due to incomplete preservation, individuals Nos 12 and 40 were excluded from assessments of total surface area, volume, and maximum height.

There is no statistically significant relationship between the biological distance of biologically related individuals and the degree of frontal sinus similarity based on total surface area (p = .133; Figure S2), volume (p = .060; Figure S3) or width (p = .304; Figure S4). However, based on maximum height, the degree of frontal sinus similarity was statistical significant (p = .031; Figure 3). The greater the degree of biological relationship, the greater the degree of similarity. In any case, this does not always apply. Within closely related individuals, there may be a greater degree of similarity between some cousins (r = .125, logR = −0.90309) than among other close relatives, such as parents and children (r = 0.5, log = −0.30103) or grandparents and grandchildren (r = .25, log = −0.60206). For example, cousins Nos 8 and 31 (DS^h = 85.7) and mother No. 8 and daughter No. 17 (DS^h = 66.7), cousins Nos 1 and 7 (DS^h = 100.0) and male No. 1 and his son No. 3 (DS^h = 44.4), or cousins Nos 1 and 7 (DS^h = 100.0) and male No. 7 and his grandsons Nos 20 and 21 (DS^h = 75.0, 85.7 respectively).

Figures S5–S8 show the degrees of similarity of all evaluated individuals using cluster analysis based also on total surface area,

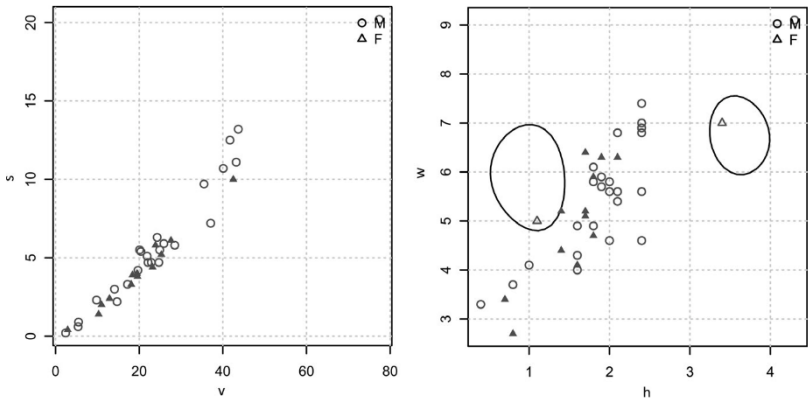


FIGURE 2 Graphical expression of total surface area (s), volume (v), and maximum height (h) and width (w) data by sex of individuals (F = females, M = males). The right graph shows two correctly classified females

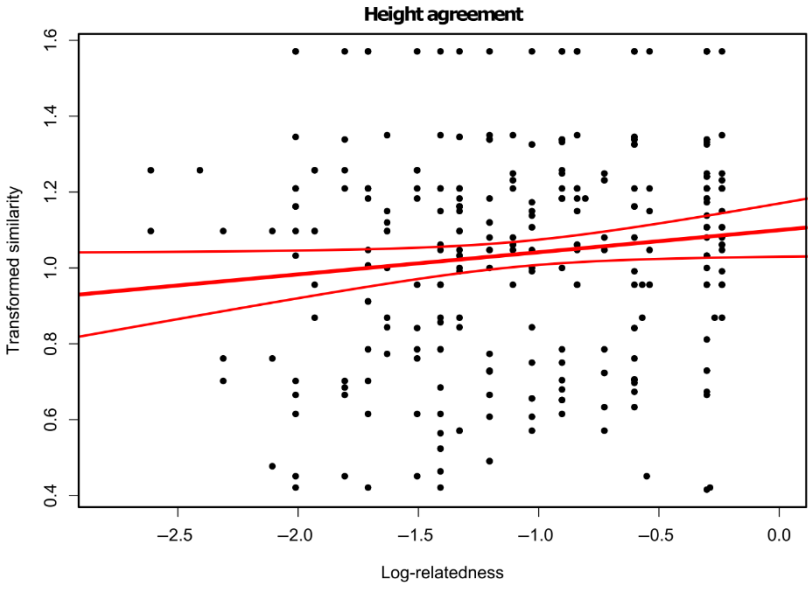


FIGURE 3 Relationship of the similarity between biologically related individuals based on the maximum height of the frontal sinuses (DS^h, y-axis) by log-relatedness of individuals (x-axis). The red line represents the regression line. Note: log-relatedness disregards unrelated individuals [Colour figure can be viewed at wileyonlinelibrary.com]

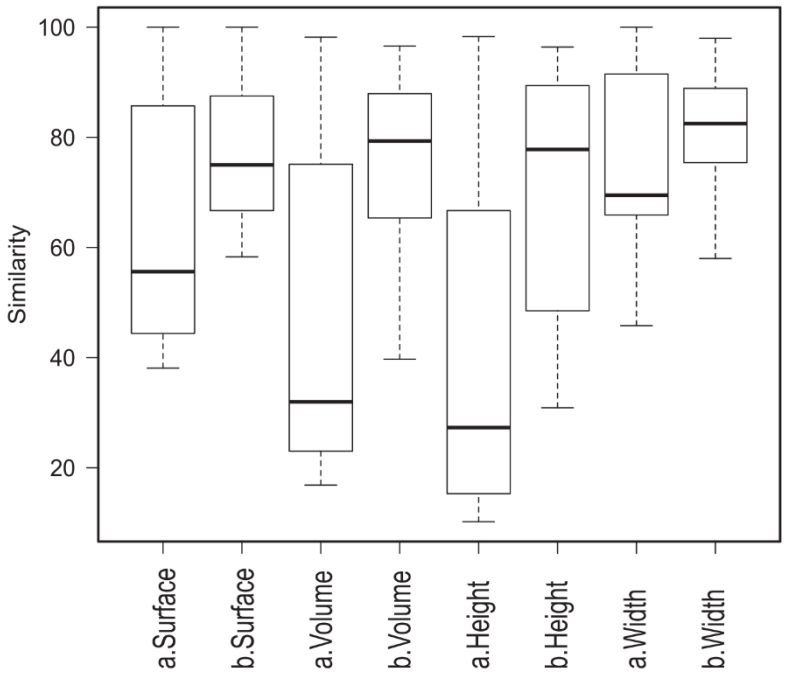


FIGURE 4 Variability in the degree of similarity between family with common degrees of consanguinity (a) and family with very close consanguineous relationships (b) for frontal sinus total surface area, volume, and maximum height and width

volume, and maximum height and width. The results reflect some known genetic relationships.

In total surface area, the closest are father No. 18 and son No. 19, father No. 7 and son No. 9, and mother No. 23 and daughter No. 24 (Figure S5).

In total volume, the closest are grandfather No. 3 and grandson No. 6, grandmother No. 31 and granddaughter No. 37, and brothers Nos 9 and 11 (Figure S6).

In maximum height, the closest are brothers Nos 18 and 22, grandfather No. 32 and grandson No. 36, and siblings Nos 11 and 14 (Figure S7).

Finally, in maximum width, the closest are father No. 18 and son No. 19, grandmother No. 17 and grandson No. 19, and mother No. 34 and son No. 36 and daughter No. 37 (Figure S8).

Furthermore, there is greater variability in the degree of similarity among the parallel family with common degrees of consanguinity (group A) compared with the family with very close consanguineous relationships (group B) in all dimensions (total surface area, volume, and maximum height, width) (Figure 4). Each group contains 17 pairs of individuals. Statistically, there are significant differences only in total surface area (p = .031) and volume (p = .012); there is no significant difference in height (p = .116) or width (p = .380).

3.3 | Morphological similarity

Figures showing the frontal sinus are provided in Supporting Information No. 3 (Figures S9–S13). The figures are divided by family. Based on a simple visual description of sinus morphology, several specific features and several configurations among close relatives have been identified. There is the laterally stretched arcade separated by a wide notch in the right sinus in the inbred brothers Nos 9, 11 and 13 or a specific notch (mirror-occurring) with pointed peaks in the same brothers, Nos 9 (near medial border of the left sinus) and 11 (near lateral border of the right sinus). Mirror similarity can also be observed in their double biologically related parents Nos 7 and 8 (first cousins and second cousins) in the overall character of their sinuses: the right sinus of male No. 7 and the left sinus of female No. 8 have a fan shape (even a ginkgo shape) with arcade category 5, and the left sinus of male No. 7 and the right sinus of female No. 8 are irregularly lobed, with arcade category 3 (Figure S10). Similarly, we found a distinct lateral extension in male No. 39 (right side) and his son No. 41 (left side). These similarities are shown in Figure 5.

Manifestations of the morphological similarities of all evaluated individuals are shown in Supporting Information No. 2 (Table S5). Seven individuals whose patterns could not be completely evaluated had to be excluded from the evaluations due to poor preservation (Nos 11, 12, 19, 32, 39, 40, 45). The difference in the degree of frontal sinus morphological similarity (Table S6) and the biological distance of biologically related individuals (p = .034) is statistically significant; that is, the greater the degree of biological relationship, the greater the degree of morphological similarity (Figure 6). However, as with the maximum height of the frontal sinus, within closely related individuals, there may be a greater degree of similarity between some cousins (r = .125, log = −0.90309) than among other close relatives, such as parents and children (r = .5, logR = −0.30103) or grandparents and grandchildren (r = .25, logR = −0.60206), such as cousins Nos 8 and 31 (MS = 0.86) and mother No. 31 and daughter No. 34 (MS = 0.77), cousins Nos 1 and 7 (MS = 0.89) and male No. 1 and his son No. 3 (MS = 0.88), or cousins Nos 1 and 7 (MS = 0.89) and male

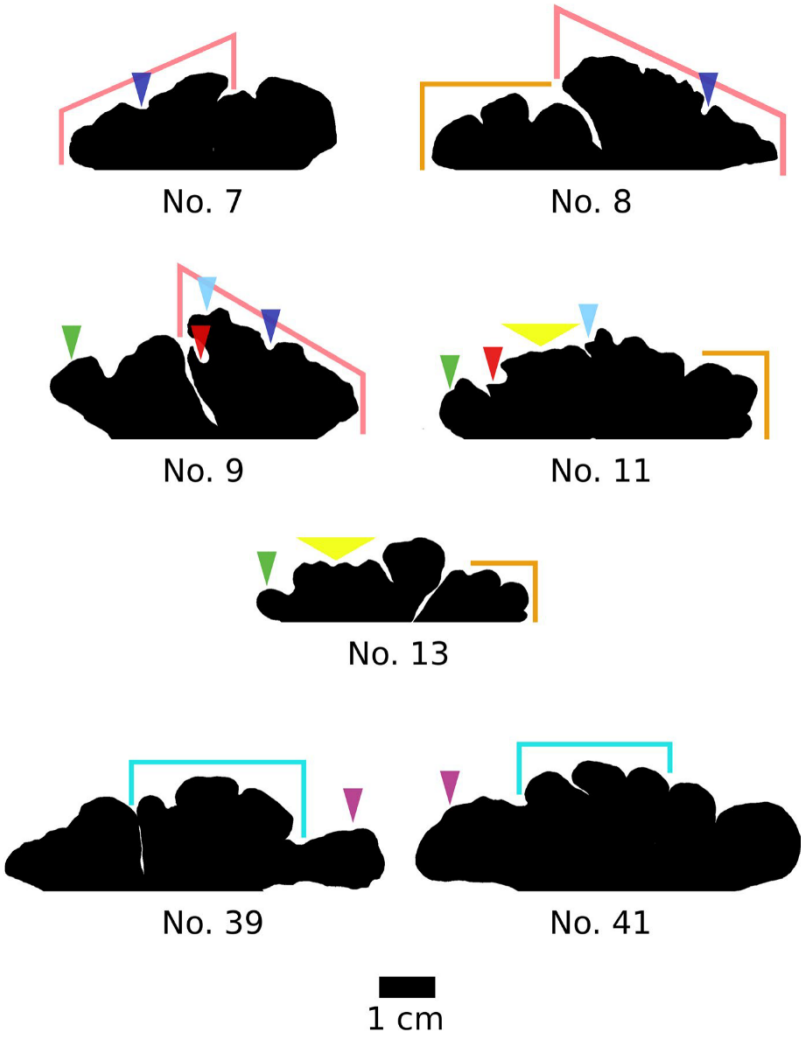


FIGURE 5 Examples of various morphological similarities (arrows, clamps) shared by closely related individuals (spouses and first cousins Nos 7 and 8, their sons Nos 9, 11 and 13, and father No. 39 with son No. 41). Shared similarities are marked with the same colors [Colour figure can be viewed at wileyonlinelibrary.com]

No. 7 and his grandsons Nos 20 and 21 (MS = 0.75). Some of these cases are also evident when we talk about their overall morphology in the picture, such as cousins Nos 1, 7 and 39 (Figures S9, S10 and S13), and male No. 1 and his son No. 3 (Figure S9). There may also be a case where completely unrelated individuals are very similar, even more than to their children, such as spouses Nos 1 and 2 and their son No. 3 (Figure S9). The only case of full agreement in frontal sinus morphological pattern is shown in brothers Nos 20 and 21 (Table S5).

Figure S14 shows the degrees of similarity of all evaluated individuals using cluster analysis based on morphology. This figure reflects only some relationships. The greatest degrees of similarity are between uncle No. 33 and niece No. 44 and between half-siblings Nos 7 and 31.

There is greater variability in the degrees of similarity among parallel family with common degrees of consanguinity (group A) than family with very close consanguineous relationships (group B) in morphology (whiskers), as shown in Figure 7. Group A contains 17 pairs of individuals, and group B contains 11 pairs of individuals due to the exclusion of individual No. 19. However, there are also outlying values, such as the similarity between mother No. 4 and son No. 5, and grandmother No. 4 and grandson No. 6 (group A) and the similarity between great-grandfather No. 7 and great-grandson No. 20 (group B). There is no statistically significant similarity between these groups (p = .890).

4 | DISCUSSION

For this study, we used a new method of frontal sinus demarcation using the 'external supraorbital line' (ESOL). This approach better reflects the preservation limits of bioarchaeological and forensic material. Frontal sinuses are protected by the solid frontal bone; for that reason, we can evaluate them even when remains are poorly preserved (Cameriere *et al.*, 2008; Goyal *et al.*, 2013), though some parts of the frontal bone are often exposed to decomposition processes and artificial interventions. In our material, this is particularly true for the thin sinus base, the ethmoidal labyrinth and the orbital roofs. In addition to postmortem damage or deformation (24.4% of

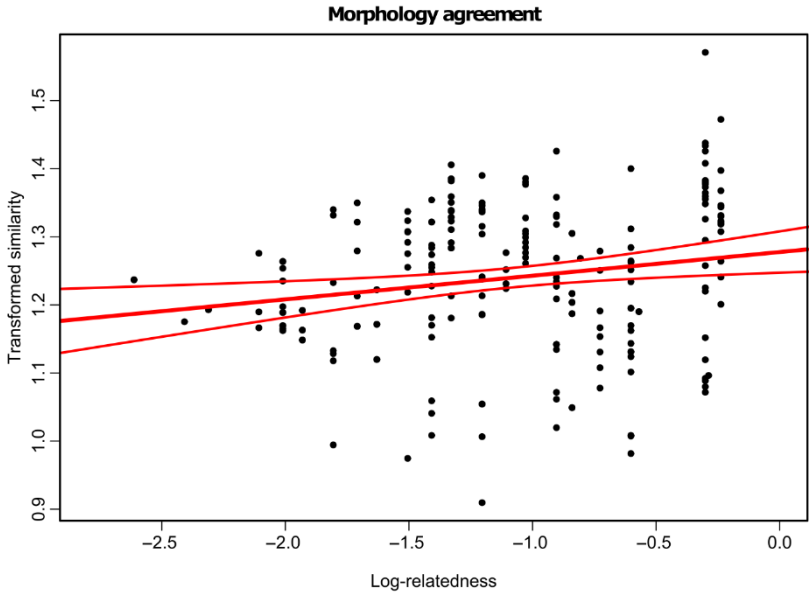


FIGURE 6 Relationship of the similarity between biologically related individuals based on the morphology of the frontal sinuses (MS, *y*-axis) by log-relatedness of individuals (*x*-axis). The red line represents the regression line. Note: log-relatedness disregards unrelated individuals [Colour figure can be viewed at wileyonlinelibrary.com]

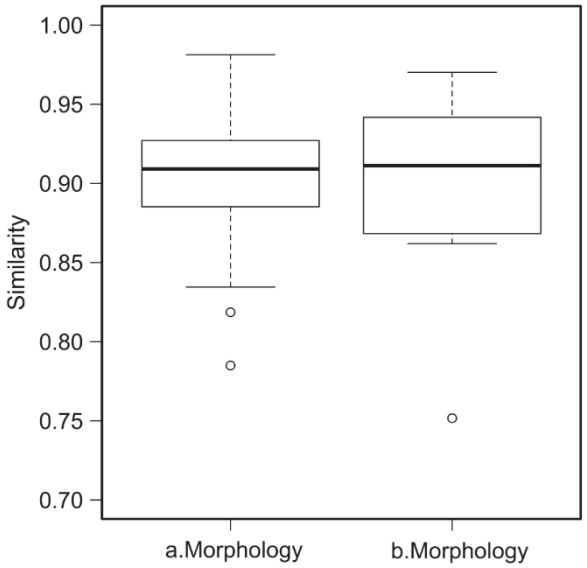


FIGURE 7 Variability in the degree of similarity between family with common degrees of consanguinity (a) and family with very close consanguineous relationships (b) for frontal sinus morphology

individuals), the sinuses are often subjects of investigation for pathological changes during autopsy (19.5% of individuals). This represents a very significant limitation for our work, as almost half of the whole sample is impacted. Thus, past approaches, such as using the 'supraorbital line' (SOL) (Christensen, 2004; Cameriere *et al.*, 2008; Nikam *et al.*, 2015; Nikolova *et al.*, 2018) or evaluation of complete sinuses, including the base (Čechová *et al.*, 2019), cannot be used for our purposes. Our proposed approach bypasses these limitations. In addition, the results do not seem to be particularly dependent on the method used and are comparable to methodically different studies if the defined procedure is always followed. This will be discussed below.

4.1 | Sex differences

There were no significant differences in any of the studied sinus dimensions between males and females. This suggests that although there are sex differences evident within normal populations, i.e. in randomly selected unrelated individuals (Uthman *et al.*, 2010; Sai Kiran *et al.*, 2014; Benghiac *et al.*, 2015; Michel *et al.*, 2015; Akhlaghi *et al.*, 2016; Hamed *et al.*, 2016; Motawei *et al.*, 2016; Choi *et al.*, 2018), the sex differences are not noticeable within families. We do not have to take into account the sex of individuals when investigating family relationships.

There are significant differences between males and females in the total volume and surface area of the frontal sinuses in randomly selected individuals from the contemporary Czech population, which is genetically similar to our sample (Čechová *et al.*, 2019). At the same time, the success rate of sex prediction was substantially decreased when using contemporary sex standards. The cited study also showed that there are greater variabilities in volume and surface area of the frontal sinuses of Czech males than females. This is also suggested by our results. However, a comparison of linear dimensions is currently unavailable. Further verification will be required for other genealogically documented material.

4.2 | Dimensional similarity

According to our results, there are no significant relationships between biological distance and size similarity based on total surface

area, volume or width. However, we found a significant relationship with maximum height. According to Kjaer *et al.* (2012) who evaluated lateral radiographs of 42 pairs of monozygotic twins (18 male and 24 female pairs) from the recent Danish population, significant differences between two individuals were found only within one female twin pair and within two male twin pairs in the maximum height of the frontal sinus. There were no significant differences within twin pairs in other studied cases. Although different approaches were used, we can consider these results to support our study in that kinship affects the similarity of frontal sinus maximum height. At the same time, it turns out that the simple formula originally designed by Szilvássy (1974) for expression of frontal sinus side asymmetry is appropriate for our purposes.

Comparison information from past studies is lacking. Other studies using linear dimensions (especially Szilvássy *et al.*, 1987; Slavec, 2004, 2005) lack numerical expressions of degrees of similarity between related individuals and statistical data processing. Also, comparison of their surface area and volume are missing. This is mainly because the frontal sinuses previously could only be evaluated using X-ray images (Slavec, 2005; Kjaer *et al.*, 2012). However, according to our results, radiographs seem to be quite sufficient for the detection of related individuals. Although the relationship between the width of the frontal sinus and biological distance has not proved significant, our study suggests that linear dimensions could be more important than surface area and volume. At the same time, unlike modern imaging methods, X-ray examination makes it easier to evaluate even incomplete remains where it would be meaningless to use surface models.

Considering all of the dimensions examined, only a few documented family relationships were reflected in the cluster analysis; less than shown by non-metric traits (Cvrček *et al.*, 2018). However, the closest pairs are again the closest relatives, such as father and son, mother and daughter, siblings, and grandparents and grandchildren.

4.3 | Morphological similarity

There is a statistically significant relationship between biological distance and morphological similarity in the frontal sinuses. The same conclusions reported for the measured dimensions above apply to the results of the morphological trait cluster analysis. The closest pairs in the cluster analysis are closest relatives (father and son, mother and daughter, siblings, and grandparents and grandchildren).

The scoring systems designed by Szilvássy (1986), Yoshino *et al.* (1987), Reichs (1993) and other authors (Kim *et al.*, 2013) allow the use of frontal sinus morphology for kinship analysis. We came to the same conclusion in our analysis of non-metric traits on the same sample with known genealogical data (Cvrček *et al.*, 2018); that is, the greater the degree of biological relationship, the greater the degree of morphological similarity. However, we emphasize that the degree of morphological similarity of biologically related individuals based on the frontal sinuses is less than that using non-metric traits (Cvrček *et al.*, 2018). In addition, identification of exact genealogical position in the family tree of an individual using frontal sinus analysis is impossible (Cameriere *et al.*, 2008). The same can be seen in skeletal non-metric traits (Ricaut *et al.*, 2010).

The overall morphology of the sinuses can vary, even if they have the same pattern, such as in two brothers in our sample (Nos 20 and 21). Although we can consider categorical evaluation to be more objective (Cameriere *et al.*, 2008) than visual assessment of overall morphology (Asherson, 1963), in our opinion, it is possible to find a subjective level of similarity even using categorical evaluation. This is especially true for assessing the number of arcades. In some cases, however, it may be difficult to determine which arcades should be counted, and whether the small arcades are large enough and their boundaries are well enough marked. Therefore, the simple verbal description of overall character of the frontal sinuses including specific traits (Szilvássy *et al.*, 1987) is still not negligible for expressing similarity among individuals. For non-metric traits, statistical evaluation does not allow for the capture of some of the markers that further highlight the biological relationships of individuals (e.g. inbred brothers Nos 9, 11 and 13). We consider the mirror similarity of sinuses as one of the most important markers that can indicate biological affinity. In the past, this phenomenon was observed in twins (Asherson, 1963), making it apparent that such sinus development is genetically influenced. However, in that case, the authors argued that the twins were not similar (Asherson, 1963). Although this conclusion cannot be completely denied, we should always consider that this feature may occur, and we should take this possibility into account. The importance of the perception of possible side asymmetry in trait expression, which serves to verify similarity on opposite sides, has already been demonstrated in non-metric osteological traits (Cvrček *et al.*, 2018). In this study, this is especially apparent in the case of doubly biologically related spouses Nos 7 and 8, their sons Nos 9 and 11, or in male No. 39 and his son No. 41. With emphasis only on the character and peculiarities of the sinuses, we can reduce the subjectivity of the descriptive approach (Szilvássy *et al.*, 1987). The use of both scoring systems and morphological descriptions should be combined (Slavec, 2004, 2005), as supported by our results.

Combined assessment of dimensions and morphology is useful for identification of biological affinity (Slavec, 2004, 2005). Our results confirm that there are similarities between biologically related individuals in both dimensions and morphology. In addition to combining approaches to the evaluation of sinus morphology, we consider it important to compare sinuses (dimensions, morphology) and non-metric traits where possible. We have shown the obvious similarity of unrelated spouses Nos 1 and 2 and their son No. 3, who is not as similar to them as is the son of No. 38. Their identity is undoubtedly certain, and non-metric traits confirm that there is a greater degree of similarity between female No. 2 and her son No. 3 than between female No. 2 and her husband No. 1 (Cvrček *et al.*, 2018).

4.4 | The rate of similarity between cousins and the influence of inbreeding on variability

It is not absolutely true that the greater the degree of biological relationship, the greater the degree of dimensional or morphological similarity. This has been confirmed in several previous studies using osteological non-metric traits (Gemmerich-Pfister, 1999; Velemínský and Dobisíková, 2005; Cvrček *et al*., 2018). Within closely related individuals, a greater degree of similarity may exist between first cousins or grandparents and grandchildren than between more closely related parents and children or siblings, as illustrated in the well-known case of the physical resemblance of first cousins King George V, Tsar Nicholas II and Prince Henry of Prussia (Hall, 2018). Previously, based on a small number of studies and limited materials, it was suggested that this resemblance could be an accident or an error due to the limitations of statistical evaluation (Velemínský and Dobisíková, 2005; Cvrček *et al*., 2018). However, this phenomenon appears to be common among relatives, and the occurrence of such cases may be relatively constant.

Our results suggest that inbreeding can affect the degree of frontal sinus dimensional and morphological variability by reducing it. Although the differences are mostly not statistically significant, similar to the situation for non-metric traits (Cvrček *et al*., 2018), they can clearly be seen when presented graphically. Therefore, we consider it important to combine these approaches to identify such differences.

Focusing on single dimensional variables and morphological features showed that the height and morphology of the frontal sinuses correspond to biological family relationships. However, the introduction of this new approach for the identification of relatives would in practice require that a comparative sample of unrelated individuals from a similar population and period be analyzed as a next step, with a focus on comparing calculated similarity with a sample of biologically related individuals, including questioning how often two people might show a certain level of morphological similarity purely by chance.

5 | CONCLUSIONS

There were no significant sex differences between males and females within families based on frontal sinus dimensions. On the other hand, the relationship between the degree of biological relationship and the degree of similarity according to total height and morphology of the frontal sinus has been demonstrated. The greater the degree of biological relationship, the greater the degree of similarity for both variables. At the same time, however, there were cases of greater similarity between first cousins or grandparents and their grandchildren than among much closer relatives such as siblings or parents and their children. Use of total height and morphology for the detection of biological affinity between individuals is possible, but we cannot use frontal sinuses to identify the exact relationships of individuals, or their exact genealogical position in the family tree. Although the differences in the assessed variables of the frontal sinus between a family with a common degree of consanguinity and a family with very close consanguineous relationships are not always significant, the reduced variability where there is a greater degree of consanguinity is evident.

ACKNOWLEDGEMENTS

The authors declare no competing interests. The study was financially supported by the Charles University Grant Agency (grant numbers GAUK 1276217, 1590218) and the Ministry of Culture of the Czech Republic (DKRVO 2019-2023/7.I.b, 00023272). The authors are very grateful to M. Horák (Department of Radiology, Hospital Na Homolce, Prague) for creating the CT scans, J. Dupej (Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague; Department of Science and Computer Science Education, Faculty of Mathematics and Physics, Charles University, Prague) for his help with statistical analysis, and L. Vostrý (Czech University of Life Sciences Prague) for help with calculating the coefficients of relationships. We would also like to thank to P. Uhlík Spěváčková (Department of Anthropology, University of West Bohemia, Plzeň) for her help with the graphical processing of the results. Thanks to A. Millar for correcting the text. We would like to thank two reviewers for their useful comments, which have contributed to improving the text.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: CvrčekJ, Rmoutilová R, Čechová M, et al. Biological relationships and frontal sinus similarity in skeletal remains with known genealogical data. *J. Anat.* 2020;237:798–809. https://doi.org/10.1111/joa.13246

Received: 10 August 2020 | Revised: 26 October 2020 | Accepted: 27 October 2020

DOI: 10.1002/ar.24564

FULL LENGTH ARTICLE

The Anatomical Record | WILEY

Frontal sinus anatomy of the noble Swéerts-Sporck family and verification of their biological relationships using similarity analysis

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Funding information
Charles University Grant Agency, Grant/Award Number: GAUK 1276217; Ministry of Culture of the Czech Republic, Grant/Award Number: DKRVO 2019-2023/7.I.c, 00023272

Abstract

The evaluation of frontal sinus similarity is one way to detect biological relationships, especially in small groups, including families of historically known personalities. However, possibilities for studying this issue are currently limited. This contribution deals with the frontal sinuses of a rare osteological sample with known genealogical data, members of the noble Swéerts-Sporck family from the 17th to 20th centuries. The aim is to verify whether the frontal sinuses reflect documented family relationships. Basic dimensions of the frontal sinus such as total surface area and volume, and maximum height and width, and also morphology and anatomical features were evaluated using computed tomography scans. The portions of the frontal sinus above the "external supraorbital line" were analyzed. The degree of similarity between biologically related individuals was determined for each variable and compared with their known biological distance. The degree of similarity based on dimensions was evaluated using both the unadjusted measured data and standardized data adjusted to size. For the unadjusted dimensions, a positive correlation between morphological similarity and biological relatedness was apparent. On the other hand, no positive correlation was apparent for most of the standardized data. Only total volume showed a very weak indication of a positive trend in the standardized data, but this was weaker than in the original values. A positive quantifiable relationship between morphological patterns and biological distance is not clearly indicated. However, nonmetric features do support the documented relationships of the individuals.

KEYWORDS

aristocracy, CT examination, frontal sinus development, genealogically documented sample, inbreeding

1 | INTRODUCTION

In addition to studying osteological or dental anatomical variants, better known as nonmetric traits, the evaluation of the frontal sinus is another possible approach for detecting biologically related individuals (Stojanowski & Schillaci, 2006; Szilvássy, 1986) when DNA analysis cannot be used (e.g., Slavec, 2005). Several studies of living

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people have shown that monozygotic twins have a pronounced degree of frontal sinus similarity, greater than that of dizygotic twins (Leicher, 1928; Schwarz, 1928; Turpin, Tisserand, Bernyer, & Caspar-Fonmarty, 1942). On this basis, it is assumed that genetics is involved in frontal sinus anatomy, and can be used to detect biologically related individuals. This is particularly significant from the forensic perspective in the process of individual identification (Cameriere, Ferrante, Molleson, & Brown, 2008), or in bioarchaeology in small groups or in situations where the relationships are archaeologically indicated (e.g., Breitinger, 1980; Szilvássy, 1982), or as an aid to individual identification inside genealogically documented osteological samples, such as members of families of historically known personalities, mostly nobility (Slavec, 2004, 2005; Vlček, 1997). However, the inheritance of frontal sinus development and the possible influence of nonhereditary factors is still under discussion, not only in the mentioned areas, but also in terms of evolution and in clinical and anatomical studies (Ito, Kawamoto, Hamada, & Nishimura, 2015; Latiff, Das, & Othman, 2009; Márquez, Lawson, Mowbray, Delman, & Laitman, 2015). In previous studies, the expression of morphological similarity between related individuals has mostly been based on a simple visual description of overall similarity and the detection of individual anatomical features. This approach has been used mainly in small groups of individuals, or where archaeological context suggested biological kinship. It has brought promising results (Szilvássy, Kritscher, & Teschler-Nicola, 1987), but could be subjective; the term "similarity" in particular can be considered vague (Asherson, 1963).

To avoid subjectivity, frontal sinus dimensions have also been used. The combination of morphology and size has also been used to detect family relationships (Slavec, 2004, 2005), although there is no clear coincidence in sinus size, even in monozygotic twins (Kjær, Pallisgaard, & Brock-Jacobsen, 2012). On the other hand, with some exceptions, variations in sinus size were not significantly different within twin pairs. This may indicate the influence of both genetics and environmental exposure. Even where no statistical significance has been found in the intrapair similarity in any of the studied parameters of monozygotic and dizygotic twins, it has been assumed that the degree of paranasal sinus pneumatization may be influenced by genetic factors, while the development of anatomical variations of some other paranasal structures is affected more by environmental influences than genetic dispositions (Chaiyasate, Baron, & Clement, 2007).

At the same time, it turns out that the degree of similarity between biologically related individuals is not so strong that, for example, it would significantly adversely affect the process of individual identification using morphological patterns (Cameriere et al., 2008). The importance of the frontal sinus for individual identification thus remains valid, similar to a fingerprint (e.g., Christensen & Hatch, 2018; Nambiar, Naidu, & Subramaniam, 1999; Ubelaker, 1985), because frontal sinus patterns are so highly variable and each configuration is considered unique (Schaeffer, 1916). It is however possible to identify biological affinity between individuals, although frontal sinuses cannot be used to identify exact relationships (Cameriere et al., 2008). Furthermore, there may be cases of a high degree of coincidental similarity even between biologically related individuals, which must be taken into account (Cvrček et al., 2020).

Evaluating the degree of similarity between individuals can be complicated by sex differences. Significant differences in frontal sinus size have been found between males and females from different populations (Akhlaghi, Bakhtavar, Moarefdoost, Kamali, & Rafeifar, 2016; Čechová et al., 2019; Choi, Duailibi-Neto, Beaini, da Silva, & Chilvarquer, 2018). In a genealogically documented sample from the 19th to 20th centuries with a predominance of biologically related individuals, however, there were no such differences between adult males and females (Cvrček et al., 2020). Given this, a significant positive relationship was found between biological relatedness and the degree of similarity of individuals based on the frontal sinus total height and morphology, which matches the findings of previous studies (Kjær et al., 2012; Szilvássy et al., 1987).

Unfortunately, the number of studies dealing with this issue, remains small. This is mainly due to limited research opportunities, and a lack of material. There are also ethical restrictions on the research of living people (Kjær et al., 2012), mainly in terms of X-ray dosage without medical indication. In the case of osteologically documented material, there is only a small number of samples worldwide with preserved genealogical documentation (Carson, 2006; Gavrus-Ion et al., 2017; Ricaut et al., 2010).

The authors had the opportunity to study the rare osteological sample of biologically related members of the noble Swéerts-Sporck family. The goal of this study is to analyze frontal sinus similarity in the sample of Swéerts-Sporck family members with known genealogical data using computed tomography (CT) scans, and to compare our results with a previous study of their skeletal nonmetric traits. We address the following questions: Is there a positive relationship between frontal sinus dimensional and morphological similarity and the biological distance between individuals? Does the degree of morphological similarity of individuals reflect their documented relationships? Are there any anatomical features, which support documented relationships?

2 | MATERIALS AND METHODS

2.1 | Materials

Kuks hospital, with the 1707-17 Church of the Holy Trinity, in the Hradec Králové region of the Czech Republic, is an important Baroque monument associated from its foundation with the person of Francis Anthony, Count Sporck (1662–1738) and his descendants of the Swéerts-Sporck family (Hrdina & Kuchařová, 2017; Svatoš, 2011). Beneath the church lies the family crypt. In the early 1990s, it was necessary to repair the coffins of several family members; this provided an opportunity for their osteobiographical investigation (Dobisíková, Vyhnánek, Velemínský, Kuželka, & Čejková, 1999; Velemínský, Dobisíková, & Kuželka, 2000). For this study, the data from the skeletal remains of nine members of the Sporck and Swéerts-Sporck family from the 17th to 20th centuries were available:

1. General Johann Nepomuk, Count Sporck (Jan. 6th, 1600–Aug. 6th, 1679)
2. Eleonora Maria Catharine, Countess Sporck, née Fineck (Feb. 10th, 1639–Sept. 3rd, 1674)
3. Francisca Elizabeth Apollonia, Countess Sporck, née Baroness (i.e., Freifrau) from Reist and Swéerts (Jan. 11th, 1667–Apr. 22nd, 1726)
4. Cavalry Captain Joseph Philipp Friedrich Karl, Count Swéerts-Sporck, Baron (i.e., Freiherr) from Reist, Laaken, and Blauenthurn (Feb. 10th, 1809–Nov. 12th, 1848)
5. Barbara, Countness von Rothkirch and Panthen, née Countess Swéerts-Sporck, Baroness from Reist, Laaken, and Blauenthurn (Feb. 9th, 1810–Oct. 7th, 1873)
6. Joseph, Count Swéerts-Sporck, Baron from Reist, Laaken, and Blauenthurn (Jul. 7th, 1788–Feb. 11th, 1855)
7. Moritz Gustav, Count Swéerts-Sporck, Baron from Reist, Laaken, and Blauenthurn (May 12th, 1821–Nov. 21st, 1882)
8. Céline Pauline, Countess Swéerts-Sporck, Baroness from Reist, Laaken, and Blauenthurn, née de Noblée (Aug. 10th, 1832–Jul. 13th, 1914)
9. Philipp Benitus, Count Swéerts-Sporck, Baron from Reist, Laaken, and Blauenthurn (Feb. 9th, 1810–July 4th, 1817)

The family tree shows their biological relationships (Figure 1); we can see both close and distant kinship, and division into three groups is possible: (A) spouses nos. 1 and 2, and their daughter-in-law (generations nos. 1–2); (B) their descendants after five/four generations nos. 4, 5, 6, and 9 (siblings, including a pair of dizygotic twins, and first cousins, generation no. 7); (C) close family of individuals nos. 6, 7, and 8 (father, son, and his wife, generations nos. 7–8).

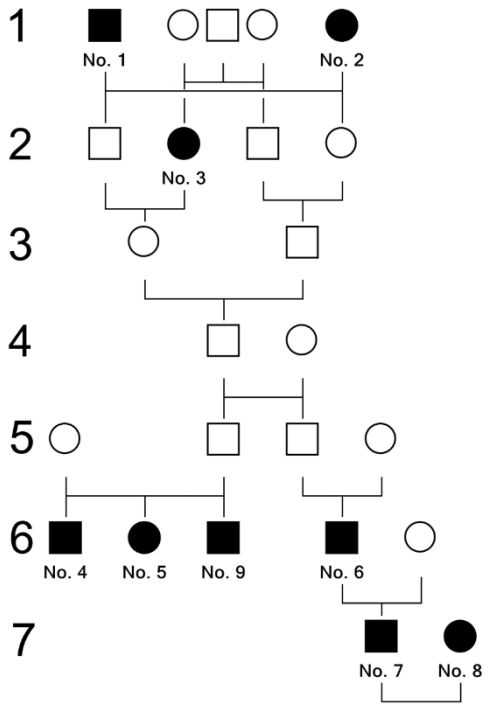


FIGURE 1 The family tree of the studied individuals according Wurzbach (1880) and Dobřenský z Dobřenic (1841–1919)

There is also a consanguineous marriage in generation No. 3: the mother of the groom and the father of the bride were siblings, and the father of the groom and the mother of the bride were half-siblings (Dobřenský z Dobřenic, 1841–1919; Wurzbach, 1880).

Genealogical data came from the State Regional Archives in Zámrsk, Litoměřice, Prague, and Plzeň, the Prague City Archive, the National Museum Archive in Prague, the parish registers of Vienna, and the Central State Historical Archives of Ukraine-City of Lviv. Identification of the individuals was based on inscriptional platelets found in the coffins. The identities of some individuals are also evidenced by the injuries found, which are known from written sources (Dobisíková et al., 1999; Otruba, 1898). Finally, DNA analysis and also analysis of blood groups supported documented relationships between individuals (Mazura, Dobisíková, & Beran, 1997). All data were stored in the archive of the Department of Anthropology of the National Museum in Prague.

2.2 | Methods

2.2.1 | CT scans processing

For individuals Nos. 1 and 2, only anteroposterior radiographs of the skull without scale were available. Therefore, their use is limited.

For the other individuals, Nos. 3–9, in addition to radiographs, CT images were also available. This allows for a more accurate assessment. Virtual surface models of their sinuses were segmented from the CT scans in Morphome3cs software (www.morphome3cs.com), (e.g., Musilová, Dupej, Velemínská, Chaumoitre, & Brůžek, 2016), and further processed in Meshlab (Visual Computing Lab, Italian National Research Council), Avizo 7.1 (Visualization Sciences Group, SAS) and Geomagic Studio 2012 (3D Systems, Inc.) in order to obtain water-tight virtual surface models of the frontal sinuses. The models were cut along a plane that passes through the upper external orbital margins and is parallel to the Frankfurt plane (Borovanský, 1936; Nussen, 2007), this limit being defined by the external supraorbital line ("ESOL"), (Cvrček et al., 2020). A portion superior to this plane was further examined (Figure 2). The mesh was cut along this plane in Avizo 7.1. This is a modification of the supraorbital line (SOL; e.g., Nikolova, Toneva, Georgiev, & Lazarov, 2018); we propose its use especially for historical osteological material where the orbital roof is poorly preserved (Cvrček et al., 2020).

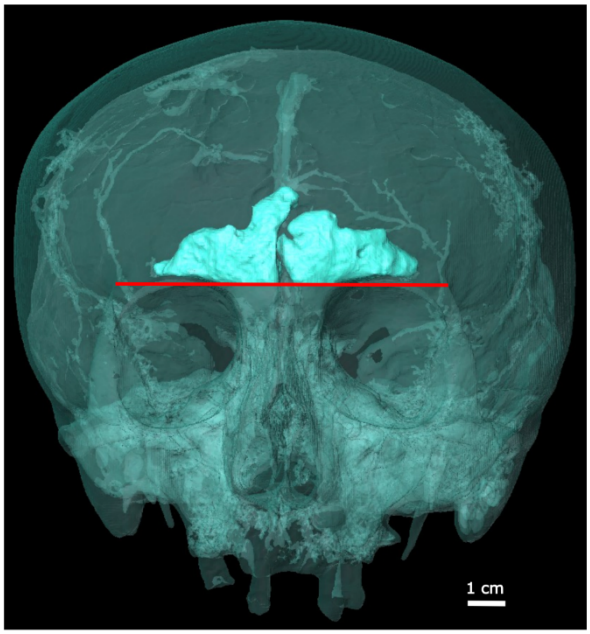


FIGURE 2 CT scan of a skull with a highlighted frontal sinus and "external supraorbital line" (ESOL, red line), individual No. 3: Francisca Elizabeth Apollonia, Countess Sporck, née Baroness from Reist and Swéerts (Jan. 11th, 1667—Apr. 22nd, 1726)

2.2.2 | Evaluation of dimensions

Four basic dimensions of the frontal sinuses were measured in individuals Nos. 3–8 based on their virtual surface models: total surface area (measured without the base created from the ESOL plane), total volume (the amount of space contained within the shape), and maximum height and width. The dimensions of individuals Nos. 1 and 2 could not be detected, as explained above. Similarly, the dimensions of individual No. 9 were not evaluated, as he is immature and the development of his sinuses is not complete (Cameriere et al., 2008; Moore & Ross, 2017).

Initially, whether there is a pattern in the metric dimensions related to the sex of the individuals was assessed (Cvrček et al., 2020). Sex classification was performed in Morphome3cs software using fitting support vector machines on the combinations of the total surface area and volume and the maximum height and width of the frontal sinuses. Equations created for the contemporary Czech population were used on this sample (Čechová et al., 2019) due to the temporal and geographical affinity of the samples.

Further, for each variable, the degree of dimensional similarity ("DS") between two individuals was calculated based on a formula originally designed by Szilvássy (1974) for the expression of frontal sinus side asymmetry:

$$DS = 100 \times \frac{A}{B},$$

where A is an individual with a smaller value, and B is an individual with a larger value of the given variable. The higher the DS value, the greater the degree of similarity. Finally, we used the resulting DS values of each assessed dimension, which represent the basic data for graphical representation of the degree of similarity of individuals.

The relationship between the degree of similarity and the biological distance of individuals was expressed using the coefficient of relationship (logarithm of biological distance) for each possible pair in the sample (15 combinations). The coefficient was estimated using a tabular method (Falconer & Mackay, 1996) based on the method of VanRaden (1992). Its logarithm eliminates entirely unrelated individuals (their coefficient of relationship is always zero, so the logarithm is not defined). Hereafter, the variable log-relatedness is used for the graphical expression of the relationship between the degree of similarity and biological distance for biologically related individuals (Cvrček et al., 2020; Cvrček, Velemínský, Dupej, Vostrý, & Brůžek, 2018).

In addition, variation in the similarity of the Swéerts-Sporck family members was compared to the variation of biologically unrelated pairs from a genealogically documented sample from the 19th to 20th centuries, which was evaluated similarly and comes from a very close

geographical region and time period. This sample includes 41 adult individuals (25 males, 16 females), with a predominance of members of one family over four generations. In addition, however, it also contains individuals which have become family members through marriage and allow the creation of 504 combinations of biologically unrelated pairs (for more information about this sample see Cvrček et al., 2020).

Subsequently, principal component analysis (PCA) was performed to analyze morphological similarity based on the whole set of four dimensions. For the total surface area and volume the second, and cube root of the measured values were used, respectively.

To analyze the degree of similarity between individuals without the possible effect of size sexual dimorphism, measurements were transformed by the geometric mean and logarithmization (Mosimann, 1970; Rmoutilová et al., 2018). In this case, too, the second, and cube root of the measured values were used for the total surface area and volume. The resulting values were evaluated in the same manner as the unadjusted ones, as described above: that is, via calculation of the degree of similarity with the graphical representation using the logarithm of the biological distance, and PCA.

2.2.3 | Evaluation of morphology

2D images (silhouettes) of the obtained sinuses of adult individuals were created from radiographs in Inkscape 0.91 (Nos. 1–2,), and from CT scans in Avizo 7.1 (Nos. 3–8). Using a simple visual evaluation (Szilvássy et al., 1987), morphologically identical or analogous structures were observed, and were graphically highlighted. The images of No. 9 were not used because of his low age (Cameriere et al., 2008) and the relationship of the sinuses to the ESOL being unfinished.

The frontal sinus morphological patterns of all the individuals were expressed using eight categories after Szilvássy (1986), Yoshino, Miyasaka, Sato, and Seta (1987), and Reichs (1993), (Table 1). Most have been shown to be independent (Cameriere et al., 2005), and the use of this set of features has previously proven to be useful for this purpose (Cvrček et al., 2020). We were able to evaluate all adult individuals, regardless of the imaging method used (x-ray, CT), although the first two categories are unrated in individuals Nos. 1 and 2, because their x-ray images do not have a scale. These categories can only be estimated and are shown in brackets. In this evaluation, unlike with the dimensions, we were also able to describe some basic features of individual No. 9, even if he is not an adult. The degree of the morphological similarity ("MS") of individuals based on class numbers of the categories above (patterns) was calculated using cosine similarity (Singhal, 2000). To graphically express the resulting similarity between individuals in relation to their biological distance, we used the same procedure as for the dimensions.

3 | RESULTS

3.1 | Dimensional similarity

In individuals Nos. 3–8, size differences between males and females were not clearly visible, although the sex classification was relatively successful. The combination of total surface area and volume yielded successful classification of all individuals, while the combination of maximum height and width yielded unsuccessful classification of male No. 4 (Figure 3).

Concerning individual variables, there is no total agreement between any two individuals in either volume or surface area (Table 2). There was a similarity in the total surface area and the volume of the right sinus between male No. 4 and his sister No. 5, and in the volume of the left sinus between male No. 4 and male No. 7, who is the son of his first cousin (male No. 6).

In the linear dimensions, there is greater total similarity than in the total surface area and the volume (Table 3). There is agreement in the height of the left sinus between father No. 6 and son No. 7, and in the width of the right sinus between male No. 4 and his sister No. 5. Furthermore, we can see the similarity in height of both sinuses of male No. 4 and the right sinus of his sister No. 5, and both sinuses of their first cousin male No. 6; there is a similarity in total height between these three individuals. A similarity in total width between father No. 6 and son No. 7 is also visible.

Among the calculated degrees of similarity between individuals, in terms of the total surface area of the frontal sinuses the greatest degree of similarity is between father No. 6 and son No. 7. In contrast, the similarity between siblings is not so obvious (Table 4: section A). In the graphical representation, there is a hint of a relationship between the degree of similarity and the distance of biologically related individuals Nos. 3–7 (Figure 4).

In terms of total volume, there is no clear agreement between closely related individuals (Nos. 4, 5, 6, and 7), as with the total surface area (Table 4: section B). This is also clearly visible on the graphical expression of the degree of similarity and biological distance (Figure 4).

Furthermore, for maximum height, the greatest degree of similarity is between siblings Nos. 4 and 5, and their first cousin No. 6 (Table 4: section C). The relationship between similarity and biological distance is also

TABLE 1 Visual features and their classifications for expression of frontal sinus morphology

Category	Classification	Class number
Total size in cm^2	Small (0–6)	1
	Middle (6–12)	2
	Large (12–18)	3
	Very large (>18)	4
Bilateral asymmetry	Symmetry (100–80)	1
	Slight asymmetry (80–60)	2
	Moderate asymmetry (60–40)	3
	Strong asymmetry (40–20)	4
	Extreme asymmetry (<20)	5
Superiority	Absent	0
	Left equal to right	1
	Left greater than right	2
	Right greater than left	3
Outline of upper border for left side and right side separately	Absent	0
	Smooth	1
	Scalloped with 2 arcades	2
	Scalloped with 3 arcades	3
	Scalloped with 4 arcades	4
	Scalloped with above 5 arcades	5
Partial septa	Absent	0
	Present in the left side	1
	Present in the right side	2
	Present in the both sides	3
Supraorbital cells	Absent	0
	Present in the left side	1
	Present in the right side	2
	Present in the both sides	3
Shape for left side and right side separately	Bean shape	1
	Leaf shape	2
	Fan shape	3
	Pyramid shape	4
Lateral range for left side and right side separately (own category)	Absent	0
	<1/3 border of orbit	1
	<2/3 border of orbit	2
	>2/3 border of orbit	3
	>lateral border of orbit	4

more pronounced, more so than for total surface area and volume (Figure 4).

In the case of maximum width, we again see the greatest degree of similarity between father No. 6 and son No. 7. Siblings Nos. 4 and 5 are in their relative proximity but the similarity between them is missing (Table 4: section D). The relationship between similarity and biological distance is visible again (Figure 4).

Figure 5 shows the degrees of similarity between biologically related members of the Swéerts-Sporck family and in a control sample of biologically unrelated individuals, for the whole set of four dimensions. In some cases,

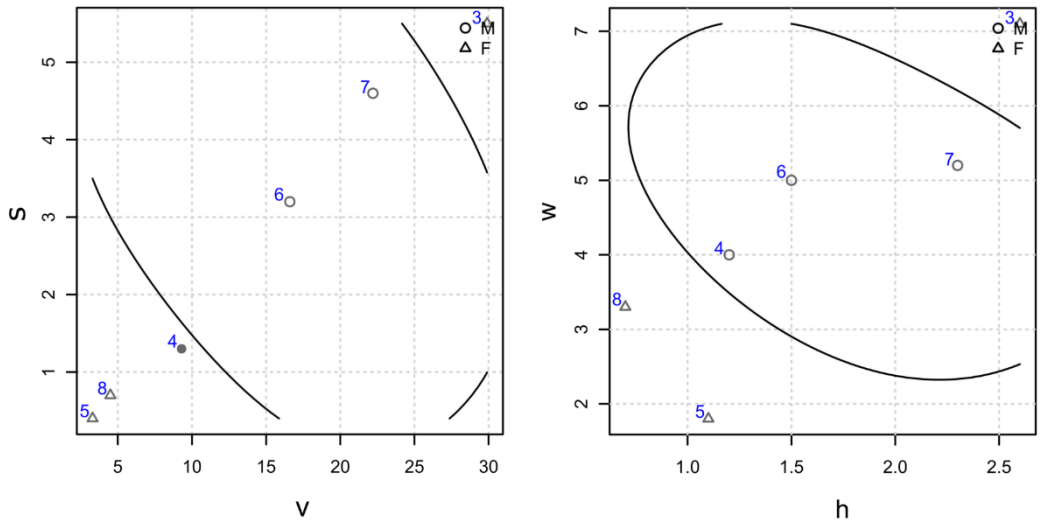


FIGURE 3 Plots of frontal sinus metric dimensions of individuals Nos. 3–8: total surface area (s) and volume (v), maximum height (h), and width (w). F, females, M, males. The ovals delimit correctly classified males. Misclassified individuals are highlighted in gray

TABLE 2 Frontal sinus total surface area (cm^2) and volume (cm^3) of individuals Nos. 3–8 based on virtual surface models from CT images

Individual no.	Right sinus surface area	Left sinus surface area	Total surface area	Right sinus volume	Left sinus volume	Total volume
3	14.8	15.1	29.9	2.6	2.9	5.5
4	3.6	5.7	9.3	0.5	0.8	1.3
5	3.3	0.0	3.3	0.4	0.0	0.4
6	8.2	8.4	16.6	1.5	1.7	3.2
7	17.6	4.6	22.2	3.7	0.9	4.6
8	1.7	2.8	4.5	0.3	0.4	0.7

TABLE 3 Frontal sinus linear dimensions (cm) of individuals Nos. 3–8 based on virtual surface models from CT images

Individual no.	Right sinus height	Left sinus height	Total height	Right sinus width	Left sinus width	Total width
3	2.6	2.0	2.6	3.9	3.7	7.1
4	1.0	1.2	1.2	1.8	2.2	4.0
5	1.1	–	1.1	1.8	–	1.8
6	1.5	1.3	1.5	2.5	2.5	5.0
7	2.3	1.3	2.3	3.8	1.6	5.2
8	0.5	0.7	0.7	1.2	1.6	3.3

reduced variability in biologically related individuals is indicated, most often at the maximum height.

PCA did not show a more pronounced clustering of biologically closely related individuals, except for the relative proximity of the first cousins males Nos. 4 and 6 in the first component, which explains most of the total variance (Figure 6).

After filtering out the overall size, the degree of similarity based on each of the metric dimensions shows a negative or not clearly positive trend with regard to

TABLE 4 The degree of frontal sinus similarity of individuals Nos. 3–8 by frontal sinus dimensions (Sections A–D)

Section A: Total surface area						
	3	4	5	6	7	8
3	–	31.1	11.0	55.5	74.2	20.3
4	31.1	–	35.5	56.0	41.9	48.4
5	11.0	35.5	–	19.9	14.9	73.3
6	55.5	56.0	19.9	–	74.8	27.1
7	74.2	41.9	14.9	74.8	–	20.3
8	20.3	48.4	73.3	27.1	20.3	–
Section B: Total volume						
	3	4	5	6	7	8
3	–	23.6	7.3	58.2	83.6	12.7
4	23.6	–	30.8	40.6	28.3	53.8
5	7.3	30.8	–	12.5	8.7	57.1
6	58.2	40.6	12.5	–	69.6	21.9
7	83.6	28.3	8.7	69.6	–	15.2
8	12.7	53.8	57.1	21.9	15.2	–
Section C: Maximum height						
	3	4	5	6	7	8
3	–	46.2	42.3	57.7	88.5	26.9
4	46.2	–	91.7	80.0	52.2	58.3
5	42.3	91.7	–	73.3	47.8	63.6
6	57.7	80.0	73.3	–	65.2	46.7
7	88.5	52.2	57.8	65.2	–	30.4
8	26.9	58.3	63.6	45.7	30.4	–
Section D: Maximum width						
	3	4	5	6	7	8
3	–	56.3	25.4	70.4	73.2	46.5
4	56.3	–	45.0	80.0	76.9	82.5
5	25.4	45.0	–	36.0	34.6	54.5
6	70.4	80.0	36.0	–	96.2	66.0
7	73.2	76.9	34.6	96.2	–	63.5
8	46.5	82.5	54.5	66.0	63.5	–

biological relatedness (Figure 7). Unlike the unadjusted maximum height, the size-adjusted height has the most negative trend. In terms of total volume, there is an indication of a positive trend, but it is very weak.

For comparison with previous results obtained on the basis of primarily measured dimensions of frontal sinuses, PCA based on standardized data adjusted to size did not show the clear clustering of any biologically closely related individuals in the first component (Figure 8).

3.2 | Morphological similarity

Silhouettes showing the individual frontal sinuses are provided in Figure 9. Using simple visual description of morphological features, the similarity of closely related individuals is apparent. For example, there is clear morphological similarity in the right sinus between first male cousins Nos. 4 and 6, or structural similarity between father No. 6 and son No. 7, or first cousins female No. 5 and male No. 6. There is also beveled left sinus and medial partial septum in the right sinus between female No. 3 and her great-great-grandson No. 4 (the septum is also shared with great-great-grandson No. 6).

Female No. 5 has a rudimentary left sinus, below the ESOL line (hypoplasia). There is a supraorbital cell at the left sinus site, which imitates the true sinus (see Figure 9). It faces the ventrocranial direction, and has the shape of a long, narrow rhomboid. We found the same variation in her brother No. 5 behind his right sinus. This supports their biological relationship.

The unrelated individual No. 8 also has a distinctly different morphology to most of the individuals, but a similarity to unrelated individual No. 1 is apparent.

Overall positive relationship between the degree of similarity and biological distance among biologically related individuals is not clearly visible when using a numerical expression of frontal sinus morphological patterns (Tables 5 and 6; Figure 10), despite the fact that there are similarities of closely related individuals in the evaluated categories, as in the overall visual assessment mentioned above: between siblings Nos. 4 and 5 (size, partly the presence of supraorbital cells, and also shape and lateral range), father No. 6 and son no. 7 (superiority, absence of supraorbital cells, presence of partial septa, partly also shape, and lateral range), first cousins Nos. 4 (5 respectively) and 6 (size, superiority, partly the number of arcades, or partly also shape), and finally between female No. 5 and male No. 7 (a great degree of asymmetry with a predominance of the right sinus).

The frontal sinuses of individual No. 9, dizygotic twin of No. 5, were not analyzed using CT images because he was under 15 years old and the sinuses were not yet complete (Cameriere et al., 2008). Based on X-ray images, however, the right sinus is much bigger than the left, there is also right superiority, and the right sinus indicates four arcades, and the left three. Given his low age we leave the other parameters without evaluation (Table 5); their overall morphological character, however, corresponds to that of other close individuals Nos. 4, 5, 6, and 7.

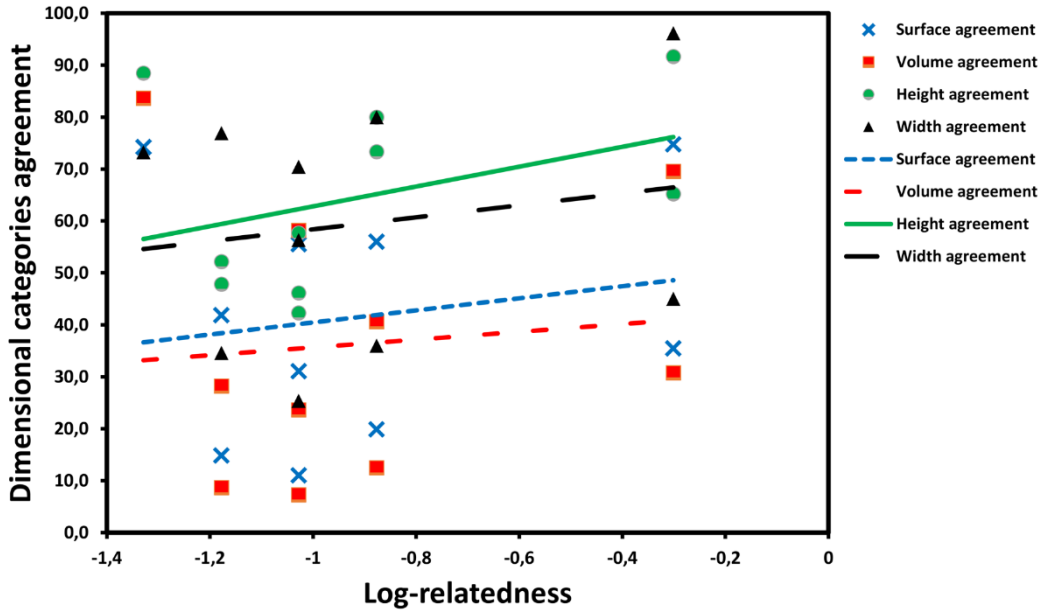


FIGURE 4 Relationship of the similarity (agreement) between biologically related individuals Nos. 3–7 based on dimensions of the frontal sinuses (*y*-axis) by log-relatedness of individuals (*x*-axis). The lines represent regression lines.*Note*: log-relatedness disregards unrelated individual No. 8

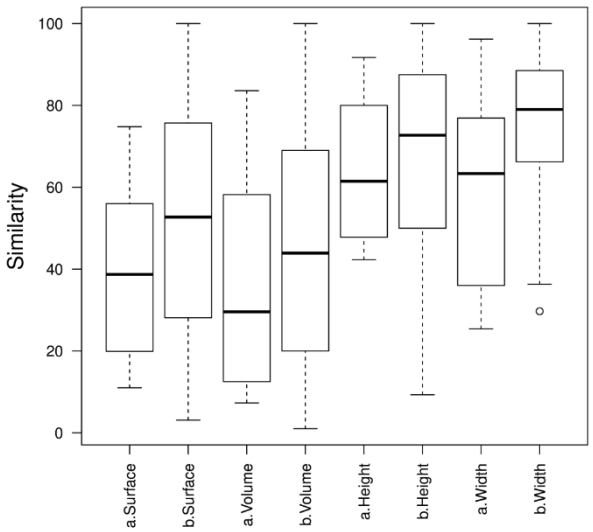


FIGURE 5 Variability in the degree of similarity between biologically related members of the Swéerts-Sporck family (a,$N = 10$ pairs) and biologically unrelated individuals (b,$N = 504$ pairs) based on data from the study by Cvrček et al. (2020) for frontal sinus total surface area, volume, and maximum height and width

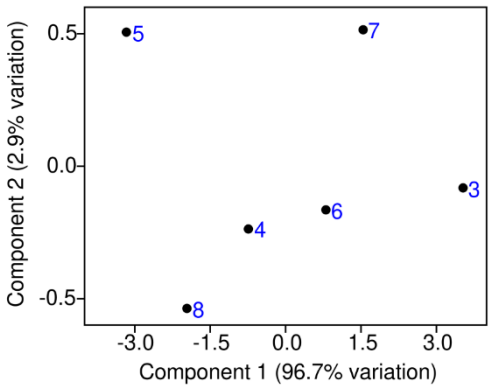


FIGURE 6 Principal component analysis performed on unadjusted metric dimensions of evaluated individuals Nos. 3–8

4 | DISCUSSION

The influence of biological relatedness on the frontal sinuses can be analyzed using two approaches: (1) an assessment of dimensions (e.g., Kjær et al., 2012) and (2) a morphological evaluation using their anatomical configuration (e.g., Szilvássy et al., 1987) or morphological patterns using scoring systems (Cameriere et al., 2008). As many studies of different populations have shown, dimensions could be adversely affected by sexual dimorphism (e.g., Akhlaghi et al., 2016; Čechová et al., 2019; Choi et al., 2018), which can skew the interpretation of the degree of similarity between evaluated individuals. However, it has already been shown that

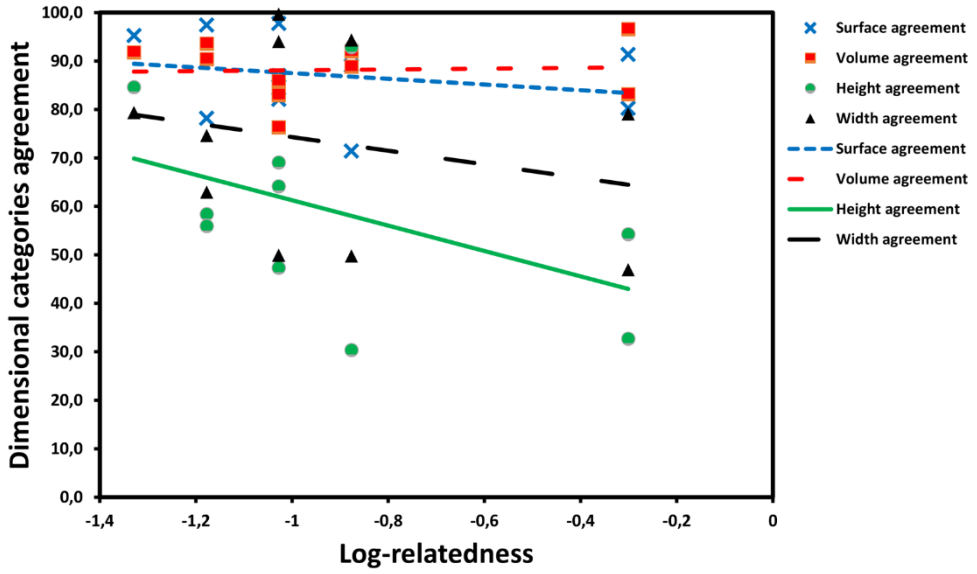


FIGURE 7 Relationship of the similarity (agreement) between biologically related individuals Nos. 3–7 based on dimensions of the frontal sinuses (*y*-axis) by log-relatedness of individuals (*x*-axis). The lines represent regression lines.*Note*: log-relatedness disregards unrelated individual No. 8

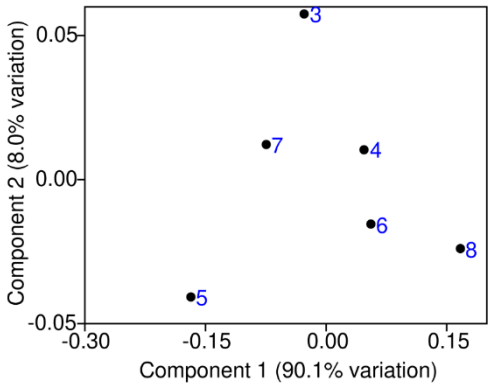


FIGURE 8 Principal component analysis performed on size-adjusted metric dimensions of evaluated individuals Nos. 3–8

there was no pattern and no significant differences existed between adult males and females from the 19th to 20th centuries in a genealogically documented sample in any of the dimensions that we have evaluated in the Swéerts-Sporck family. The Wilcoxon signed-rank test (Siegel, 1956) was used to compare these differences, and the resulting *p*-values were adjusted by Holm-Bonferroni correction (Bonferroni, 1936; Holm, 1979). The *p*-values are .263 for total surface area, .215 for total volume, .101 for maximum height, and .285 for maximum width (calculations based on data taken from Cvrček et al., 2020). The explanation is proposed that while the aforementioned studies, and many others describing sex differences, evaluated randomly selected, that is, biologically unrelated, individuals from a population, differences between biologically related individuals may be erased by genetic influence (Cvrček et al., 2020). Due to the small number of Swéerts-Sporck family members evaluated, however, an adequate statistical evaluation of sex differences was not possible, and so a graphical representation of individuals divided by sex based on combinations of measured dimensions was employed. Even in this case, no clear pattern was found between males and females.

Despite the absence of size differences related to sex, the metric dimensions were evaluated in both forms: unadjusted and adjusted to size (Mosimann, 1970). PCA based on standardized data without size effect did not show an obviously different, or rather greater, degree of similarity between individuals than the PCA based on unadjusted dimensions, where there is a more obvious similarity between the first cousins.

More interesting results are obtained by analyzing individual variables. When using unadjusted dimensions, all showed a positive relationship between the degree of similarity and the degree of biological distance between individuals. This means that more closely related individuals showed a greater degree of similarity. For the most part, the greatest degree of similarity was found between father and son, first cousins, and siblings. This was also observed by Velemínský and Dobisíková (2005) using skeletal nonmetric traits for the same sample; this study

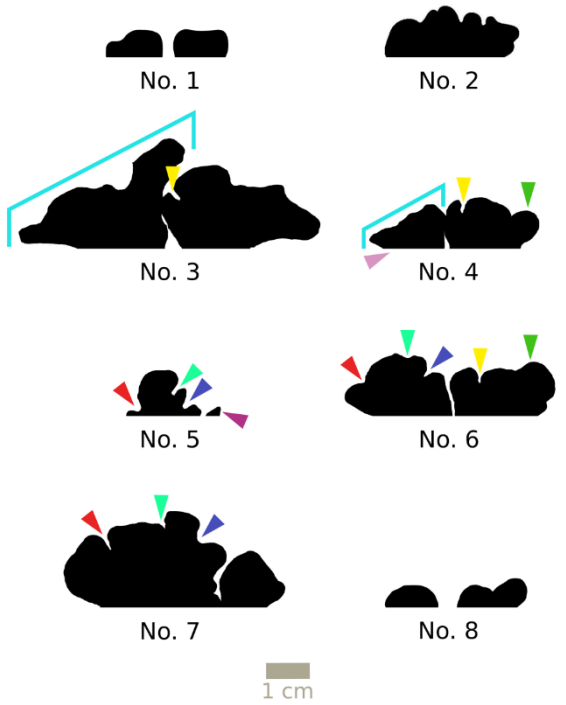


FIGURE 9 Anteroposterior views of the frontal sinus morphology of all of the studied adult individuals based on X-rays (Nos. 1–2) and CT images (Nos. 3–8) above their ESOL line. Various shared morphological similarities (arrows, clamps) are marked with the same colors. The image of No. 9 was not used because his low age means that the relationship of his sinuses to the ESOL line is not stable

TABLE 5 Frontal sinus morphological patterns of all the studied individuals (Nos. 1–9)

Individual no.	Frontal sinus pattern
1	- - 111012211
2	- - 225003312
3	21334204323
4	13233133311
5	15340013111
6	11344303322
7	24352203222
8	12212002211
9	- - 34320- - - -

Note: Given the low chronological age of individual No. 9 some parameters are left without evaluation.

found the greatest morphological similarity between father and son, while there was also a higher degree of similarity among first cousins. Similarity among siblings, however, was not so obvious, and as the authors also stated, the

TABLE 6 The degree of similarity of individuals Nos. 3–8 by frontal sinus morphology

	3	4	5	6	7	8
3	–	0.85	0.65	0.97	0.87	0.91
4	0.85	–	0.81	0.85	0.86	0.88
5	0.65	0.81	–	0.67	0.91	0.79
6	0.97	0.85	0.67	–	0.89	0.86
7	0.87	0.86	0.91	0.89	–	0.87
8	0.91	0.88	0.79	0.86	0.87	–

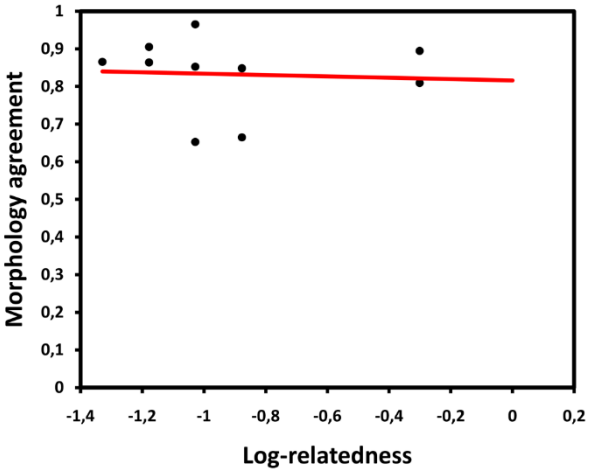


FIGURE 10 Relationship of the similarity (agreement) between biologically related individuals Nos. 3–7 based on frontal sinus morphology (*y*-axis) by log-relatedness of individuals (*x*-axis). The red line represents the regression line. Note: log-relatedness disregards unrelated individual No. 8

greatest difference was observed among biologically unrelated family members, that is, females who married into the family (Velemínský & Dobisíková, 2005).

On the other hand, when standardized data adjusted to size were used, such a clearly positive trend was not visible for any dimension. The most negative trend can be observed at total height, but this did not visibly outweigh the positive trend of unadjusted data. The situation was similar for the maximum width, but for the total surface area, the positive trend for unadjusted dimensions was stronger than the negative trend for standardized dimensions without size. In terms of total volume, there was a very weak indication of a positive trend in the standardized data, but this was weaker than in the original values. This would suggest that genetics does affect the size of the frontal sinuses. At the same time, in comparison with biologically unrelated individuals, the variability

of similarity in biologically related members of the Swéerts-Sporck family based on the evaluated dimensions in their original form was shown to be lowest at the maximum height. Some past studies have also shown the significant effect of genetics on the maximum height of frontal sinuses (Cvrček et al., 2020; Kjær et al., 2012).

In the case of this family, the degree of similarity between individuals based on dimensions reflects better documented relationships than the results based on the assessment of morphological similarity. Nevertheless, similarity in individual morphological features was found among close relatives, which supported the documented biological relationships of individuals. In a previous study using a larger sample, and using the same evaluated variables as in the Swéerts-Sporck family, the relationship between the degree of morphological similarity and the degree of biological distance of individuals proved to be significant (Cvrček et al., 2020), while individual traits supporting their biological affinity were also found (Szilvássy et al., 1987). It can therefore be assumed that the slightly negative trend found in the Swéerts-Sporck family is influenced by the small sample size.

The evaluation approaches described have so far mostly been used separately. Nevertheless, each has its advantages and limits. The basic dimensions do not allow capturing of the shape, and vice versa. Metric evaluation, however, can be considered more objective than morphological evaluation, which is generally considered subjective (Asherson, 1963). It later became apparent, however, that simple "visual" morphological analysis may not be as subjective or unreliable as often argued, compared to more "objective" metric methods. Even a slight change in the frontal sinus appearance can negatively affect the measurement result, as has been demonstrated in the case of individual identification (e.g., Besana & Rogers, 2010; Nikolova et al., 2018), but this fact should be kept in mind when comparing the degree of similarity of individuals in terms of possible kinship relationships. This is especially true for radiographs. Recently, there have also been attempts to make the simple visual morphological evaluation of frontal sinuses more objective using a scoring system (Besana & Rogers, 2010; Cameriere et al., 2005; Cameriere et al., 2008), but this approach too has its drawbacks. Even if we use a scoring system that could be considered objective and can be used regardless of the imaging method (X-ray, CT), and does not require complete preservation of the skeletal remains, the overall morphology of the sinuses can vary significantly, even if they have the same pattern (Cvrček et al., 2020). In our opinion, therefore, the visual assessment of overall similarity cannot be completely ignored, even though it may be subjective. If we also draw attention to the shared occurrence of some deviations and traits, such as those used in the process of individual identification (e.g., Christensen & Hatch, 2018; Kullman, Eklund, & Groudin, 1990), we can reduce the degree of subjectivity. It follows that these approaches should be combined (e.g., Slavec, 2004, 2005), and this is confirmed by our results. The similarities between biologically related individuals are found in both dimensions and morphology. At the same time, we agree with Cameriere et al. (2008) that we cannot use the frontal sinuses to identify the kinship of an individual, even if we use a scoring system. The same finding can also be found in skeletal nonmetric traits (e.g., Ricaut et al., 2010). Morphological methods do not allow the absolute reconstruction of a family tree. At the same time, the possibility of accidental similarity between biologically unrelated individuals must be considered (Cvrček et al., 2020). Despite this, taking into account the previous results of the analysis of skeletal nonmetric traits (Velemínský & Dobisíková, 2005), we can confirm that the analysis of frontal sinuses can be seen as a useful supplement to the analysis of nonmetric traits (Slavec, 2005; Szilvássy et al., 1987). This approach has also been applied, for example, in an effort to reveal kinship relationships in several generations of princes and later kings of the Přemyslid dynasty (Vlček, 1997).

There is also one consanguineous marriage in the Swéerts-Sporck family (generation No. 3, see Figure 1). Our genealogical research has shown that the mother of the groom and the father of the bride were siblings, and the father of the groom and the mother of the bride were half-siblings (e.g., Wurzbach, 1880). In contrast, most of the available sources have neglected this, and the fathers of the groom and the bride were taken as full siblings (e.g., Hrdina & Kuchařová, 2017). According to calculations of the coefficients of relationships (r), the influence of this consanguineous marriage is also evident in the generations of Nos. 6 and 7: for example, first cousins Nos. 4/5 and 6: $r = .131$ (standardly $r = .125$), or individuals Nos. 4/5 and 7: $r = .0654$ (standardly .0625). Due to the nature of the sample, however, it is not possible to comment on the influence (negative or positive) of inbreeding on the dimensions and morphology of the frontal sinuses. The number of individuals is small, and they are also separated by several generations. Unfortunately, it was not possible to examine more members of the family. A comparison of the degree of similarity of another family with an increased degree of consanguinity and a parallel family with normal relationships based on the same evaluated dimensions and morphological categories, however, showed a higher degree of similarity and at the same time a lower degree of variability of individuals from the family with an increased degree of consanguinity, but a significant difference was found only in the total surface area and volume (Cvrček et al., 2020).

The low number of individuals and the considerable generational distance of some of them also explain why statistical processing was not performed in this study. The power of statistical significance tests, in particular, would be low (Ellis, 2010); furthermore, they should be considered only as indicative (e.g., Spence, 1996; Velemínský & Dobisíková, 2005). Nevertheless we have arrived at similar conclusions to some prior studies (e.g., Kjær et al., 2012; Slavec, 2005). Although we fully recognize that the potential for the diverse growth patterns of the frontal sinus is so highly variable that each configuration is considered unique (Schaeffer, 1916), like fingerprints (e.g., Christensen & Hatch, 2018), and a number of nonhereditary factors such as for example, craniofacial configuration, hormonal growth factors, different diseases or age-related changes may also be involved in their forming (Nambiar et al., 1999; Shapiro & Schorr, 1980), genetic factors are one of the non-negligible factors involved in their development (Chaiyasate et al., 2007; Cvrček et al., 2020; Kjær et al., 2012; Leicher, 1928; Schwarz, 1928; Szilvássy et al., 1987; Turpin et al., 1942).

5 | CONCLUSION

This contribution provides a complex similarity analysis of frontal sinus dimensions and morphological properties in relation to the biological family relationships of individuals with known genealogical data. The degree of similarity based on frontal sinuses corresponds to the degree of their similarity based on nonmetric traits. Although the possibilities for interpretation could be limited by the small number of studied individuals, the results confirm that the analysis of frontal sinus similarity is a useful approach to estimate family relationships, particularly when DNA analysis is not feasible. Also combining with other osteological features may be recommended. Currently, small osteological samples with documented relationships provide a very rare opportunity to extend our knowledge of the effect of biological kinship on osteological features, and enhance possibilities for the study of kinship in the area of bioarchaeology and forensic anthropology.

ACKNOWLEDGMENTS

The authors sincerely thank M. Horák (Department of Radiology, Hospital Na Homolce, Prague) for creating the CT scans, O. Naňka (Institute of Anatomy, First Faculty of Medicine, Charles University, Prague) for help with assessment of the anatomical structures of the sinuses, F. Fortin (the Czech Genealogy and Heraldry Society in Prague), M. Běličová (National Museum Archive, Prague), and P. Mareš (National Archives, Prague, Czech Republic) for their help with the genealogical research, M. Čechová (Department of Anthropology and Human Genetics, Přírodovědecká Fakulta, Univerzita Karlova, Prague, Czech Republic) for help with a graphical representation of the sexual dimorphism of the sinuses, and L. Vostrý (Czech University of Life Sciences Prague) for help with calculating the coefficients of relationships. They would also like to thank K. Cvrček for his help with the graphical processing of the results, and A. Millar for revising the text. They would also like to thank associate editor T. D. Smith and both reviewers for the comments that led to the improvement of this contribution.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Jan Cvrček: Conceptualization; data curation; methodology; data analysis; author of the text. **Petr Velemínský**: Supervisor; material and data collection; co-author of the text. **Tomáš Jor**: Conceptualization; methodology; data analysis; graphic processing. **Rebeka Rmoutilová**: Conceptualization; methodology; data analysis; co-author of the text. **Jaroslav Brůžek**: Supervisor, conceptualization; co-author of the text.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: Cvrček J, Velemínský P, Jor T, Rmoutilová R, Brůžek J. Frontal sinus anatomy of the noble Swéerts-Sporck family and verification of their biological relationships using similarity analysis. *Anat Rec*. 2021;304:1717–1731. https://doi.org/10.1002/ar.24564

NÁRODNÍ MUZEUM

Journal of the National Museum (Prague), Natural History Series
Vol. 189 (2020), ISSN 1802-6842 (print), 1802-6850 (electronic)

DOI: 10.37520/jnmpnhs.2020.004

Původní práce / Original paper

Contribution to the study of frontal sinus familial similarity based on genealogically documented individuals (Bohemia, 19th to 20th centuries)

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Cvrček J. & Velemínský P., 2020: Contribution to the study of frontal sinus familial similarity based on genealogically documented individuals (Bohemia, 19th to 20th centuries). – Journal of the National Museum (Prague), Natural History Series 189: 21–30.

Abstract: The issue of the influence of biological relatedness on frontal sinus patterns is still the subject of discussion. Research possibilities worldwide are however limited by the lack of genealogically documented skeletal remains. This study presents the results of analyses of seven identified 19th-20th century skulls from two families, discovered in the collections of the Hrdlička Museum of Man. Anteroposterior X-rays were created and the morphology of the frontal sinuses was evaluated using two approaches: A) a simple visual assessment indicating shared or similar features; and B) calculation of the degree of similarity based on a scoring system. With both approaches, the results of family relationship assessments were best reflected in the first family, between individuals with the smallest degree of biological distance. In the second family, which had more distant relationships compared to the first, the positive relationship between biological distance and degree of similarity was not clearly apparent. Although this is a small sample with a small number of generations, our findings support the conclusions of previous studies, i.e. that the degree of similarity between individuals decreases with their increasing biological distance.

Keywords: frontal sinus, morphology, X-ray, inbreeding, 19th–20th century

Received: September 8, 2020 | Accepted: October 1, 2020 | Published on-line: October 15, 2020

Introduction

Osteological samples with known family relationships, which allow study of the possible influence of genetic factors on morphological and metric features of the human skeleton, are very rare worldwide (Gavrus-Ion et al. 2017). They are, however, of irreplaceable significance, because the conclusions of studies based on such material can be applied to anonymous, (pre)historic burial grounds with individuals whose relationships are unknown or are indicated by archaeological findings (Adachi et al. 2006, Meyer et al. 2012, Brown 2015, Velemínský et Cvrček 2020). One of the features that helps reveal possible biological rela-

tionships between individuals is the frontal sinus, its morphology and dimensions being displayed using both radiographs and CT scans (e.g. Szilvássy 1986, Cvrček et al. 2020).

Morphological similarities of the frontal sinus between closely related individuals were observed in the first half of the 20th century (Leicher 1928, Schwarz 1928, Turpin et al. 1942). Although the formation of the frontal sinus is unique in each individual, like fingerprints, and there is no absolute agreement even between monozygotic twins (Asherson 1963, Christensen et Hatch 2018), it is possible to identify the biological affinity of individuals; at the same time, however, frontal sinuses cannot be used to identify their exact relationship (Cameriere et al. 2008). The evaluation of morphological features can, though, be considered subjective (Asherson 1963). Therefore, the degree of similarity of individuals is also determined from the dimensions of the frontal sinuses (e.g. Slavec 2005), and in particular a significant relationship between their maximum height and the biological distance of individuals has been demonstrated (Kjaer et al. 2012, Cvrček et al. 2020). Sex differences in frontal sinus size between biologically related individuals have not been found (Cvrček et al. 2020).

Nevertheless, it has been shown that the evaluation of dimensions can be negatively affected by even a slight change in the setting of the skull during the taking of an X-ray image (Nikolova et al. 2018), and that therefore a morphological evaluation might ultimately be "more objective", especially if a scoring system is used (Besana et Rogers 2010). This approach has already proven useful for this purpose (Cameriere et al. 2008), a significant relationship between the degree of morphological similarity and the degree of biological distance between individuals having been found (Cvrček et al. 2020), i.e. the smaller the biological distance, the greater the degree of similarity. It turned out, however, that a simple visual assessment of the morphology of the frontal sinus (Szilvássy 1986) was irreplaceable, because statistical methods do not allow the capture of specific shared features, especially if the osteological material is incompletely preserved, or the number of individuals is very small or even just a pair, where a comparison of the values of the degree of morphological similarity cannot be used (Cvrček et al. 2020).

The authors were given the opportunity to examine two small samples of genealogically documented skulls from the collections of the Hrdlička Museum of Man in the Faculty of Sciences of Charles University in Prague. The aim of this contribution is 1) to answer the question as to what extent the morphological similarity of the frontal sinuses corresponds to documented family relationships; and 2) assess whether there are shared individual features between individuals that support these relationships.

Material and methods

Material

The material consists of seven skulls from two generations of biologically unrelated families (families A and B) dating to the 19th and 20th centuries (Fig. 1); all the familial relationships and other biographical data were known. For ethical reasons, only their initials and years of birth and death are given here:

Family A

No. 1: Male *J. R.* (1835–1892), Prague goldsmith. Died of exhaustion.

No. 2: Female *E. R.* (1831–1887), biologically unrelated wife of J. R. Died of kidney inflammation.

No. 3: Male *E. R.* (1867–1889), son of J. R. and E. R. Died of pulmonary tuberculosis and spewing blood.

Family B

No. 1: Male *J. R.* (1816–1894), one of most important businessmen in the Austro-Hungarian Empire. He died of marasmus senilis.

No. 2: Female *M. A. R.* (1819–1855), the first cousin and also first wife of J. R. (their fathers being brothers). She died of paralysis of the lungs due to typhus.

No. 3: Female *J. C. R.* (1836–1920), the second and biologically unrelated wife of J. R. She died of pneumonia.

No. 4: Female *C. R.* (1857–1930), wife of one of the inbred sons of first cousins J. R. and M. A. R., and at the same his first cousin again (her mother was both sister of his mother and first cousin of his father). She had three sons and one daughter with her husband, but according to available archive documents all remained childless, and this branch of the family became extinct. She died of tuberculosis and atherosclerosis.

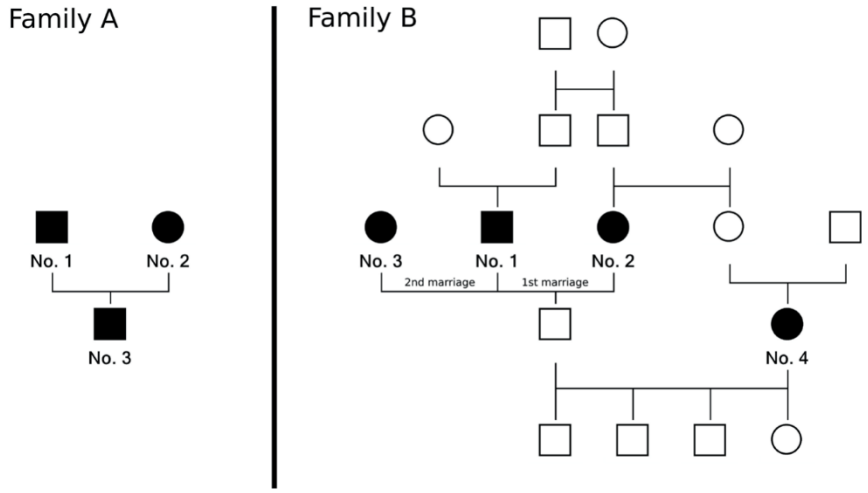


Fig. 1. Graphical expression of family relationships of evaluated individuals from families A and B.

Biographical data were verified by genealogical research in the Prague City Archives, and the State District Archives in Most (a branch of the State Regional Archives in Litoměřice, Czech Republic). The skulls are part of the collections of the Hrdlicka Museum of Man. The tombs of both families were looted in the second half of the 20th century, and the material was probably obtained for the museum by its director, the anthropologist prof. Vojtěch Fetter (1905–1971). Although the skulls were saved and their identification preserved, archival sources indicate that the postcranial parts of the associated skeletons, and even the whole bodies of other family members, were cremated or lost, e.g. the remains of the sons of individuals Nos. 1 and 2 from family B. Only the mummified forearm and hand of female No. 4 from family B were preserved. Incidentally, the skulls of all the members of family B were also more or less covered with remnants of mummified soft tissues and hair, so analysis of cranial non-metric traits (e.g. Cvrček et al. 2018) could not be included in this study, while analysis of dental non-metric traits was made impossible by the intra-vital loss of most teeth in all individuals from both families.

Methods

An anteroposterior radiograph of the skull oriented in the Frankfurt Plain was taken for each individual. Radiographs were performed using computed radiography (CR) at the Department of Anthropology, National Museum, Prague. A Bucky grid was used, the standard distance between the focal point and the CR cassette was 1.2 m, with tube voltages ranging between 80–85 kw and beam intensity between 3.5–5 mAs. The skulls were stabilized with

Category	Classification	Class number
Total size	Small (0–6) Middle (6–12) Large (12–18) Very large (> 18)	1 2 3 4
Bilateral asymmetry	Symmetry (100–80) Slight asymmetry (80–60) Moderate asymmetry (60–40) Strong asymmetry (40–20) Extreme asymmetry (< 20)	1 2 3 4 5
Superiority	Absent Left equal to right Left greater than right Right greater than left	0 1 2 3
Outline of upper border for left side and right side separately	Absent Smooth Scalloped with 2 arcades Scalloped with 3 arcades Scalloped with 4 arcades Scalloped with above 5 arcades	0 1 2 3 4 5
Partial septa	Absent Present in the left side Present in the right side Present in the both sides	0 1 2 3
Supraorbital cells	Absent Present in the left side Present in the right side Present in the both sides	0 1 2 3
Shape for left side and right side separately	Bean shape Leaf shape Fan shape Pyramid shape	1 2 3 4
Lateral range for left side and right side separately (own category)	Absent < 1/3 border of orbit < 1/2 border of orbit > 1/2 border of orbit > lateral border of orbit	0 1 2 3 4

Tab. 1. Visual features and their classifications for expression of frontal sinus morphology by Szilvássy (1986), Yoshino et al. (1987), and Reichs (1993).

a standard pad; the distance from the face of the skull to the detector surface was about 3 cm, so partially geometric magnification was taken into account by the enclosed scale.

The skulls were completely preserved, without autopsy cuts or significant post-mortem destruction, so all of the displayed frontal sinuses could be cut along a plane that passes through the upper orbital margins and is parallel to the Frankfurt plane. This limit defined the supraorbital line (SOL; Nikolova et al. 2018). A portion superior to this plane was further examined (Fig. 2). 2D images (silhouettes) of the obtained sinuses of individuals were created in Inkscape 0.91.

Using a simple visual assessment (Szilvássy et al. 1987), morphologically identical or analogous structures were observed, which were then graphically highlighted. The frontal sinus morphological patterns of all the individuals were expressed using eight categories

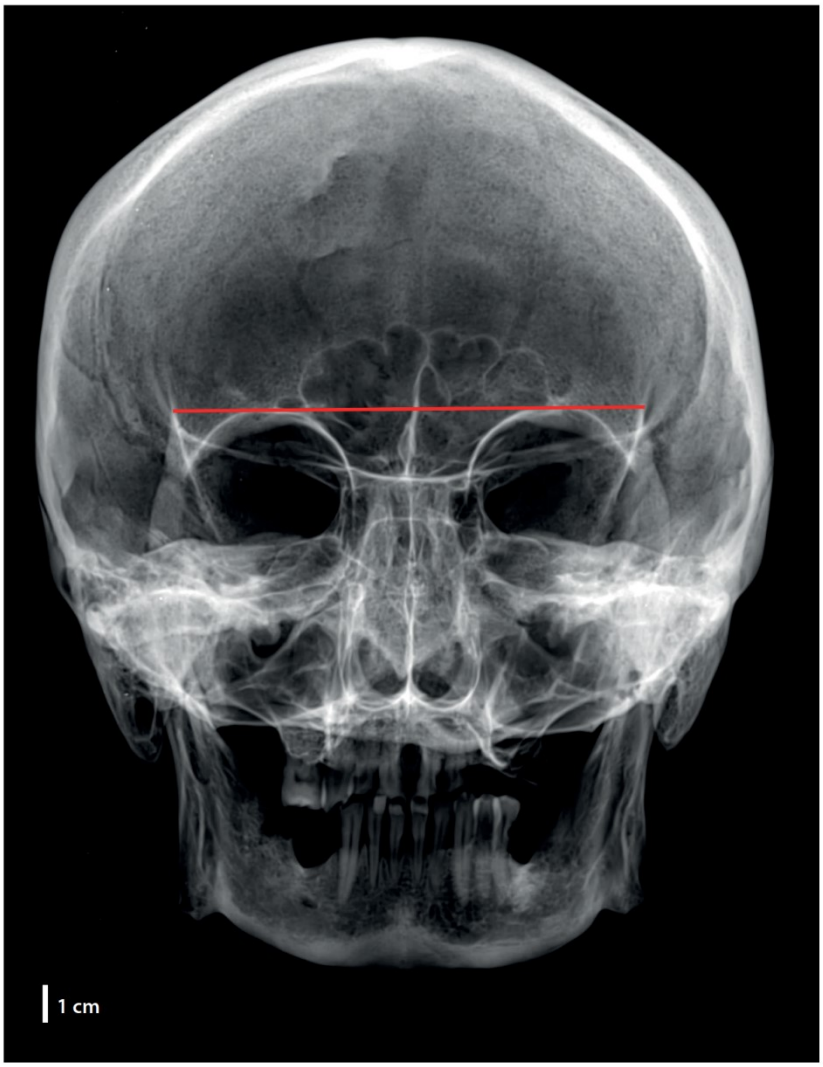


Fig. 2. Anteroposterior radiograph of the skull with "supraorbital line" (SOL, red line), family A, individual No. 1.

after Szilvássy (1986), Yoshino et al. (1987), and Reichs (1993) (Tab. 1). Most of these have been shown to be independent (Cameriere et al. 2005), and the previous use of this set of variables (Cvrček et al. 2020) demonstrated their applicability for this purpose. The degree of the morphological similarity ("MS") of individuals based on class numbers of the categories above (patterns) was calculated using cosine similarity (Singhal 2000, Cvrček et al. 2020). This method measures the cosine of the angle between two *n*-dimensional vectors of an inner product space. The vectors are individuals within a family. In space, vectors are determined using the values of individual morphological features (Tab. 1). The similarity between individuals is calculated using cosine and can be converted to a distance.

Results

Family A

Based on simple visual assessment, a greater similarity is visible between father No. 1 and son No.3, with a greater number of shared features between them than between mother No. 2 and son No. 3 (Fig. 3). The most characteristic feature that is shared by father and son, and which clearly supports their documented relationship, is a small separated lobe above the right orbit (Fig. 3, blue arrow).

The degree of the morphological similarity calculated using cosine similarity confirmed the finding based on the simple visual assessment. The greatest value is between father No. 1 and son No. 3, similarity is also apparent between mother No. 2 and son No. 3, and the least similarity is between unrelated spouses Nos. 1 and 2 (Tab. 2).

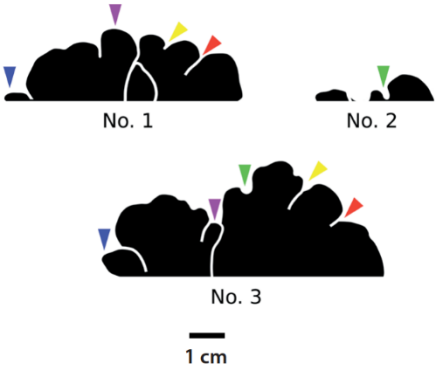


Fig. 3. Anteroposterior views of frontal sinuses above their SOL, from X-rays of closely related individuals Nos. 1–3 from family A. Examples of various shared morphological similarities (arrows) are marked with the same colors.

	1	2	3
1	–	0.82	0.84
2		–	0.97
3			–

Tab. 2. The degree of similarity of individuals Nos. 1–3 from family A by frontal sinus morphology, calculated based on cosine similarity. The higher value, the darker color.

Family B

A simple visual assessment shows no visible similarity between biologically related individuals in this family, such as first cousins and spouses Nos. 1 and 2, or these individuals and their niece/daughter-in-law No. 4 (Fig. 4). Female No. 4, unlike the other individuals, shows hypoplasia of the right frontal sinus, and her left sinus is greatly reduced.

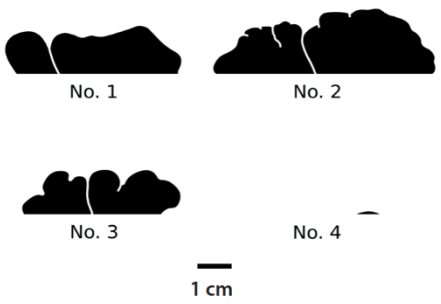


Fig. 4. Anteroposterior views of frontal sinuses above their SOL from X-rays of distantly related individuals Nos. 1–4 from family B. without visible shared morphological similarities.

	1	2	3	4
1	–	0.82	0.84	0.76
2		–	0.97	0.55
3			–	0.52
4				–

Tab. 3. The degree of similarity of individuals Nos. 1–4 from family B by frontal sinus morphology, calculated based on cosine similarity. The higher value, the darker color.

In this case too, the calculated similarity corresponds to the simple visual assessment. There is no positive relationship between the degree of similarity and the degree of biological distance between individuals. Although there is a relatively higher value between first cousins Nos. 1 and 2, the highest value occurs between biologically unrelated individuals Nos. 2 and 3, and Nos. 1 and 3. (Tab. 3).

Discussion

The results of visual assessment showed several identical or similar features between biologically related individuals in family A, unlike the biologically related individuals in family B, among whom no clear similarities were found. This is most likely due to the fact that there are smaller biological distances in family A, i.e. a greater degree of relatedness, than between the members of family B. This relates to a father and son or mother and son (coefficient of relationship r = 0.5) on the one hand, and an aunt/niece (r = 0.25), first cousins (r = 0.125), and cousin of mother/daughter of cousin (r = 0.0625) on the other. This finding was confirmed by the calculated degree of similarity between individuals, in family A the values correspond more to documented family relationships than in family B. In family B, there is even greater similarity between biologically unrelated individuals Nos. 1 and 3, or Nos. 2 and 3, than between individuals with biological relationships, such as individuals Nos. 1 and 4, or 2 and 4. This may be the result of chance, such cases have already been described (Cvrček et al., 2020). Although significant similarities were found in some cases even between first cousins in previous studies, both on the basis of the evaluation of features and according to calculations of similarity, nevertheless the most obvious similarities were primarily between closer relatives, such as parents and children, siblings, and grandparents and grandchildren (Cvrček et al. 2020). This also applies, for example, to osteological non-metric traits (Velemínský et Dobisíková 2005, Cvrček et al. 2018). The results of this study therefore confirm these earlier conclusions. A graphical expression of the relationship between similarity and relatedness (Cvrček et al. 2018, 2020) is impossible in these families, due to the small number of individuals and the small number of generations.

From a methodological point of view, this study confirms that a simple visual assessment of similarity may not be so burdened with subjectivity as assumed (Asherson 1963), even with respect to a scoring system (Besana et Rogers 2010), because similar results were arrived at on the basis of both approaches (Cvrček et al. 2020). Furthermore, a simple visual assessment (Szilvássy 1986), unlike scoring systems (e.g. Cameriere et al. 2005, 2008; Besana et Rogers 2010), allows the assessment of incompletely preserved skeletal remains, for which the missing parts cannot be exactly reconstructed (Kuželka 2018, Drtikolová Kaupová et al. 2020). At the same time, the situation may arise where the same frontal sinus pattern exists in two individuals based on the scoring system, but they differ visually (Cvrček et al. 2020). Although efforts have recently been made towards exact research of the frontal sinuses using computer tomography (CT) and/or focussing on their dimensions (Chaiyasate et al. 2007, Kjaer et al. 2012), X-ray examination with a focus on morphology appears to be sufficient, even with regard to preservation limits. The main advantage of morphological evaluation over dimensional assessments remains the variability of frontal sinus patterns. This is so great that although it is possible to detect the biological affinity between individuals (Cameriere et al. 2008), sinus formation is unique to each individual, similar to a fingerprint (Christensen et Hatch 2018).

Even though there are two proven consanguineous marriages in family B, their possible consequences on the morphology of the frontal sinuses cannot be studied in this case. For this knowledge, it would be necessary to have the skulls of the children of the parents and at the same time the first cousins Nos. 1 and 2, or the children of their son and related daughter-in-law No. 4; unfortunately these have not been preserved. It has however previously been found that inbreeding can affect some parameters of the frontal sinus, reducing their variability, albeit not always statistically significantly (Cvrček et al. 2020).

Although the studied samples include a small number of individuals from only two generations, this study significantly contributes to knowledge of the possible influence of family relationships on frontal sinus pattern inheritance, because this topic is still under discussion (Latiff et al. 2009, Ito et al. 2015).

Acknowledgements

The authors greatly thank Z. Schierová and M. Galetová (Hrdlicka Museum of Man, Faculty of Science, Charles University, Prague) for the opportunity to study documented osteological collections, M. Jantač (Department of Anthropology, National Museum, Prague) for making radiographs of the skulls, T. Jor (Department of Zoology, Faculty of Science, Charles University, Prague) for help with calculations of degrees of similarity, F. Fortin (Czech Genealogy and Heraldry Society in Prague) for his help with the genealogical research, and A. Millar for revising the text.

We would also like to thank both reviewers, Alena Šefčáková (Slovak National Museum) and Zdeněk Tvrdý (Moravian Museum), for the comments that led to the improvement of this contribution.

Grant sponsorship

Charles University Grant Agency (Grant Number: GAUK 1276217); Ministry of Culture of the Czech Republic (DKRVO 2019–2023/7.I.b, 00023272).

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Received: 22 December 2020 | Revised: 18 March 2021 | Accepted: 22 May 2021

DOI: 10.1002/oa.3004

SHORT REPORT

A reflection of the unfavorable living conditions caused by the First World War in the diet and health of a 10-year-old boy (1907–1917) from Vetlá cemetery, Bohemia

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Funding information
Ministry of Culture of the Czech Republic, Grant/Award Number: DKRVO 2019-2023/7.II.c, 00023272; Czech Science Foundation, Grant/Award Number: GACR 19-13265S; Charles University Grant Agency, Grant/Award Number: GAUK 1276217

Abstract

Although First World War battles did not directly affect the territory of all participating states, the conflict brought suffering in the form of a decline in their inhabitants' economic situation. This was also true in the Czech Lands. Opportunities for studying the impact of the period's difficult living conditions on the human body are very limited. This study presents pilot research into the possible influence of the wartime environment on children's lives through the example of a 10-year-old boy (1907–1917) from a family of landowners. The circumstances of his death and burial, demonstrated in coffin adjustment, confirm that the war influenced conditions at the time. We therefore considered the possible osteological and nutritional manifestations of the stress of that time, such as difference between biological and chronological age, the presence of skeletal developmental anomalies, signs of nonspecific stress in the form of linear enamel hypoplasia and Harris lines, and diet, also in the family context. The results showed no significant difference between the boy's biological and chronological ages nor body height disorder. There were seven developmental anomalies on his skeleton, some also found in other family members, which supports documented family relationships. Co-occurrence of signs of nonspecific stress and isotopic shifts were also found, indicating the possible presence of nutritional stress before his wartime death.

KEYWORDS
burial, coffin, diet, First World War, stable isotopes, stress

1 | INTRODUCTION

The First World War (WW1) brought severe economic distress to the Czech Lands. Male breadwinners were enlisted, and high military conscription resulted in goods shortages and high prices. Hunger was accompanied by repeated epidemics of infectious diseases (Lenderová et al., 2015). This clearly stressful environment could have had a negative impact on the growth and health of juveniles (e.g., Atta et al., 2016).

In bioarchaeology, carbon and nitrogen isotopic values are routinely used to define some basic characteristics of consumed foods (Lee-Thorp, 2008). Some studies suggest that human, especially juvenile, isotopic values may also be affected by nutritional or physiological stress (Beaumont et al., 2018). Markers of physiological stress (linear enamel hypoplasia [LEH] and Harris lines [HL]), reflecting episodes of growth arrest, are regularly examined (Lewis & Roberts, 1997).

Opportunities for applying this approach to documented skeletal remains are very limited on a global scale. However, during research on a 19th to 20th century family tomb in Vetlá, the funeral of a 10-year-old boy whose death (1917) took place during WW1 was exhumed. The official cause of death was diffuse peritonitis from

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gangrene of the appendix and heart failure. In a family chronicle, his older brother commented: "A tragic event that affected our whole family in November 1917, our 10-year-old boy died of appendicitis. In wartime, the hospital was full of soldiers and so he was transported to the Podolí sanatorium in Prague, but late, and thus a promising life was thwarted." The hospital was 7 km from his home, but the sanatorium 63 km away. He was buried in a double metal coffin, its use being explained by the long distance to transport the body for the funeral in his village, as required by hygiene regulations. Although he was prepubertal, he was buried in an adult coffin, and three oddities were found in the outer coffin and inset: (1) the inset is unusually small compared with the outer coffin, a short plank being used to prevent movement of the inset, so their mismatch was reflected at the funeral; (2) there are remains of sawdust and plant residues between the coffins, but the inset is intact; and (3) the outer coffin is damaged in several places and deformed outward. This all indicates that unlike the previously unused inset, the outer coffin was used secondarily for this funeral. This matches the contexts of the economic consequences and life of the civilian population during the WW1 in the Czech Lands and the known circumstances of death.

The following questions are raised: (1) Are there osteological manifestations of stress from that time, such as a discrepancy between biological and chronological age, the presence of developmental anomalies, and LEH and HL as indicators of nonspecific stress on the skeleton? (2) Is it possible to prove nutritional stress through analysis of carbon and nitrogen stable isotopes?

TABLE 1 Basic biographical data of the family sample

Individual No.	Sex	Age (years)
1	Male	74 (1791–1865)
2	Female	48 (1793–1842)
3	Female	33 (1823–1857)
4	Male	66 (1820–1886)
5	Male	68 (1844–1912)
6	Female	57 (1834–1891)
7	Female	86 (1848–1934)
8	Male	10 (1907–1917)

2 | MATERIAL AND METHODS

2.1 | Material

The tomb belongs to a regionally significant landowner's family. The skeletal remains of eight family members from four generations (Table 1) with biographical data were available (Figure 1). Their exhumation was carried out at the request of the descendants for the purpose of the necessary renovation of their family tomb, and the anthropological research was conducted with their written legal consent. Biographical data comes from the family's private archive, the State Regional Archives in Litoměřice, and the Prague City Archive. The remains were completely preserved (Figure 2).

2.2 | Methods

2.2.1 | Biological age and stress signs

The boy's biological age was estimated by X-ray based on development of the permanent teeth (Smith, 1991; Štepanovský et al., 2017). Skeletal developmental anomalies and growth abnormalities (Scheuer & Black, 2000), including nonspecific stress indicators on the frontal permanent teeth (LEH; King et al., 2005) and long limb bones (HL; Lewis & Roberts, 1997; Kulus & Dąbrowski, 2019), were evaluated, also using X-ray (Department of Anthropology, National Museum, Prague). The age at LEH formation was estimated using Reid and Dean's (2000) standards for the timing of anterior tooth growth. Following best practice in bioarchaeology, only coforming LEH

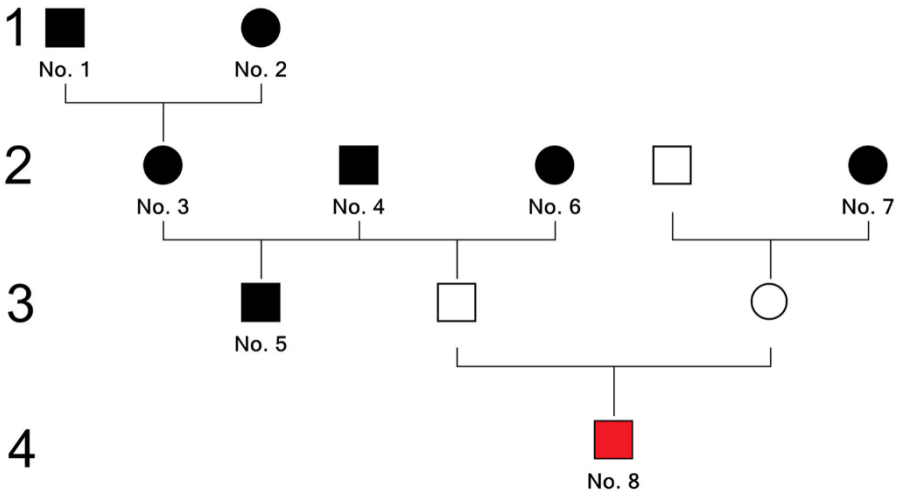


FIGURE 1 Family tree of a genealogically documented sample (generation Nos. 1–4). All individuals in the sample have a number, individuals included in this study (8) are shown in black, and the subject (No. 8) is highlighted in red color [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 2 Double metal coffin of boy No. 8, with Buxus wreaths (left) and his skeletal remains (right) [Colour figure can be viewed at wileyonlinelibrary.com]

present on at least two teeth was taken into account. Body height was estimated based on Stewart (Olivier, 1960) using the femur diaphysis, and the resulting value was compared with population growth data for boys from 1895, 1951, and the recent period (Matiegka, 1927; Vignerová et al., 2006). The body height of the boy's adult relatives was estimated, where this was possible, using the method of Černý and Komenda (1982) for adults, based on the population of the 1930s; the resulting values were compared with data for both sexes from the same sample and from 1951 (Vignerová et al., 2006).

2.2.2 | Stable isotope analysis

The serial sampling methodology for incremental dentine collagen enabled creation of detailed isotope profiles for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), which show temporal changes in the isotope ratios throughout the subject's life. Tooth collagen was extracted using Method 2 described by Beaumont et al. (2013), as modified by van der Haas et al. (2018).

Rib and femur cortical bone collagen was extracted using the method of Longin (1971) modified by Bocherens (1992). As bone undergoes constant turnover, its isotopic composition reflects the diet of some period before death (Hedges et al., 2007). Bone samples were also taken from five of the subject's relatives. Although it would be unfeasible to make dietary interpretations from the comparison of tooth dentine versus adult bone collagen data (Beaumont et al., 2018), a knowledge of the family's long-term dietary norm could be extremely useful when looking at the dentine data as a result of a complex interaction between diet and physiology. This may help to distinguish short-term deviations from this norm, for which either a dietary or a physiological cause might be found in the history of the course of the boy's life.

Finally, two samples of hair were analyzed to describe diet during the last months before death using a modified version of the O'Connell and Hedges (1999) method as used by Cooper et al. (2016).

All of the sample preparations were carried out at the Department of Anthropology, National Museum, Prague. Elemental analysis–isotope ratio mass spectrometry (EA-IMRS) was performed at Iso-Analytical, Crewe. Details of samples preparation and measurement are provided in Appendix S1.

3 | RESULTS

3.1 | Biological versus chronological age, skeletal anomalies, and nonspecific stress indicators

The subject died 9 days after his 10th birthday. The dental age estimate was 9.7 years. Body height was estimated around 141 cm. The average body stature of adult males from his family was estimated at 170 ± 4.15 cm (grandfather No. 4 and half-uncle No. 5) and the height of females at 156 ± 4.51 cm (both grandmothers, Nos. 6 and 7).

Seven skeletal developmental abnormalities were found (Figure 3): (1) bilateral open foramens of the transverse process of the first cervical vertebra (usually closed at the age of 3–4 years); this anomaly was also found in his half-uncle No. 5; (2) bilateral incomplete closure of the transverse process of the sixth cervical vertebra (especially evident on X-ray; usually closed at 3–4 years); (3) partial cleft of the posterior part of the body of the sixth thoracic vertebra; (4) unfused arch of the fifth lumbar vertebra (fusion usually occurs at about 1 year); (5) developmental asymmetry of the ischiopubic ramus:

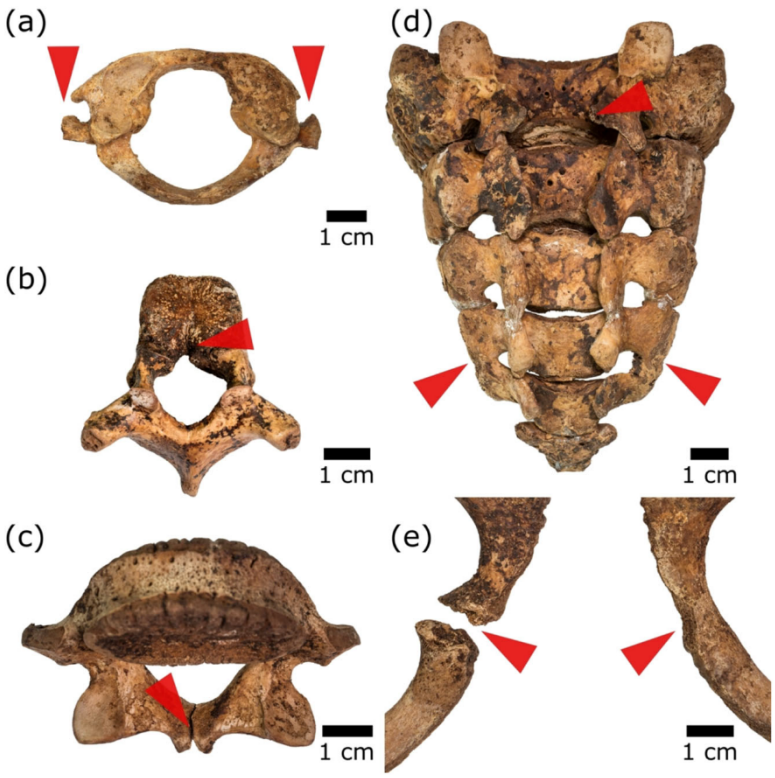


FIGURE 3 Some of the skeletal developmental anomalies found in the skeletal remains of the boy (arrows): (a) bilateral open foramens of the transverse process of the first cervical vertebra, (b) partial cleft of the posterior part of the body of the sixth thoracic vertebra, (c) unfused arch of the fifth lumbar vertebra, (d) open vertebral canals of all sacral vertebrae and bilateral fusion of the lateral segments of the fourth and fifth sacral vertebrae, and (e) developmental asymmetry of the ischiopubic ramus: left, fused; right, unfused [Colour figure can be viewed at wileyonlinelibrary.com]

left, fused; right, unfused (fusion occurs at 5–8 years); (6) open vertebral canals of all sacral vertebrae (usually closed by around 10 years, development completed in puberty), localized incomplete fusion of the vertebral arches of the sacrum also being confirmed in his grandfather No. 4 and half-uncle No. 5; and (7) bilateral fusion of the lateral segments of the fourth and fifth sacral vertebrae (usual at the age of 12 years). The ontogenetic development of other parts of the skeleton showed no changes.

Two signs of possible nonspecific stress were detected. (a) LEH on the front permanent incisors (best preserved Nos. 21 and 41 were selected): two lines were observed at upper 21 whereas six lines were observed at lower 41, with the age-at-formation estimates spanning from 1.7 to 2.9 years. One line was found to conform on both teeth to the age-at-formation estimate of approximately 2.5 years. The permanent canines had not yet erupted and therefore could not be evaluated. (b) Growth arrest HL: these are present on multiple bones but most prominent on the distal metaphyses of the tibiae and developed from age 7–8 years until death.

3.2 | Diet based on stable isotope analysis

All samples of the boy's tooth dentine ($N = 18$), his bone ($N = 2$), and bone from his relatives ($N = 5$) met the criteria for good collagen preservation (DeNiro, 1985; van Klinken, 1999). Table 2 presents the complete isotopic data. Across the dentine profile, the maximum variation in $\delta^{13}C$ values during life was 0.6‰, ranging from −20.7‰ to −20.1‰. The maximum variation in $\delta^{15}N$ across the dentine profile was 1.7‰ ranging from 10.3‰ to 12.0‰. $\delta^{15}N$ value is lowest in the earliest sample (slice CAS_13a, forming approximately at 0.0–0.4 years). Two main peaks occur during life: the first culminating around the age of 3 years (CAS_13e, 1.8–2.2 years) and the second at 8–9 years (CAS_13m, 7.6–9.4 years; CAS_12h, 8.0–8.6 years). The latter is followed by a sudden drop in $\delta^{15}N$ during the last year of life (CAS_12j, 9.3–10 years). Apart from the earliest slice, the dentine isotopic values are within the variation observed among the subject's relatives (Figure 4).

Carbon isotopic values show an opposite trend, albeit that negative peaks show slightly earlier timing than in nitrogen: the $\delta^{13}C$ value is high in the earliest sample (CAS_13a) from 0 to 0.4 years. The first negative peak occurs around 1 year (CAS_13c, 0.9–1.3 years) and the second at 7 years (CAS_13l, 6.8–7.6 years; CAS_12f, 6.6–7.3 years). The negative covariation with $\delta^{15}N$ disappears around the time of death, where another drop in $\delta^{13}C$ appears. During all three peaks, the subject's values fall below the variation observed in his family (Figure 4).

The carbon and nitrogen isotopic values of rib and femur bone are almost identical and close to the values observed in the last dentine slice. Considering that collagen is slightly enriched (by 0‰

TABLE 2 Complete isotopic data of the studied individual (No. 8) and his family (Nos. 3–7)

Sample code	Sample detail	Individual No.	Collagen yield[a]	Sample location[b]	Median age (min–max)[c]	Nitrogen content (%)	Carbon content (%)	C:N	$\delta^{15}N_{AIR}$ (‰)	$\delta^{13}C_{V\text{-}PDB}$ (‰)
CAS_13a	36	8	120.4	Ci-Crc	0.2 (0.0–0.4)	16.3	43.6	3.1	10.3	−20.1
CAS_13b	36	8	120.4	Ci-Crc	0.7 (0.4–0.9)	14.9	39.3	3.1	11.7	−20.3
CAS_13c	36	8	120.4	Ci-Crc	1.1 (0.9–1.3)	17.0	44.8	3.1	11.3	−20.7
CAS_13d	36	8	120.4	Ci-Crc	1.5 (1.3–1.8)	17.8	46.7	3.1	11.9	−20.6
CAS_13e	36	8	120.4	Ci-Crc	2.0 (1.8–2.2)	16.7	44.1	3.1	12.0	−20.4
CAS_13f	36	8	120.4	Crc-R1/2	2.6 (2.2–3.0)	17.2	45.5	3.1	11.9	−20.1
CAS_13g	36	8	120.4	Crc-R1/2	3.4 (3.0–3.8)	16.6	44.1	3.1	11.7	−20.1
CAS_13h	36	8	120.4	Crc-R1/2	4.1 (3.8–4.5)	16.5	44.0	3.1	11.7	−20.2
CAS_13i	36	8	120.4	Crc-R1/2	4.9 (4.5–5.3)	16.6	44.0	3.1	11.6	−20.2
CAS_13j	36	8	120.4	R/1/2-A1/2	5.7 (5.3–6.1)	16.7	44.7	3.1	11.4	−20.3
CAS_13k	36	8	120.4	R/1/2-A1/2	6.5 (6.1–6.8)	15.9	42.1	3.1	11.4	−20.3
CAS_13l	36	8	120.4	R/1/2-A1/2	7.2 (6.8–7.6)	15.5	40.9	3.1	11.4	−20.5
CAS_13m	36	8	120.4	A1/2-Ac	8.5 (7.6–9.4)	14.5	38.7	3.1	12.0	−20.4
CAS 12f	25	8	149.0	Crc-R1/2	6.9 (6.6–7.3)	14.5	38.5	3.1	11.4	−20.5
CAS 12g	25	8	149.0	Crc-R1/2	7.6 (7.3–8.0)	15.3	40.8	3.1	11.8	−20.5
CAS 12h	25	8	149.0	Crc-R1/2	8.3 (8.0–8.6)	14.8	39.4	3.1	12.0	−20.4
CAS 12i	25	8	149.0	Crc-R1/2	9.0 (8.6–9.3)	13.2	35.3	3.1	11.9	−20.3
CAS 12j	25	8	149.0	Crc-R1/2	9.7 (9.3–10.0)	7.0	19.1	3.2	10.9	−20.6
CAS 10/3a	Hair—prox	8	x	x	9.9–10.0	16.0	45.9		10.5	−20.9
CAS 10/3b	Hair—dist	8	x	x	9.8–9.9	15.3	44.0		10.3	−21.0
CAS 15	Rib	8	226.9	x	8–10	16.1	42.6	3.1	11.1	−20.7
CAS 16	Femur	8	199.8	x	8–10	16.7	44.5	3.1	11.1	−20.6
CAS 01	Femur	3	207.4	x	Several decades	16.8	44.9	3.1	11.3	−19.6
CAS 03	Femur	4	189.3	x	Several decades	17.2	45.2	3.1	11.6	−19.7
CAS 05	Femur	5	202.9	x	Several decades	15.9	42.4	3.1	11.0	−20.1
CAS 07	Femur	6	211.2	x	Several decades	15.4	40.9	3.1	12.1	−20.3
CAS 09	Femur	7	186.8	x	Several decades	16.0	42.3	3.1	10.6	−20.1

[a]In teeth samples, the average collagen yield was counted as the sum of collagen weights from all the slices divided by the total weight of unmineralized dentine before slicing.
[b]Tooth developmental stages according to Smith (1991).
[c]Approximate age at formation of a particular sample (in years); for teeth, average age at completion of developmental stages according to Smith (1991); for subadult bone, the bone turnover rate calculated by Tsutaya and Yoneda (2013); and for hair, the growth rate used by Cooper et al. (2016) were used for estimates.

to 1‰; O'Connell & Hedges, 1999) relative to keratin, the same is true for the $\delta^{15}N$ of the hair samples. However, as collagen is enriched by 1‰ to 2‰ relative to keratin in $\delta^{13}C$, it seems that the subject's diet or his nutritional status changed briefly before death in the opposite direction. There was no significant difference between the hair segments in $\delta^{15}N$ and $\delta^{13}C$.

4 | DISCUSSION

4.1 | Biological versus chronological age and skeletal anomalies

There was no significant discrepancy between biological and chronological age according to the development of permanent teeth and no

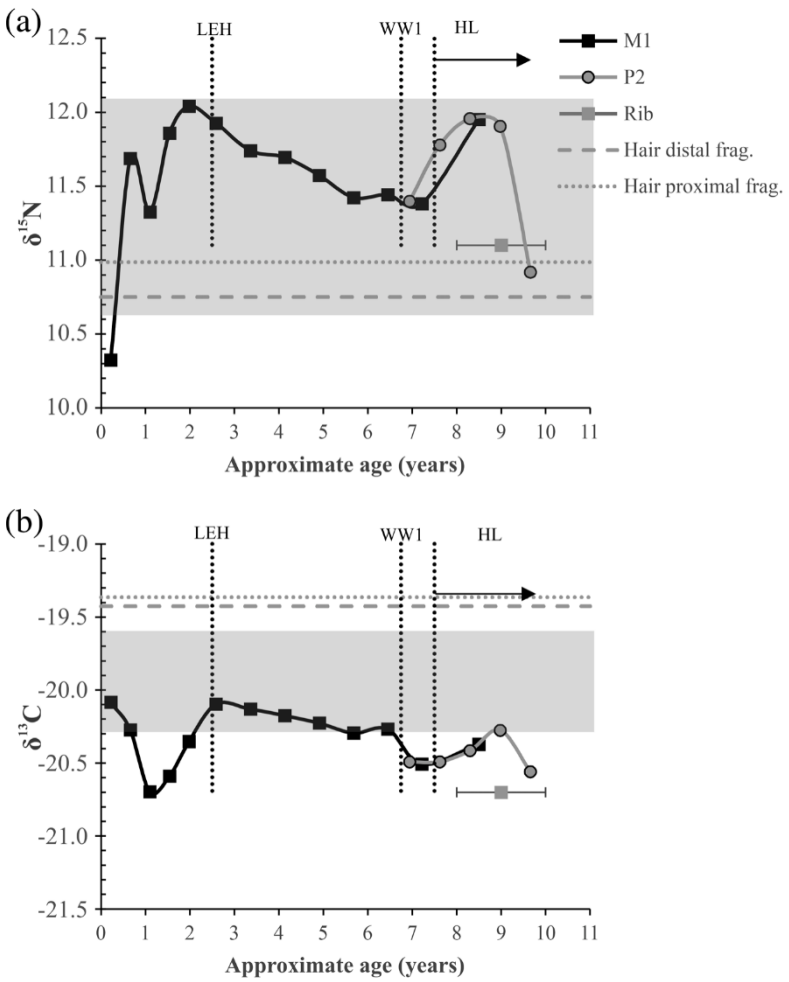


FIGURE 4 Carbon and nitrogen isotopic values of the boy. Concerning the hair values, a value of 0.5 for $\delta^{15}N$ and 1.5 for $\delta^{13}C$ was added to compensate for the collagen-keratin isotopic offset. The gray box represents the isotopic variation observed in the boy's family. LEH = approximate age at formation of linear enamel hypoplasia; WW1 = beginning of the First World War; HL = period of Harris line formation marked with an arrow. The midpoint for each age range was used as the value for the *x*-axes instead of an actual time span, for the sake of simplicity. For the age ranges represented by particular samples, see Table 2

significant impairment of body height. Height was closer to the average of contemporary Czech boys (144 cm at age 10) than that of boys at the end of the 19th century or in the mid-20th century. It is around 13 cm more than the average for 10-year-old boys in 1895 and around 6 cm higher than the average for the same population in 1951 (Vignerová et al., 2006). The measured average height of related adult individuals (170 ± 4.15 and 156 ± 4.51 cm) is, even taking into account possible deviation, comparable with the height of the population in the 1930s (167 and 155 cm) (Černý & Komenda, 1982) and in 1951 (173 and 162 cm) (Vignerová et al., 2006). Unfortunately, for 1895, there are data for both sexes only up to the age of 14 (Matiegka, 1927). In 1848–1889, height measurements were made on young adult male recruits in the Habsburg Monarchy, for which average values ranged from 165.0 to 167.5 cm (Komlos, 2007).

The skeletal developmental variations found, which do not correspond with chronological age (Scheuer & Black, 2000), may be a result of variability, but anomalies in the first cervical vertebra and sacrum may relate to genetic factors and thus support documented family relationships (e.g., Thurzo et al., 2001). Nevertheless, their association with physiological stress cannot be excluded (e.g., Trinkaus, 2018). Unfortunately, comprehensive evaluation of skeletal developmental anomalies among the whole family was made impossible by the incomplete preservation of relatives' skeletal remains.

4.2 | Stable isotope analysis and nonspecific stress indicators

The isotopic profile of the individual lacks the typical positively covariant $\delta^{15}N$ and $\delta^{13}C$ peaks in the earliest dentine slices, which occur during breastfeeding (Beaumont et al., 2018; Fogel et al., 1989; Fuller et al., 2006). This means that either the subject was never breastfed or the period of breastfeeding was too brief to significantly affect the isotopic values of the earliest dentine slice. It is very difficult to be more particular about the length of this potential period of "hidden" breastfeeding, due to the interindividual variation in M1 development, the slight variation in timing of individual cusp formation (Jakobsen, 1975), and the unknown period needed to incorporate the isotopic signal of the new diet into the dentine collagen. However, taking into account that the apical millimeter of the tooth dentine

retains the signal from approximately 0.4 years of age, it may have lasted rather for weeks than for months. Before WW1, artificial baby nutrition existed on the market, based on hygienically treated sterilized milk distributed in bottles (Lenderová et al., 2015), and may have been available. Surprisingly, $\delta^{15}N$ values in the earliest slice are significantly lower than in the rest of life and outside the range of values typical for his family. This may suggest early introduction of plant-based foods—that is, cereals.

Throughout the rest of his life, $\delta^{15}N$ values are within the variation observed among his relatives. During dentine formation, we observe two events reflected by opposing covariance between $\delta^{15}N$ and $\delta^{13}C$, which may be seen in cases of nutritional or physiological stress (Beaumont & Montgomery, 2016). During the first peak, $\delta^{15}N$ rises from 11.3‰ to 12.0‰ and during the second from 11.4‰ to 12.0‰. Given that the analytical uncertainties in isotopic analyses are around 0.4‰ at two standard deviations, these should be considered significant. This offset is comparable with that observed by Fuller et al. (2006) during episodes of morning sickness in a sample of eight gravidae. Even in severely starved anorectic patients, Mekota et al. (2006) observed a rise in $\delta^{15}N$ of 0.6‰ to 2.2‰. Concerning dentine collagen data, Beaumont and Montgomery (2016) found the isotopic manifestation of nutritional stress in an approximately 1‰ rise in $\delta^{15}N$ in victims of the Great Irish famine. A similar rise was observed by Goude et al. (2020) in a juvenile individual suffering from tuberculosis and was interpreted as a probable result of tuberculosis-induced wasting. This suggests that the subject's isotopic values are well within the range of those observed within the context of restricted dietary intake or physiological stress.

It is true that negative peaks in $\delta^{13}C$ show slightly earlier timing than those in $\delta^{15}N$. This fits the hypothesis of Salesse et al. (2019), whose data suggested that carbon balance may become negative more rapidly than nitrogen, and thus, $\delta^{13}C$ may be more sensitive to milder forms of nutritional stress. In later stages of undernutrition, $\delta^{13}C$ may rise again as the body runs out of ^{13}C depleted fat reserves (Neuberger et al., 2013). It must however be kept in mind that carbon isotopic profiles in nutritionally and/or physiologically stressed individuals show notable variation, with no universal pattern (Fuller et al., 2006; Goude et al., 2020; Mekota et al., 2006; Neuberger et al., 2013).

This interpretation is supported by the results of the osteological examination. LEH present on both the upper and lower first permanent incisors and HL found on the distal ends of both tibiae reflect periods of physiological stress. In particular, the timing of the HL corresponds with stress events suggested by isotopic values. It is notable that the second potential stress event began during 1915. According to historical newspapers and chronicles, during 1915, the socioeconomic level, dietary, and epidemic situation of the general population worsened, even in the families of landowners. Men were drafted, increasing quantities of crops and live inventory were demanded by the state, and rationing was introduced. The amount of food per person decreased, and its quality deteriorated (change in the share of raw materials and their substitutes). Various epidemics also began to spread, especially among children (e.g., Lenderová et al., 2015). Several records in the family chronicle correspond to this general trend. While before WW1 the family homestead was expanded every year by the construction of new buildings and the purchase of agricultural machinery (1883–1914), during WW1 (1914–1918), they were forced to hand over their own horses and modern agricultural machinery. In addition, the farm's products were difficult to sell, and in 1917, the boy's older brother had to go to war, causing the family to lose one of its breadwinners. The age at LEH formation is however slightly delayed compared with the period of physiological stress suggested by stable isotopes.

While the above suggests that repeating episodes of nutritional stress affected the subject's isotopic values, an alternative explanation is possible. All the shifts may reflect dietary changes caused, for example, by age adjustment of child feeding strategy or the annual variation in staple composition. This scenario is suggested by the sharp isotopic shift during the final period of life, reflected in the last dentine slice, as well as in the bone and hair samples. This would mean that the subject's diet worsened significantly only in 1917 in the sense of decreased animal protein consumption. As a general crisis culminated in 1917, this explanation appears more plausible than the recovery of the subject from a catabolic state. Further, the individual was not stunted, as expected when experiencing repeated periods of nutritional stress. As his fatal illness was acute, it is not assumed to affect isotopic values.

Finally, $\delta^{15}N$ may fall during periods of intensive growth, though to date there has been no convincing evidence of this process in human bone or dentine tissue (Waters-Rist & Katzenberg, 2010).

Another point deserving wider discussion is the minimal difference in $\delta^{15}N$ between bone and the last dentine slice ($\Delta^{15}N = 0.2‰$). Nitrogen isotopic values in both femur and rib were actually slightly higher than in the dentine, which is in accordance with the isotopic peak observed in previous dentine samples. Taking into account the estimated bone turnover rate, which is slightly below 0.5 (Tsutaya & Yoneda, 2013) during late childhood, a slightly higher bone $\delta^{15}N$ would however be expected. As there is thus far no evidence of an isotopic offset between bone and tooth collagen, this evidence seems to support the finding of Beaumont et al. (2018) that bone fails to record the isotopic signal from a period of physiological stress in full. Interestingly, bone $\delta^{13}C$ values were slightly lower than in any of the premolar slices. However, considering the small extent of isotopic differences and the analytical precision of the instrument, this finding should not be overestimated.

Concerning the hair isotopic data, it is impossible to ignore the potential impact of diet seasonality. Taking into account that the boy died on November 9, the higher $\delta^{13}C$ observed in his hair tissue formed approximately 2 months before death may well represent seasonal shifts in the character of plant foods, such as changes in the proportion of green leafy vegetables versus cereals (Fraser et al., 2011). Regrettably, taking into account the age-at-formation estimates for the particular dentine slices (Table 2), even 1-mm increments of dentine cover too long a period to explore the potential impact of seasonality.

Some of the limits of dentine stable isotope analysis also need to be mentioned here, mainly in terms of the precision of the age

estimation for particular dentine slices: here, several sources of error inevitably occur. First, horizontal sequential dentine samples do not respect the course of the incremental growth layers, including material from several growth layers, and thus do not represent separate time spans. Moreover, time averaging differs between the crown and the root, where increments become much more convex, as well as between different tooth types. Second, mean ages at which the stages of tooth formation are completed are used to simplify age calculations, ignoring the interindividual variability in dental growth (Czermak et al., 2020). And finally, although the developmental scheme of the teeth is considered relatively stable, variation in dentine initiation ages and growth rate may be found between populations (Scharlotta et al., 2018). At least the latter two sources of error may affect also the age-at-formation estimates for LEH and HL (though in the latter, the bone growth is in issue). Moreover, the recording of bone or tooth lesions consecutively to physiological stress may well present a different timetable, and the threshold level of stress to produce the isotopic shifts and developmental disruptions may also vary. More dentine profiles of individuals with a known medical history are needed to describe the mutual relationships between these stress markers.

This however does not affect the two main findings of this study: the lack of breastfeeding and the dramatic shifts in isotopic values during the period of WW1. Knowing the exact age at death of the boy and the stage of his tooth development and considering the relative temporal and geographical proximity of the studied population to those used to create Smith's standards of tooth development, there is no doubt that the majority of his P2 root developed under the circumstances of the war.

5 | CONCLUSION

Although neither a significant discrepancy between biological and chronological age nor significant body height disorder was found in the skeleton of the examined boy, seven skeletal developmental anomalies were observed. Nevertheless, some of these relate to familial characteristics and natural variability rather than to a direct developmental disorder due to nutritional stress. On the other hand, isotopic shifts and the co-occurrence of nonspecific stress indicators (LEH and HL) suggest the presence of nutritional stress in the wartime. The results of isotopic analysis raised interesting questions for the future, rather than definitive conclusions. More individuals growing up in the context of WW1 should be sampled to explore the general norms for infant feeding and especially the impact of the stressful environment on children's lives.

ACKNOWLEDGMENTS

This study was supported by Charles University Grant Agency (Grant GAUK 1276217), Czech Science Foundation (Grant GACR 19-13265S), and Ministry of Culture of the Czech Republic (DKRVO 2019-2023/7.II.c, 00023272). We greatly thank P. Čašek and his family for supporting our research.

CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Cvrček, J., Drtikolová Kaupová, S., Půtová, L., & Velemínský, P. (2021). A reflection of the unfavorable living conditions caused by the First World War in the diet and health of a 10-year-old boy (1907–1917) from Vetlá cemetery, Bohemia. *International Journal of Osteoarchaeology*, 1–9. https://doi.org/10.1002/oa.3004

Received: 6 April 2021 | Revised: 10 June 2021 | Accepted: 10 June 2021

DOI: 10.1111/joa.13499

ORIGINAL PAPER

Journal of Anatomy ANATOMICAL SOCIETY WILEY

Familial occurrence of skeletal developmental anomalies as a reflection of biological relationships in a genealogically documented Central European sample (19th to 20th centuries)

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Funding information
Charles University Grant Agency (Grant Number: GAUK 1276217); Ministry of Culture of the Czech Republic (DKRVO 2019-2023/7.II.c, National Museum, 00023272).

Abstract

Skeletal developmental anomalies (SDA) are a subject of constant interest across scientific disciplines, but still mostly as isolates and curiosities. The aim of this study was to find out to what extent the occurrence of SDA reflects documented biological relationships. The skeletal remains of 34 individuals with known genealogical data were available, members of one family over four generations (19th to 20th centuries, Bohemia, Czech Republic), including some inbred individuals. The occurrence of 89 SDA was assessed on the basis of scopic morphological evaluation and X-ray and CT examinations. The degree of similarity between individuals was calculated using a "similarity coefficient" (SC). A linear model was used to test the relationship between positive values of the SC and the relatedness of biologically related individuals. Simultaneously, based on population frequencies of the evaluated anomalies, those that could be considered familial were recorded. A statistically significant relationship between morphological similarity and the biological distance between individuals was found. The greatest similarity was found among close relatives such as parents and children, siblings, or grandparents and grandchildren. The effect of increased consanguinity on the occurrence of anomalies was not confirmed, however. Seventeen SDA shared by closely related individuals were found in the sample, supporting the documented family relationships among them. Eleven of these were selected as possibly familial, but only five were statistically significant: an elongated styloid process, a cervical block vertebrae (arch, facet joints), hamate hamulus aplasia, anteater nose sign, and incomplete fusion of the S1 spinous process. There were also 28 cases of individual occurrences of 17 different SDA, without connection to the documented relationships between individuals.

KEYWORDS
biodistance, heredity, inbreeding, ossification, similarity coefficient

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1 | INTRODUCTION

Skeletal developmental anomalies (SDA) are related to disorders of ossification, the nonjunction of ossification centers or their absence; they are defined as structural bone variants deviating from designated standard ranges, excluding skeletal dysplasias (Barnes, 2012; Ortner, 2003). Since the end of the 19th century, a possible genetic basis for familial occurrence of SDA have been observed in clinical practice (e.g., Lemarie, 1909; Shepherd, 1893; Symmers, 1895). Such studies, however, now face ethical restrictions in terms of research on living people, especially in terms of X-ray dosage without medical indication (Kjaer et al., 2012). Therefore, interest in studying this topic has shifted to the field of osteology (Henderson & Cardoso, 2018). SDA have not hitherto been as commonly perceived of suitable tools for the detection of genetic relationships as nonmetric dental traits and the frontal sinus (Stojanowski & Schillaci, 2006), although identical patterns in the incidence of these abnormalities have also been observed in closely related individuals such as parents and children, and siblings or first cousins within genealogically documented osteological samples (e.g., Rösing, 1986; Giuffra & Fornaciari, 2013; Vlček, 2000). It has even been found that these must not always be identical anomalies, and that similarity based on the same defect at different places is also possible (Thurzo & Beňuš, 2003).

Despite the potential value of SDA, most of the research on SDA has unfortunately been through case studies rather than population studies, and they have been taken as curiosities (e.g., Drozdová, 2006; Fornaciari et al., 2007; Masnicová & Beňuš, 2003). Populational studies remained rare for a long time (Titelbaum, 2020). Recently, however, the approach to them has begun to change, and systematic research has come to the fore (e.g., Partiot et al., 2020; Tur et al., 2019). The possible role of inbreeding and reduced genetic variability in the incidence of SDA has also been discussed (Trinkaus, 2018); it is assumed that the consequences of inbreeding are ultimately reflected in skeletal morphology through their increased number (Ríos et al., 2015, 2019). On the other hand, these findings were based on studies of extinct Neanderthal populations, and some are of the opinion that the prevalence of certain SDA in Middle and Upper Pleistocene hominin populations may be higher than in modern humans (Palancar et al., 2020; Ríos & Cardoso, 2020). However, this does not exclude the likelihood that genetic diversity may have been low in these populations. Study of this issue in modern humans is still lacking.

Despite this increasing attention to genetic influences on the occurrence of SDA, the fact remains that, with some exceptions, the application of mathematical and statistical methods to SDA with possible familial occurrence still lags (e.g., Burnett & Case, 2011; Case, 2003; Case et al., 2017; Offenbecker & Case, 2012). So far, SDA frequencies have been compared between populations, and based on a reference group of individuals with known biological relatedness, such SDA were sought as reflected these documented relationships well and at the same time had a low frequency. Their increased occurrence in (pre)historic burial grounds would then help identify possible genetic relatives (Case et al., 2017).

The authors had the opportunity to analyze the occurrence of SDA in a recent genealogically documented osteological collection, including some inbred individuals. The aim of this paper was to show the frequency of monitored SDA in this sample, and to use new statistical approaches to answer the following questions: 1) Are there significant positive relationships between the degree of similarity based on SDA and the biological distance of biologically related individuals? Is there an increased degree of similarity and a higher incidence of anomalies in families with increased consanguinity compared to members of parallel-related families with common degrees of consanguinity? 2) Are there any specific anomalies the occurrence of which supports the documented family relationships?

2 | MATERIALS AND METHODS

2.1 | Materials

Thirty-four adult individuals (21 males and 13 females, numbered Nos. 1–38) from a recent genealogically documented osteological collection were selected based on their state of preservation (Cvrček et al., 2018). Individuals Nos. 26, 27, and 29 were excluded from the study because their remains preserved only fragments of some long bones. They are aged 20–90 years, members of four different branches of one family over four generations in the 19th to 20th centuries (generations Nos. 4–7, see Supporting information No. 1, Figure S1), including two cases of consanguineous marriages. The first case is spouses Nos. 7 and 8 (first cousins and second cousins, generation No. 4), whose five children (generation No. 5) are also in the sample: sons Nos. 9, 11, and 13 and daughters Nos. 14 and 17; the next two generations Nos. 6 and 7 (males Nos. 19, 20, and 21) from daughter No. 17 were also evaluated. The second case is spouses Nos. 13 and 37 (generation No. 5/6, the mother of female No. 37 was the half-first cousin of male No. 13).

The material was made available by repairs to their family tombs and coffins, and the research was supported by descendants of the studied individuals (Cvrček et al., 2018, 2020). The genealogical data were verified using written documents and vital records from the family, regional registry offices, hospitals, and state institutions (State Regional Archives in Prague, Třeboň, and Litoměřice; Prague City Archive; Archive of the Prague Archbishopric; Vatican Secret Archives). Family archives, cemetery lists and the position of the bodies in the tombs were also used for the identification of individuals. After the conclusion of the study, the skeletal remains were re-buried in their tombs. All data are stored in the archive of the Department of Anthropology of the National Museum in Prague.

Three skeletal samples were used to determine reference data for this study. These samples were chosen for their chronological, geographical, and socioeconomic similarity to the genealogically documented collection, and the almost certain unrelatedness of the

individuals according to written records (information on the bones, or vital records from the Prague City Archive).

The first sample is the Pachner collection (P) of the Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague, and the Institute of Anatomy, First Faculty of Medicine, Charles University, Prague (Pachner, 1937), which consists of adult individuals who lived in the second half of the 19th century and the first third of the 20th century. The collection comprises 143 skulls (78 males and 65 females, aged 20–88 years) and a larger number of postcranial skeletons (485 individuals). Due to the incomplete preservation of the sample, anomalies of the skull, pelves and limb bones, the first cervical vertebra and the ribs were evaluated in particular, the same bones in which nonmetric traits have already been evaluated (Cvrček et al., 2018). Regarding the fixed number of skulls in the collection (N = 140), approximately the same number of postcranial bones were evaluated, adequate to the skulls (see Supporting information No. 2, table S4).

The second sample is from the 18th century, from the crypt of the Church of Our Lady Victorious in Prague, held at the Department of Anthropology of the National Museum, Prague (Stloukal, 2013). It was possible to evaluate 107 adult individuals (49 males and 53 females with 5 unknowns, aged 20–60 years with 24 adults of unknown age) and preserved isolated adult bones in this sample; for this study, we evaluated bones that could not be evaluated at all or sufficiently in the Pachner collection, especially spines, sternums, and patellae. These are also the same bones in which nonmetric traits have previously been evaluated (Cvrček et al., 2018).

The third, unnamed recent sample (R) comes from the Department of Anthropology of the National Museum, Prague, and Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, and consists of 45 adult individuals (22 males and 23 females older than 20 years) who also lived in the second half of the 19th century and the first third of the 20th century. These are complete assembled skeletons, which in particular allowed for evaluation of the complete bones of the hands and feet, the development of the rib 12 and some anomalies of the sternum and sacrum, that is, bones or their parts which in archaeological samples are often missing or damaged postmortem.

2.2 | Methods

A total of 89 SDA were investigated (Barnes, 2012; Chapman, 2007; Velemínský, 2000) (see Supporting information No. 2, Table S1). They were evaluated using morphological assessment, and X-ray and CT examinations. X-ray examination was performed using digital radiography (direct flat panel detector) at the Department of Anthropology, National Museum, Prague. All bones were captured in anteroposterior and lateral views, and vertebrae in axial view as well. Bones were placed directly on the CR cassette, so the geometric magnification of the resulting images is negligible. The standard distance between the focal point and the CR cassette was 1.5 m, with tube voltages ranging between 48 and 55 kV and beam intensity between 8 and 20mAs. Computed tomography (CT) was performed at the Radiology Department of the Homolka Hospital in Prague using a Somatom Definition Flash 2 x 128 Siemens 2010. Data were processed based on axial scans with a symmetric matrix of 512 × 512 points. Acquisition parameters were optimally set with a voxel size of 0.49 mm, a slice thickness of 0.6 mm, and a slice increment of 0.3 mm.

The presence (+) or absence (-) of anomalies was recorded. If a bone was damaged or missing at the site, the anomaly could not be evaluated (0). We used a similarity coefficient (SC) to express the degree of similarity between individuals (Cvrček et al., 2018; Supporting information No. 2, Table S2). The SC of two individuals without the presence of at least one anomaly from among those investigated is 0, the SC of two individuals without any of the investigated anomalies is NA (non-applicable). Pairs of biologically related individuals with SC = 0 and with NA were excluded from further analyses. Due to the rare occurrence of most anomalies (e.g., Case, 2003; Case et al., 2017; Ríos et al., 2019), only with biologically related pairs with a positive degree of similarity in the interval of 1–100 were considered, and these values were applied as follows:

1. First, a logarithm of the coefficient of relationship for each pair of individuals in the sample was calculated (561 combinations). The coefficient of relationship was estimated using a tabular method (Falconer & Mackay, 1996) based on that of VanRaden (1992). The use of logarithms of this coefficient disregards biologically unrelated individuals in this step. Their coefficient of relationship is always zero, so the logarithm is not defined (Cvrček et al., 2018, 2020). Linear model was used to test the relationship between the positive SC and the logarithm of the biological distance for all biologically related pairs in the sample.
2. Next, all positive SCs between individuals regardless of their kinship were transformed to distances as d=100-sc. Points corresponding to each individual in a 2D parametric space were reconstructed with a multidimensional scaling (MDS) approach from these distances. MDS was calculated with a stress majorization algorithm (SMACOF) (de Leeuw & Mair, 2009). K-means clustering was used to group the resulting points into four clusters.
3. Finally, the related families were compared in the positive SC of their members: the family with common degrees of consanguineous relationships (group A, including individuals Nos. 1, 2, 3, 4, 5, 6, and 38), the genealogically and temporally parallel-related family with very close consanguinity (group B, the primogeniture branch of the previously mentioned family including individuals Nos. 7, 8, 9, 17, 18, 19, and 20), and the parallel and partially related family to the previous group with common degrees of consanguinity (group C, including individuals Nos. 31, 21, 33, 34, 35, 36, and 37). Differences were tested using the analysis of variance.

Finally, independently of the previous analyses, the anomalies were selected which permitted the capture of traits that supported the biological affinity of individuals and could therefore

be considered familial. Their selection was conditioned by a population frequency of less than 10% (e.g., Cvrček et al., 2018; Gemmerich-Pfister, 1999) based on the aforementioned reference samples, either incidence by individual or incidence by side, and simultaneously their occurrence in at least two biologically related individuals. For the chosen traits, the difference in their occurrence between the genealogically documented sample and the reference samples was tested using Fisher's exact test (Fisher, 1922). For multiple tests, p-values were adjusted according to the Benjamini–Hochberg false discovery rate method (BH-fdr) (Benjamini & Hochberg, 1995). At the same time, the differences were tested in the average frequency of chosen possible familial anomalies found in the genealogically documented sample and in the reference samples, both on individual and on side using the Wilcoxon signed-rank test (Siegel, 1956).

All calculations were performed in the R program (core set of packages; R Core Team, 2018) with a significance level of $\alpha = 0.05$. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

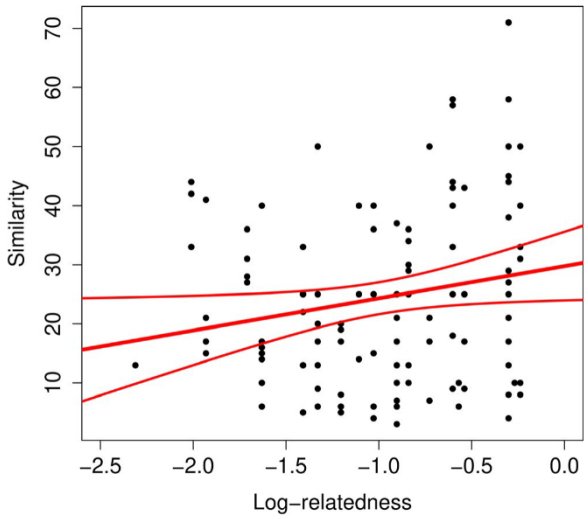


FIGURE 1 Relationship between the similarity coefficient (SC) of biologically related individuals (y-axis) and log-relatedness of individuals (x-axis). The red line represents the regression line. Note: log-relatedness disregards unrelated individuals

3 | RESULTS

3.1 | Similarity of biologically related individuals

The resulting SC values between all individuals based on all SDA according a numerical expression of agreement and disagreement are given in Supporting information No. 2 (Table S3). There is statistical significance between the degree of similarity and the biological distance of biologically related individuals (p-value = 0.024). That is, the greater the degree of biological relationship, the higher the incidence of shared SDAs (Figure 1).

The greatest degree of similarity in biologically related individuals is between father No. 19 and son No. 20 (SC = 71). A high degree of kinship was also found for example between father No. 1 and son No. 3 (SC = 44), mother No. 2 and sons Nos. 3 (SC = 25) and 38 (SC = 29), father No. 18 and son No. 19 (SC = 58), father No. 32 and daughter No. 34 (SC = 44) etc., as well as among siblings, for example, brothers Nos. 20 and 21 (SC = 50), brother No. 36 and sister No. 37 (SC = 45), or between grandparents and grandchildren, who in some cases are more similar than parents and children, for example, father No. 32 and daughter No. 34 (SC = 44) versus grandfather No. 32 and grandson No. 36 (SC = 57). At the same time, some cases were found in which first cousins are more similar than closely related individuals, for example, cousins Nos. 1 and 7 (SC = 25) versus father No. 1 and son No. 38 (SC = 4) or father No. 7 versus son No. 13 (SC = 10), and cousins Nos. 19 and 24 (SC = 21) versus mother No. 23 and daughter No. 24 (SC = 13). There is also a clear resemblance between some family members with a high degree of consanguinity, for example, father No. 7 and sons Nos. 9 (SC = 40) and 11 (SC = 50), brothers Nos. 9 and 11 (SC = 33) and 13 (SC = 31), or uncle No. 9 and nephew No. 19 (SC = 43).

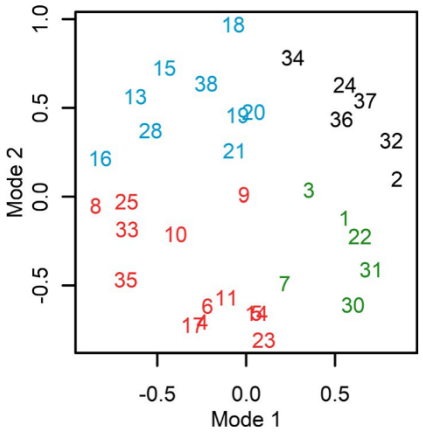


FIGURE 2 A scatter plot of the multidimensional scaling coordinates assigned to each individual by SMACOF (stress majorization algorithm). Colors indicate cluster assignment

The graph of MDS (Figure 2) reflects the documented family relationships well, and the distinct proximity of several closely biologically related individuals is apparent. The first cluster (marked in blue) includes father No. 18, son No. 19, and two grandsons Nos. 20 and 21, as well as male No. 13, who is the uncle of male No. 19 and half-nephew of female No. 28 (simultaneously, as she is half-grandaunt of male No. 19). In the second cluster (black), closely related members of one family are gathered: male No. 32, his daughter No. 34, his grandson No. 36, and granddaughter No. 37. At the same time, it is clear that the grandchildren are in a position between the father and his daughter, so in some cases, the grandchildren may be closer to

the grandparents than the children to their parents, as shown above. In the third cluster (red), there are two groups of close relatives: A) female No. 4, her son No. 5, and grandson No. 6 (again, the grandparent and grandchild are closer than the parent and child) and B) female No. 8 and her children Nos. 9, 11, 14, and 17, among which there is increased consanguinity. Finally, there is closeness between father No. 1 and son No. 3, first cousins Nos. 1 and 7, and male No. 30 and his aunt No. 31 in the last cluster (green).

The difference between the three related and parallel families based on the positive SC of their biologically related members (group A = 7 pairs, group B = 10 pairs, and group C = 8 pairs) is not statistically significant (p-value = 0.183), although it can be observed that their medians differ (Figure 3). Group A has the lowest median, group C the highest. Group A has the smallest variability, group B the largest.

3.2 | Anomalies shared by biologically related individuals

There are 17 anomalies shared by biologically related individuals in the sample, especially between close relatives such as parents and children, grandparents and grandchildren, siblings, or first cousins (Tables 1 and 2).

Besides the above described anomalies shared between closely related individuals, the increased incidence of congenital blocks of the cervical and thoracic vertebrae (anomalies Nos. 23–26) deserves special attention. However, the description of the occurrence of this anomaly is more difficult, so it is summarized in the following table (Table 2). In particular, there are first cousins Nos. 1 and 7 and their male descendants (Nos. 3, 9, 11, and 21). The second and third

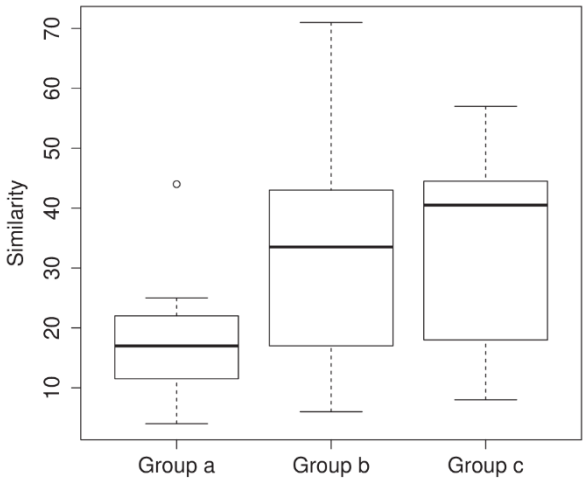


FIGURE 3 Variability in the degree of similarity for skeletal developmental anomalies in three related family branches from the sample: family with common degree of consanguinity (a), parallel family with very close consanguineous relationships (b), and family unrelated to the first family and half-related to the second family with a common degree of consanguinity (c)

cervical vertebrae are most often affected (70% of affected individuals), mostly their arch and facet joints on the left side (85.7%). Male No. 3 is placed between father No. 1 and mother No. 2 in the localization of a block. Next there is granduncle No. 22 and grandnephew No. 21, and female No. 31, half-sister of male No. 7, and their (half) nephew No. 30.

Based on the population frequency of the evaluated anomalies (see Supporting information No. 2, Table S4), 11 possible familial anomalies were found, four unilateral and seven bilateral (Table 3). According to the Wilcoxon signed-rank test, there was a statistically significant difference in the average frequency of all 11 possible familial anomalies between the genealogically documented sample and the reference samples, both by the individual (p-value = 0.001) and by the side (p-value = 0.016) (Figure 4). According to Fisher's exact test with Benjamini–Hochberg adjustment, there was a statistically significant difference in the occurrence of five anomalies between the genealogically documented sample and the reference samples. One of them, incomplete fusion of spinous process S1, is unilateral, and the other four are significant both by the individual and by the side: elongated styloid process, cervical block vertebrae (arch, facet joint), hamate hamulus aplasia, and anteater nose sign (Table 3, Figure 5). Although the cervical block vertebrae-body was not statistically significant compared to the reference sample, it can be assumed that this is only a different manifestation of the same condition as in the arch and facet joints; a division into two areas merely gave us an opportunity to pay more attention to the similarities or differences between individuals in calculation of their similarity. If we consider them together, their occurrence is also statistically significant in the genealogically documented sample compared to the reference sample (p-value = 0.001).

Finally, there are 28 cases of the individual occurrences of 17 different SDA, which have no connection to the documented relationships between individuals (Table 4).

4 | DISCUSSION

4.1 | 1. Similarity of biologically related individuals

The finding of a statistically significant relationship between biological distance and the degree of similarity of individuals in this study corresponds to the results of two previous analysis of nonmetric osteological traits and the maximum height and morphological patterns of the frontal sinus that were previously performed in the same sample (Cvrček et al., 2018, 2020). However, even in this study, it was shown that in some cases there may be a greater degree of similarity between grandparents and grandchildren or first cousins than between parents and children or siblings (Cvrček et al., 2018, 2020; Gemmerich-Pfister, 1999; Velemínský & Dobisíková, 2005). Nevertheless, in some cases the degree of similarity between individuals could be negatively affected by the incomplete preservation of the remains of some individuals, together with the very low

TABLE 1 Skeletal developmental anomalies shared by closely related individuals Nos. 1–38

Anomaly No.	Definition	Individuals	Relationships	Side
15	Elongated styloid process	No. 1	Father	Bilateral
		No. 3	Son	Bilateral
		No. 19	Father	Right side
		No. 20	Son	Right side
		No. 21	Son	Right side
		No. 22	Father	Bilateral
		No. 24	Daughter	Bilateral
		No. 36	Brother	Bilateral
		No. 37	Sister	Bilateral
21	Bifid spinous process S1	No. 2	Mother	–
		No. 38	Son	
		No. 30	Nephew	–
		No. 31	Aunt	
22	Incomplete fusion of spinous process S1	No. 1	First cousin of No. 7	–
		No. 9	Son of No. 7	
		No. 19	Nephew of No. 9	
		No. 20	Son of No. 19	
		No. 36	Half-grandnephew of No. 7	
32	Cranial shift at the C-T border	No. 32	Father	–
		No. 34	Daughter	
		No. 36	Grandson	
		No. 37	Granddaughter	
39	Caudal shift at the S-C border	No. 9	Uncle	–
		No. 19	Nephew	
		No. 20	Son of No. 19	
		No. 21	Son of No. 19	
		No. 38	Second cousin of No. 9	
		No. 28	Aunt	–
		No. 34	Niece	
42	Cervical ribs (C7)	No. 32	Father	Right side, class II
		No. 34	Daughter	Bilateral, class II
		No. 36	Grandson	Right side, class II
		No. 37	Granddaughter	Left side, class I
47	Hypoplasia of rib 12	No. 9	Granduncle	Bilateral
		No. 21	Grandnephew	Right side
		No. 22	Granduncle of No. 21	Right side
		No. 37	Related to Nos. 9 and 21	Left side
51	Manubriosternal fusion	No. 13	Half first cousins	–
		No. 34		
64	Hamate hamulus aplasia	No. 7	Father	Right side
		No. 11	Son	Right side (uncertain)
		No. 19	Grandson	Left side
		No. 20	Great-grandson	Right side

(Continues)

TABLE 1 (Continued)

Anomaly No.	Definition	Individuals	Relationships	Side
70	Incomplete acetabular synostosis	No. 18	Father	Bilateral (trace)
		No. 19	Son	Bilateral
		No. 21	Grandson	Right side (trace)
76	Anteater nose sign	No. 2	Mother	Left side
		No. 38	Son	Bilateral
		No. 18	Father	Bilateral
		No. 19	Son	Left side
		No. 20	Grandson	Bilateral
		No. 21	Grandson	Bilateral
81	Accessory navicular (partial)	No. 31	Mother	Right side
		No. 34	Daughter	Bilateral
		No. 37	Granddaughter	Bilateral
89	Foot symphalangism (fifth distal IP joint)	No. 20	Brothers	Bilateral
		No. 21		Bilateral

population frequency of most of the observed anomalies (e.g., Case, 2003; Case et al., 2017; Ríos et al., 2019), as shown by the frequent occurrence of a zero degree of similarity.

Although some studies suggest a possible effect of increased consanguinity on the incidence of skeletal anomalies and an increased degree of similarity between individuals (Ríos et al., 2015, 2019), this has not been shown in the present study. Pairs with a positive SC in the family with increased consanguinity did not show less data variability than parallel relatives with a normal degree of consanguinity. Nor was the median of their degree of similarity the largest, as might be expected given that inbreeding leads to reduced variability and to the strengthening of characteristics in the family, whether positive or negative (e.g., Álvarez & Ceballos, 2015; Habicht et al., 2015; Tayebi et al., 2010). However, this result may be due to the small number of pairs with positive SC in the families being compared, and again the preservation of the skeletal remains may also have played a role.

Calculation of a SC based on SDA was originally developed for nonmetric osteological traits (Cvrček et al., 2018). However, this study shows that this approach can also be applied to other variables for which presence or absence can be assessed.

4.2 | Anomalies shared by biologically related individuals

In total, the occurrence of 17 SDA was recorded in the genealogical documented sample, the presence of which support the documented family relationships of the individuals. Based on an SDA population frequency of less than 10% (Cvrček et al., 2018; Gemmerich-Pfister, 1999), 11 were selected as possible familial anomalies, which are present in at least two biologically related individuals. However, only five of them exhibited frequencies that were statistically significant compared to the reference frequencies, a result that is similar to the nonmetric osteological traits in this sample (Cvrček et al., 2018).

The first of these, an elongated styloid process, has previously been observed among close relatives, mostly in clinical practice (e.g., Morrison et al., 2012), because this anomaly may adversely affect surrounding organs and can have significant manifestations in the form of the so-called Eagle syndrome (Eagle, 1937). However, we have no reports of clinical manifestations in the individuals studied here (valid for individuals Nos. 3, 20, 21, 24, 36, and 37 who died in the second half of the 20th century, and for whom there are detailed health data and testimonies from their offspring and witnesses, including audio and video recordings in some cases), although we cannot rule out that they had such clinical manifestations but kept them secret from their doctors and families.

Secondly, a statistically significant incidence was observed in the cervical block vertebrae in their arch and/or facet joints, arch and/or facet joints and body together, respectively. The possible effect of biological kinship on the multiple occurrence of this anomaly is also known (e.g., Barnes, 2012; Titelbaum, 2020). The presence of even a single cervical block vertebrae is taken as an argument for Klippel–Feil syndrome (type II) (Barnes, 1994; Gunderson et al., 1967). However, at the same time, we have no relevant information about any of the possible clinical manifestations associated with this syndrome in the affected individuals. There are not even any visible abnormalities in their portrait photographs that clearly show the entire neck region. Further, the autopsy protocols of some affected individuals in the sample (males Nos. 9 and 21) do not indicate any organ changes (e.g., Gunderson et al., 1967; Thomsen et al., 1997). On the other hand, in the case of inbred male No. 9, in addition to the absence of cervical lordosis due to extensive malsegmentation of the cervical vertebrae, we also recorded incomplete fusion of spinous process S1, caudal shifting at the S-C border, hypoplasia of the rib 12, and finally total aplasia of left superior cornu of the thyroid cartilage. The

TABLE 2 Occurrence of congenital blocks between the cervical (C1–7) and thoracic (T1–12) vertebrae (anomalies Nos. 23–26) in 10 closely biologically related individuals (+ = anomaly present; - = anomaly absent).

Individual	Place	C1/2	C2/3	C3/4	C4/5	C5/6	C6/7	C7/T1	T2-T12
No. 1	Arch	– –	– +	– –	+ +	– –	– –	– –	– –
	Joints	– –	– +	– –	+ +	– –	– –	– –	–
	Body	–	–	–	–	–	+	–	–
No. 2	Arch	– –	– –	– –	– –	– –	– –	– –	+ + T2-5
	Joints	– –	– –	– –	– –	– –	– –	– –	
	Body	–	–	–	–	–	–	–	–
No. 3	Arch	– –	– –	– –	– –	– –	– –	– +	– –
	Joints	– –	– –	– –	– –	– –	– –	– +	– –
	Body	–	–	–	–	–	–	–	–
No. 7	Arch	– –	– +	– –	– –	– –	– –	– –	– –
	Joints	– –	– +	– –	– –	– –	– –	– –	– –
	Body	–	+	–	–	–	–	–	–
No. 9	Arch	– –	– +	– +	– +	– –	– –	– –	– –
	Joints	– –	+ +	+ +	– +	– –	– –	– –	– –
	Body	–	–	–	–	–	–	–	–
No. 11	Arch	– –	– –	– –	– –	– –	– –	– –	– –
	Joints	– –	– +	– –	– –	– –	– –	– –	– –
	Body	–	–	–	–	–	–	–	–
No. 21	Arch	– –	+ –	– –	– –	– –	– –	– –	– –
	Joints	– –	+ –	– –	– –	– –	– –	– –	– –
	Body	–	–	–	–	–	–	–	–
No. 22	Arch	– –	+ +	– –	– –	– –	– –	– –	– –
	Joints	– –	– +	– –	– –	– –	– –	– –	– –
	Body	–	–	–	–	–	–	–	–
No. 30	Arch	– –	– –	– –	– –	– –	– –	– –	– –
	Joints	– –	– –	– –	– –	– –	– –	– –	– –
	Body	–	–	–	–	–	+	–	–
No. 31	Arch	– –	– +	– –	– –	– –	– –	– –	– –
	Joints	– –	– +	– –	– –	– –	– –	– –	– –
	Body	–	+	–	–	–	–	–	–

associated changes in the spine, ribs and voice could be further manifestations of Klippel–Feil syndrome (e.g., Clark et al., 1998; Tracy et al., 2004). Similarly, hypoplasia of rib 12 was also found in males Nos. 21 (grandnephew of No. 9) and 22 (granduncle of No. 21) with a C2/C3 block, and bifid spinous process S1 was found in Nos. 30 and 31 with a C6/7 block, and C2/3 block respectively (half-aunt and half-cousin of No. 9, and aunt and nephew among themselves). Simultaneously, in inbred male No. 11 (brother of male No. 9) we cannot rule out a slight rotation with asymmetry of the axis in the block that includes the third cervical vertebra, as a slight indication of torticollis, another possible manifestation of Klippel–Feil syndrome (e.g., Clark et al., 1998; Giuffra et al., 2009), but significant postmortem damage of this block makes a full evaluation of the changes impossible. However, the structure and size of all fused vertebrae in the other individuals is anatomically normal.

The third significant anomaly is hamate hamulus aplasia. Its occurrence is rare, only a few cases having been reported to date, and based on references we do not yet know anything about its familial occurrence. This anomaly does not appear to have any negative clinical manifestations (Seeger et al., 1988).

The fourth of the significant anomalies, anteater nose sign, is described as a tubular elongation of the anterior process of the calcaneus (Chapman, 2007). The presence of this deviation is related to the presence of calcaneonavicular coalition as its classic radiographic finding (Richer & Pawley, 2016), which is the most common type of tarsal coalition (Case & Burnett, 2010). Since the matching lesions between the anterior calcaneus and navicular could not be verified (absence of navicular bone, or postmortem damage to bone margins) or indisputably found in all cases of anteater nose sign, we distinguished anteater nose sign from true coalition when recording data. Here, however, we deal with anteater nose sign together

TABLE 3 Possible familial anomalies found in the genealogically documented sample (present in at least two biologically related individuals) and their incidence compared to the incidence of these anomalies in the reference samples (reference frequency <10%), with a statistical comparison of trait frequencies in the samples using Fisher's exact test

		Genealogical documented sample						Reference samples						Fisher's exact test		
		Incidence on individual			Incidence on side			Incidence on individual			Incidence on side			Sample	Incidence on individual	Incidence on side
Anomaly No:	Definition	N	Inc.	%	N	Inc.	%	N	Inc.	%	N	Inc.	%	Sign	*p*-value	*p*-value
No. 15	Elongated styloid process	21	9	42.9	46	15	32.6	28	1	3.6	85	3	3.5	P	0.011*	0.007**
No. 21	Bifid spinous process S1	25	4	16.0	–	–	–	44	4	9.1	–	–	–	R	0.493	–
No. 22	Incomplete fusion of spinous process S1	25	5	20.0	–	–	–	44	0	0	–	–	–	R	0.017*	–
No. 23	Cervical block vertebrae (body)	24	4	16.7	–	–	–	75	3	4.0	–	–	–	C	0.089	–
No. 24	Cervical block vertebrae (arch, facet joint)	24	8	33.3	48	10	20.8	75	8	10.7	150	14	9.3	C	0.047*	0.037*
No. 47	Hypoplasia of rib 12	10	3	30.0	22	5	22.7	45	2	4.4	90	3	3.3	R	0.068	0.052
No. 51	Manubriosternal fusion	26	2	7.7	–	–	–	45	3	6.7	–	–	–	R	1.000	–
No. 64	Hamate hamulus aplasia	12	3	25.0	32	3	9.4	45	0	0	90	0	0	R	0.021*	0.018*
No. 70	Incomplete acetabular synostosis	25	3	12.0	54	5	9.3	96	4	4.2	193	6	3.1	P	0.188	0.154
No. 76	Anteater nose sign	21	6	28.6	48	10	20.8	45	1	2.2	90	1	1.1	R	0.017*	0.011*
No. 81	Accessory navicular	20	2	10.0	49	5	10.2	45	0	0	90	0	0	R	0.126	0.107

p-values were adjusted according to the Benjamini–Hochberg false discovery rate method (BH-fdr). N = number of evaluated individuals/sides, Inc. = incidence, % = frequency, P = Pachner collection, C = crypt of the Church of Our Lady Victorious, R = recent complete assembled skeletons.

*p-value < 0.05.

**p-value < 0.01.

with calcaneonavicular coalition, which better refers to the actual condition of this anomaly. Previous studies have shown a familial occurrence of this coalition, and it appears to be a suitable indicator of biological relatedness (Case et al., 2017; Leonard, 1974). The presence of anteater nose sign in female No. 2 (large form, unilateral) and in her son No. 38 (small, bilateral), who also had a typical nonosseous calcaneonavicular coalition on the right side, supports the connection of these anomalies. We also recorded an osseous calcaneonavicular coalition on the left side in female No. 17, who is the mother of male No. 19 and grandmother of males Nos. 20 and 21, who also had anteater nose sign. However, CT and X-ray examination showed that it was secondary ankylosis, a consequence of severe rheumatoid arthritis, which this woman suffered for a long time.

As for the last of the significant anomalies, incomplete fusion of spinous process S1 (an anomaly that manifests itself as an opening in the spinous process due to its incomplete fusion, due to only apical fusion), to the best of our knowledge there is no other study available in which its familial occurrence can be traced. On the other hand, in general it is a disorder of the fusion of the sacrum arches, and in the case of complete cleft neural arches of S1–S5, there are already records of familial occurrence over several generations (Vlček, 2000). Although we recorded only individual occurrences of complete clefts in our sample without biological relationships (S1 in male No. 21 and S2–S5 in male No. 35), there is a close family relationship between these cases and individuals with significant incomplete fusion of spinous process S1 (males Nos. 9, 19, 20, and 21, and males Nos. 35 and 36).

Although the occurrence of the remaining six anomalies with possible familial occurrence is not statistically significant compared to the reference samples (bifid spinous process S1, cervical block vertebrae (body), hypoplasia of rib 12, manubriosternal fusion, incomplete acetabular synostosis, and accessory navicular), they too can be considered good indicators of the biological affinity of individuals (Case et al., 2017; Cvrček et al., 2018). Nevertheless, the incomplete preservation of the material may have been more pronounced in these results, and it is thus possible that the occurrence of some anomalies could be higher among biologically related individuals than could be observed due to postmortem losses and damage (e.g., hamate hamulus aplasia).

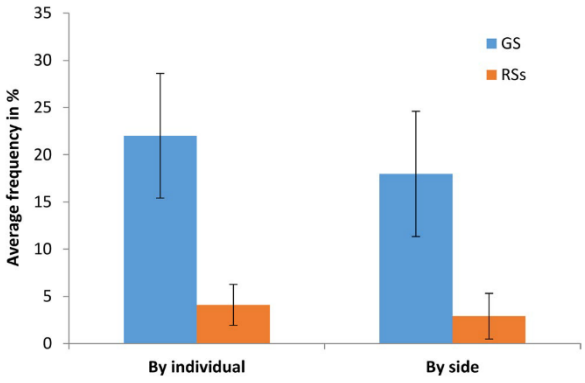


FIGURE 4 Average frequency of all possible familial anomalies (n = 11) found in the genealogically documented sample with a comparison to their incidence in the reference samples (reference frequency of SDA <10%) by the individual and by the side. GS = genealogically documented sample, RSs = reference samples

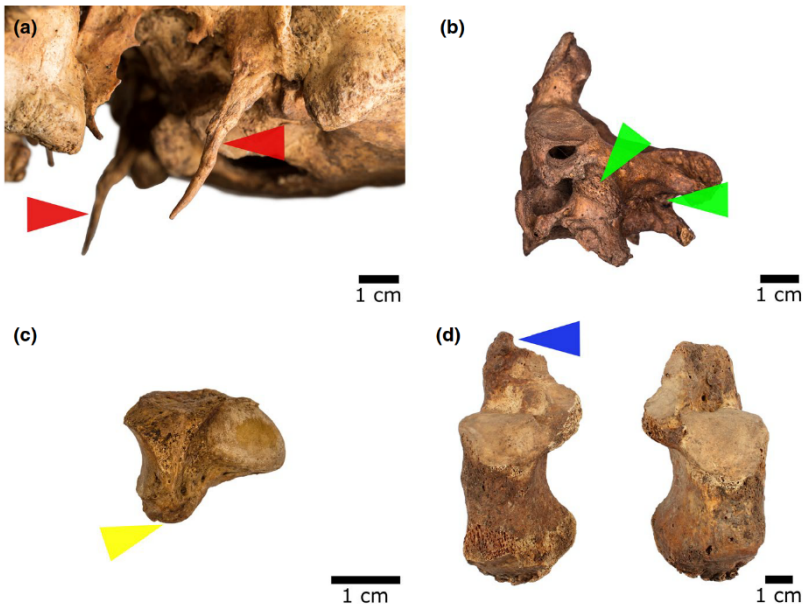


FIGURE 5 Bilateral skeletal developmental anomalies (arrows) with a statistically significant incidence in the genealogically documented sample both by the individual and by the side: elongated styloid process (a, No.1), cervical block vertebrae (arch, facet joint) (b, No. 31), hamate hamulus aplasia (c, No. 20), and anteater nose sign (d, No. 2)

TABLE 4 Skeletal developmental anomalies of all individuals Nos. 1–38 without occurrence between biologically related individuals.

Individuals	Anomaly No.	Definition
No. 2	1	Parietal thinning
No. 7	18	C2-L5: cleft neural arch (C2)
No. 8	50	Xiphoid process aperture
No. 10	7	Temporal suture agenesis (right side)
No. 15	19	C2-L5 spondylolysis (L2, left side)
	39	Caudal shift at the S-C border
	82	Os trigonum (bilateral)
	89	Foot symphalangism (left, second distal IP joint)
No. 16	28	Block of the lumbar vertebrae (arch, facet joint)
	39	Caudal shift at the S-C border
	49	Mesosternal aperture
No. 21	20	S1–S5: cleft neural arches (S1)
No. 23	38	Cranial shift at the S-C border
No. 24	42	Cervical ribs (C7, class II, left side)
	43	Lumbar ribs (right side)
No. 25	20	S1–S5: cleft neural arches (S2)
	37	Caudal shift at the L-S border
	39	Caudal shift at the S-C border
	43	Lumbar ribs (right side)
	50	Xiphoid process aperture
No. 31	49	Mesosternal aperture
No. 32	1	Parietal thinning
No. 34	41	Absence of vertebra (T1–11)
	43	Lumbar ribs (left side)
No. 35	20	S1–S5: cleft neural arches (S2–S5)
No. 38	19	C2-L5 spondylolysis (L5, bilateral)
	73	Segmented patella (left side)
	77	Calcaneonavicular coalition (right side)

Evaluation of SDA does not allow for a precise reconstruction of exact kinship relationships at the individual level, similarly to other osteological or dental features (e.g., Brown, 2015; Cameriere et al., 2008; Paul & Stojanowski, 2017). However, these results confirm that this approach can be used as a support tool for the detection of biological affinity, both in paleoanthropology and bioarchaeology (Case et al., 2017; Palancar et al., 2020; Ríos et al., 2015, 2019), and in forensic practice, for example, in the process of individual identification (Macaluso & Lucena, 2014; Verna et al., 2013, 2015).

5 | CONCLUSIONS

The results of this study show that the degree of similarity in the occurrence of SDA between individuals significantly corresponds to the degree of their biological distance. This means that the smaller their biological distance, the greater the degree of similarity between them. The greatest similarity was between close relatives such as parents and children, siblings, grandparents and grandchildren, or first cousins. At the same time, there were cases where the similarity between first cousins or between grandparents and grandchildren was greater than between siblings or parents and children. These findings have previously been demonstrated for nonmetric osteological traits, and the maximum height and morphological patterns of the frontal sinuses, in the same sample. However, the possible effect of increased consanguinity on the degree of similarity according to the evaluated anomalies was not demonstrated. Although the calculation of the SC was originally designed for nonmetric osteological traits, it turns out that it can be used for other variables, such as SDA. It is desirable to include in the analysis only pairs with a nonzero, positive degrees of similarity.

Seventeen anomalies shared by closely related individuals were found in the sample, which support the documented family relationships of the individuals. Based on their reference population frequencies, 11 of these were identified as possibly familial, as there was a statistically significant difference between their average frequency in the genealogically documented sample and the reference sample. However, among the individual anomalies of these 11, a statistically significant difference between frequency in the genealogically documented sample and in the reference samples was demonstrated in only five cases: elongated styloid process, cervical block vertebrae (arch, facet joint), hamate hamulus aplasia, anteater nose sign, and incomplete fusion of the S1 spinous process.

ACKNOWLEDGMENTS

The authors declare no competing interests. The study was financially supported by the Charles University Grant Agency (grant number GAUK 1276217), and the Ministry of Culture of the Czech Republic (DKRVO 2019/7.II.c, National Museum, 00023272). The authors greatly thank M. Jantač (Department of Anthropology, National Museum, Prague) for making radiographs and L. Vostrý (Czech University of Life Sciences Prague) for help with calculating the coefficients of relationships. We would also like to thank A. Millar for helping to revise the text and two reviewers for their useful comments that have contributed to improving the text.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Cvrček, J., Kuželka, V., Jor, T., Dupej, J., Horák, M., Naňka, O., et al (2021) Familial occurrence of skeletal developmental anomalies as a reflection of biological relationships in a genealogically documented Central European sample (19th to 20th centuries). *Journal of Anatomy*, 00, 1–13. https://doi.org/10.1111/joa.13499

International Journal of Paleopathology 34 (2021) 163–167

Contents lists available at ScienceDirect

International Journal of Paleopathology

journal homepage: www.elsevier.com/locate/ijpp

Kinship and the familial occurrence of skeletal developmental anomalies in the noble Swéerts-Sporck family (Bohemia, 17th to 20th centuries)

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ARTICLE INFO

Keywords:
Similarity coefficient
Biological distance
Genealogically documented sample
Inbreeding
Aristocracy

ABSTRACT

Objective: To determine the degree of similarity of biologically related individuals according to the occurrence of skeletal developmental anomalies (SDA), to see whether these anomalies reflect documented biological relationships.
Material and methods: The sample consists of the skeletal remains of seven members of the noble Swéerts-Sporck family from the 17th-20th centuries. Eighty-nine SDA were examined using morphological assessment, X-ray and CT. The degree of similarity was calculated using a similarity coefficient (Cvrček et al., 2018).
Results: There were three shared SDA in the sample (cranial shift at the C-T border, cervical ribs, hypoplasia of rib 12), and another fifteen individual SDA were reported. The degree of similarity between individuals supports their documented relationships. The greatest similarity was found in closely related individuals such as father/son or siblings, and the least between unrelated individuals.
Conclusions: SDA can be used as a supportive tool for detecting family relationships. The results correspond to the conclusions of earlier analyses of non-metric traits and frontal sinuses in the same sample: the smaller the biological distance between individuals, the greater the degree of their similarity.
Significance: Using unique human skeletal collections, this communication contributes to the expansion of knowledge about the familial occurrence of SDA.
Limitations: The small number of individuals limits the use of statistical approaches.
Suggestions for further research: The results call for research on this topic using a larger sample with known genealogical data and the same approaches, to confirm our conclusions.

1. Introduction

Skeletal developmental anomalies (SDA) reflect the influence of various factors on intrauterine development (Barnes, 2012). However, in paleopathology and bioarchaeology, or from an evolutionary point of view, they have hitherto been recorded mostly as individual cases (e.g., Keenleyside, 2011; Redfern and Austin, 2020), while population studies remain few (Titelbaum, 2020). On the other hand, from the second half of the 19th century, their familial occurrence began to be described (e.g., Shepherd, 1893; Symmers, 1895). Finding the hereditary basis of many of these anomalies has been used in the detection of biologically related individuals (e.g., Case et al., 2017; Tur et al., 2019). Identical or similar features have been found especially between close relatives, such as fathers and sons, mothers and daughters, siblings, or first cousins, with supporting documented kinship (e.g., Giuffra and Fornaciari, 2013; Thurzo and Beňuš, 2003; Vlček, 2000). Therefore, SDA are also monitored with regard to historically significant personalities, such as aristocrats and rulers.

The aim of this contribution is to show the degree of similarity between members of the noble Swéerts-Sporck family on the basis of SDA and to compare the results with studies of the occurrence of skeletal non-metric traits (NMT) and frontal sinus anatomy in the same samples. Previous analysis of NMT found the greatest similarity between father and son, and between first cousins of the same and opposite sex. In contrast, the similarity between siblings is not as obvious as might be expected. The least similarity was found between women who married

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https://doi.org/10.1016/j.ijpp.2021.07.005
Received 18 January 2021; Received in revised form 25 May 2021; Accepted 12 July 2021
Available online 20 July 2021
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into a family.

Cranial traits have been shown to reflect kinship relationships better than postcranial traits (Velemínský and Dobisíková, 2005). A positive relationship between relatedness and similarity was noted for the unadjusted measured dimensions of frontal sinuses (total volume and surface, and maximum height and width). Sex differences in the size of the sinuses between individuals were not pronounced, and in contrast, a relationship between relatedness and similarity was not found for standardized data adjusted to size. This suggests that genetics affects the size of the frontal sinuses. Likewise, there was no positive relationship between relatedness and similarity according to morphological patterns of the frontal sinuses. However, this could be due to the small sample size (Cvrček et al., 2020). The following questions were posed: Is there a positive relationship between similarity in anomalies and the biological distance of individuals within members of Sporck and Swéerts-Sporck noble family? Does the degree of similarity of individuals reflect their documented relationships?

2. Material and methods

2.1. Material

In the early 1990s, osteobiographical investigation of the skeletal remains of nine members of Sporck and Swéerts-Sporck noble family dating to the 17th-20th centuries was performed due to repairs of their coffins in Church of the Holy Trinity, Kuks hospital (Hradec Králové region, Czech Republic) (Dobisíková et al., 1999; Velemínský and Dobisíková, 2005). For this study, the data from the skeletal remains of seven individuals (Nos. 3–9) were available:

3) Francisca Elizabeth Apollonia, Countess Sporck, née Baroness (i.e. Freifrau) from Reist and Swéerts (Jan. 11th 1667 – Apr. 22nd 1726)
4) Cavalry Captain Joseph Philipp Friedrich Karl, Count Swéerts-Sporck, Baron (i.e. Freiherr) from Reist, Laaken and Blauenthurn (Feb. 10th 1809 – Nov. 12th 1848)
5) Barbara, Countness von Rothkirch and Panthen, née Countess Swéerts-Sporck, Baroness from Reist, Laaken and Blauenthurn (Feb. 9th 1810 – Oct. 7th 1873)
6) Joseph, Count Swéerts-Sporck, Baron from Reist, Laaken and Blauenthurn (Jul. 7th 1788 – Feb. 11th 1855)
7) Moritz Gustav, Count Swéerts-Sporck, Baron from Reist, Laaken and Blauenthurn (May 12th 1821 – Nov. 21 st 1882)
8) Céline Pauline, Countess Swéerts-Sporck, Baroness from Reist, Laaken and Blauenthurn, née de Noblée (Aug. 10th 1832 – Jul. 13th 1914)
9) Philipp Benitus, Count Swéerts-Sporck, Baron from Reist, Laaken and Blauenthurn (Feb. 9th 1810 – July 4th 1817)

The family tree shows their biological relationships (Fig. 1). Individuals Nos. 1 and 2, General Johann Nepomuk, Count Sporck (January 6, 1600 – August 6, 1679) and his wife Eleonora Maria Catharine, Countess Sporck, née von Fineck (February 10, 1639 – September 3, 1674), could not be included in this study because the postcranial skeleton of individual No. 1 was poorly preserved, and only the skull of individual No. 2 was preserved (Dobisíková et al., 1999; Velemínský and Dobisíková, 2005).

There is a consanguineous marriage in generation No. 3: the mother of the groom and the father of the bride were siblings, and the father of the groom and the mother of the bride were half-siblings (Dobřenský z Dobřenic, 1841 – 1919; Wurzbach, 1880).

Genealogical data came from State Regional Archives in Zámrsk, Litoměřice, Prague, and Plzeň, the Prague City Archive, the National Museum Archive in Prague, the parish registers of Vienna, and the Central State Historical Archives of Ukraine-City of Lviv. The identities of the individuals were confirmed by the inscribed plates found in the coffins. DNA analysis and analysis of blood groups supported the documented relationships between the individuals (Mazura et al., 1997).

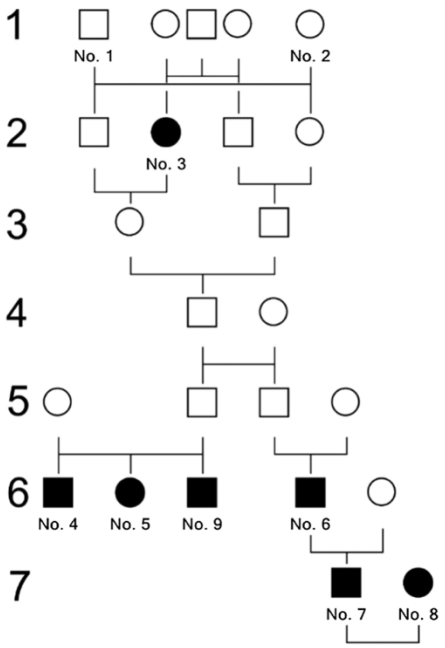


Fig. 1. The family tree of the studied individuals according to Dobřenský z Dobřenic (1841 – 1919) and Wurzbach (1880).

2.2. Methods

Eighty-nine SDA were examined in the skeletons (Barnes, 2012; Chapman, 2007; Velemínský, 2000) (see Supplemental Table A1). The morphological evaluation was verified using computed radiography (CR) at the Department of Anthropology, National Museum, Prague. The presence (+) or absence (-) of each anomaly was recorded. If a bone was damaged or missing at the site, the anomaly could not be evaluated (0). The degree of similarity of individuals was calculated using a similarity coefficient (SC) (Cvrček et al., 2018; Supplemental Table A2). If there were no common anomalies occurring in a pair of individuals, their SC was defined as 0. The values of SC and the logarithm of coefficients of relationship (r) between individuals (Falconer and Mackay, 1996; VanRaden, 1992) were used for graphic expression of the relationship between the degree of similarity and the biological distance, for biologically related individuals only. Use of a logarithm (logR) eliminates entirely unrelated individuals; their coefficient of relationship is always zero, and thus the logarithm is not defined.

3. Results

The greatest degree of similarity was between siblings Nos. 4 and 5, father and son Nos. 6 and 7, and between male No 4 and male No. 7 (No. 7 is the son of No. 6, who is first cousin of No. 4). Within the sample there was also a high degree of similarity between female No. 3 and her great-great-grandson No. 4. The least degree of similarity was between biologically related members of the family and unrelated female No. 8 (Table 1). The skeleton of individual No. 9 was not included in the calculations because it is an immature individual whose remains are very poorly preserved.

The positive relationship between similarity and biological distance is visible (coefficient of determination $R^2 = 0.4$). This means that the smaller the biological distance, the greater the degree of similarity

Table 1
The values of the similarity coefficient (SC) of individuals Nos. 3-8 by skeletal developmental anomalies.

Individual #	3	4	5	6	7	8
3	–	22	23	6	4	3
4	22	–	36	13	25	0
5	23	36	–	6	8	4
6	6	13	6	–	25	0
7	4	25	8	25	–	0
8	3	0	4	0	0	–

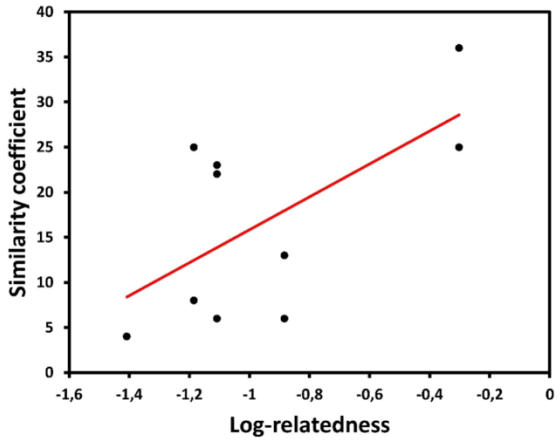


Fig. 2. Relationship between the similarity coefficient (SC) of biologically related individuals Nos. 3-7 (y-axis, black points) and log-relatedness of individuals Nos. 3-7 (x-axis). The red line represents the regression line. Note: log-relatedness disregards unrelated individual No. 8.

(Fig. 2).

There were three SDA found that were shared by relatives (Table 2) and another fifteen individual SDA in the sample (Table 3, Figs. 3 and 4). These were mostly changes in the morphology of the vertebrae and ribs.

4. Discussion

Overall, the degree of similarity between Swéerts-Sporck family members based on SDA generally corresponds to the degree of similarity found using skeletal non-metric traits (Velemínský and Dobisíková, 2005) and unadjusted measured dimensions of the frontal sinuses (Cvrček et al., 2020). This means that the greater the degree of relatedness between individuals, the greater the degree of their similarity. The most similar are siblings, father and son, and first male cousins. On the other hand, a distinct degree of similarity between more distant individuals, such as great-great-grandmother/great-great-grandson or son of the first cousin/first cousin of the father was also apparent. The least similarity between individuals in this sample was between female No. 8, who is biologically unrelated to all the others (Velemínský and Dobisíková, 2005).

It also turned out that regardless of the evaluated osteological features and methodological approaches, e.g., the Essen-Möller formula (Egeland and Mostad, 2002; Velemínský and Dobisíková, 2005), the frontal sinus asymmetry/similarity formula (Cvrček et al., 2020; Szilvássy, 1974), or the similarity coefficient, the results are comparable in this sample. The similarity coefficient used in this study was originally designed for the analysis of non-metric traits (Cvrček et al., 2018); the results showed that it can also be used for other osteological features in assessing their presence or absence.

As in previous studies of other familial osteological samples (e.g., Giuffra and Fornaciari, 2013; Thurzo and Beňuš, 2003; Vlček, 2000), several anomalies were found that could be considered familial. The familial occurrence of the cervical ribs, which is one of the manifestations within the cranial vertebral shift of the cervical-thoracic border (Barnes, 2012), has been confirmed previously based on clinical studies (e.g., Boles et al., 1982). Twelfth rib hypoplasia is also a manifestation of cranial border shifting at the thoracic-lumbar border (Barnes, 2012). In this context, it should be noted that cases of complete cranial shifts at the thoracic-lumbar border and at the sacral-caudal border, and caudal shift at the sacral-caudal border, were also found within the groups of related individuals; vertebral shifts and their manifestations can thus be considered a familial feature of the Swéerts-Sporck family.

In the case of NMT, cranial traits appear to reflect biological relationships better than postcranial traits (Velemínský and Dobisíková, 2005; Cvrček et al., 2018). However, according to previous studies (Thurzo and Beňuš, 2003; Vlček, 2000) and our results, the opposite is true for SDA. Postcranial SDAs are more numerous, appear to be more common, and reflect family relationships better than cranial ones - but the same limits apply to postcranial SDA as to the evaluation of postcranial NMT. Preservation of postcranial features generally appears to be worse than cranial features (e.g., Spence, 1996; Cvrček et al., 2018), and it is more likely that non-genetic factors may be involved in their origin (e.g., Kennedy, 1989; Capasso et al., 1999).

Although close consanguineous marriage is documented in generation No. 3, it is not possible to reliably prove whether the occurrence of SDA in the sample is influenced by the inbred relationships in this family (Merbs, 2004; Trinkaus, 2018; Ríos et al., 2019). On the one hand, the possibility of reduced variability of individuals due to inbreeding (e.g., Habicht et al., 2015) exists here despite the distance (generation Nos. 6 and 7) of the examined individuals from the marriage. Even between

Table 2
Skeletal developmental anomalies shared by closely related individuals.

Anomaly No.	Description	Individual	Side
32	Cranial shift at the C-T border	No. 3 No. 4 No. 5	–
42	Cervical ribs (C7)	No. 3 No. 4 No. 5	Left side (class IV., large) Both sides (class IV., small) Both sides (class III., small, incomplete)
47	Hypoplasia of rib 12	No. 4 No. 6 No. 7	Both sides Right side Both sides

Table 3
Individually occurring skeletal developmental anomalies of individuals Nos. 3-9.

Individual	Anomaly No.	Description
No. 3	1	Parietal thinning (trace)
	34	Cranial shift at the T-L border
	36	Cranial shift at the L-S border
	38	Cranial shift at the S-C border
No. 4	21	Bifid spinous process S1
	39	Caudal shift at the S-C border
No. 5	41	Absence of vertebra (the individual has only 11 thoracic vertebrae)
	43	Lumbar rib (left side, incomplete cranial shift at the T-L border)
	89	Foot symphalangism (fifth distal interphalangeal joint, left side)
No. 6	40	Extra vertebral segment (accessory vertebra)
	79	Tarsal-metatarsal coalition: MT3-cuneiform (incomplete, osseous, bilateral)
No. 7	–	None
No. 8	22	Incomplete fusion spinous process S1 (foramen)
	51	Manubriosternal fusion
No. 9	19	Spondylolysis (left side of the upper vertebra)
	26	Congenital block of two thoracic vertebrae

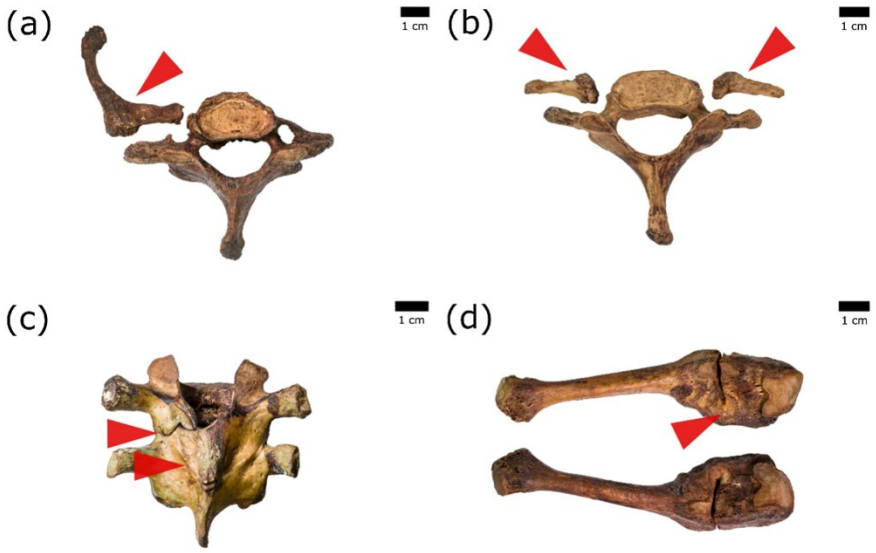


Fig. 3. Examples of skeletal developmental anomalies in the Swéerts-Sporck family (arrows): cervical ribs (C7) in No. 3 (a) and No. 4 (b); solitary congenital block of two thoracic vertebrae with spondylolysis in No. 9 (c); tarsal-metatarsal coalition: MT3-cuneiform in No. 6 (d).

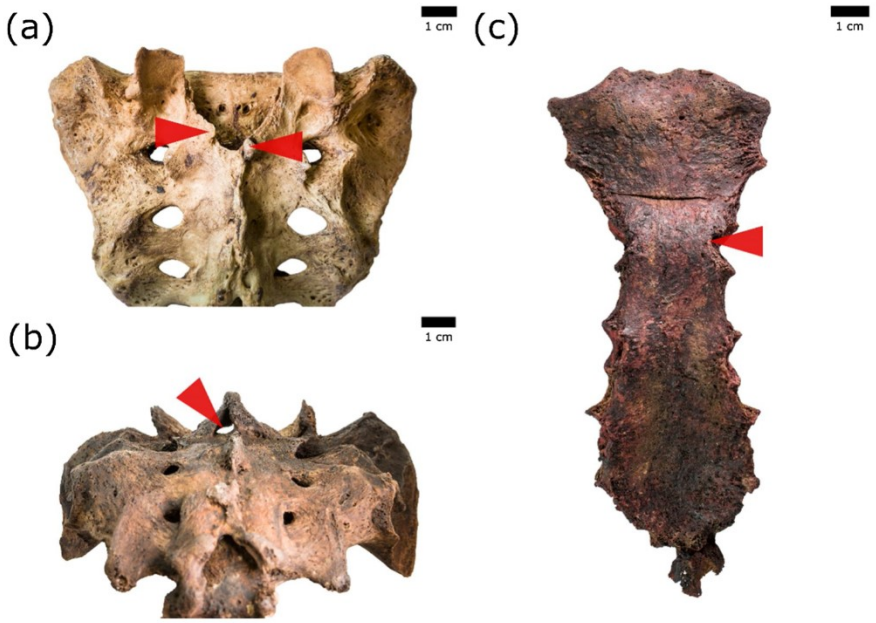


Fig. 4. Examples of skeletal developmental anomalies in the Swéerts-Sporck family (arrows): bifid spinous process S1 in No. 4 (a); incomplete fusion of spinous process S1 (foramen) in No. 8 (b); manubriosternal fusion in No. 8 (c).

some individuals from generations Nos. 6 and 7 (Fig. 1), this marriage is evident in the slightly increased values of their coefficient of relationship (Cvrček et al., 2020). On the other hand, the number of possible familial SDA found among related individuals is relatively low. The fact remains, though, that consanguineous marriages did not occur repeatedly in the family, and in such circumstances, with the increasing number of generations, the degree of consanguinity returns to normal (e.g., VanRaden, 1992; Cvrček et al., 2020). However, for more detailed results, it would be necessary to study the remains of other family members whose absence creates a gap of several generations between individuals in this study. Only in this way would it be possible to assess whether the number of anomalies occurring in a family with increasing generations has decreased since the time of the consanguineous marriage, or whether it remains approximately the same. Unfortunately, extending research to missing family members is not possible, as the burial places of some members of the family has either not been preserved (e.g., generation No. 3 in Fig. 1) or remains unknown (e.g., generation No. 5 in Fig. 1).

Similar to the aforementioned studies, the relative limitation of this study is the small number of individuals. However, the use of SDA (or other skeletal features) for verifying biological relationships is of utmost importance in numerically small groups in which individuals with

biological affinity are more likely to be detected than in larger samples (Case et al., 2017), particularly if biological relationships are also indicated, for example, by historical or archaeological contexts (e.g., Meyer et al., 2012).

5. Conclusions

The occurrence of the evaluated skeletal developmental anomalies corresponds to the documented family relationships. There is a positive relationship between the degree of biological distance and the degree of morphological similarity between individuals. This result is consistent with previous studies on the degree of morphological similarity of members of the Swéerts-Sporck noble family based on non-metric traits and frontal sinuses. It is possible to use skeletal developmental anomalies as a supportive tool for detecting family relationships. However, a study based on a larger sample is needed to confirm these conclusions.

Funding

Charles University Grant Agency (Grant Number: GAUK 1276217); Ministry of Culture of the Czech Republic (DKRVO 2019-2023/7.II.c, National Museum, 00023272). Funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report, and in the decision to submit the article for publication.

Declaration of Competing Interest

None.

Acknowledgements

The authors would like to thank František Fortin (The Czech Genealogy and Heraldry Society in Prague); Milena Běličová (National Museum Archive, Prague) and Petr Mareš (National Archives, Prague, Czech Republic) for their help with the genealogical research; Marek Jantač (Department of Anthropology, National Museum, Prague) for the X-ray documentation; Jakub Štěpán for help with photodocumentation; and Luboš Vostrý (Czech University of Life Sciences Prague) for help with calculating the coefficients of relationships. They would also like to thank Alastair Millar for helping to revise the text. They would like to thank two reviewers for their useful comments that have contributed to improving the text.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijpp.2021.07.005.

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International Journal of Paleopathology 30 (2020) 35–46

Contents lists available at ScienceDirect

International Journal of Paleopathology

journal homepage: www.elsevier.com/locate/ijpp

Multiple occurrence of premature polyarticular osteoarthritis in an early medieval Bohemian cemetery (Prague, Czech Republic)

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ARTICLE INFO

Keywords:
Skeletal dysplasia
Multiple epiphyseal dysplasia
Type II collagenopathies
Hip dysplasia
Middle Ages

ABSTRACT

Objectives: To highlight conditions that may cause early-onset degenerative joint disease, and to assess the possible impact of such diseases upon everyday life.
Material: Four adults aged under 50 years from a medieval skeletal collection of Prague (Czechia).
Methods: Visual, osteometric, X-ray, and histological examinations, stable isotope analysis of bone collagen.
Results: All four individuals showed multiple symmetrical degenerative changes, affecting the majority of joints of the postcranial skeleton. Associated dysplastic deformities were observed in all individuals, including bilateral hip dysplasia (n = 1), flattening of the femoral condyles (n = 3), and substantial deformation of the elbows (n = 3). The diet of the affected individuals differed from the contemporary population sample.
Conclusions: We propose the diagnosis of a mild form of skeletal dysplasia in these four individuals, with multiple epiphyseal dysplasia or type-II collagenopathy linked to premature osteoarthritis as the most probable causes.
Significance: Combining the skeletal findings with information from the medical literature, this paper defines several characteristic traits which may assist with the diagnosis of skeletal dysplasia in the archaeological record.
Limitations: As no genetic analysis was performed to confirm the possible kinship of the individuals, it is not possible to definitively assess whether the individuals suffered from the same hereditary condition or from different forms of skeletal dysplasia.
Suggestions for further research: Further studies on premature osteoarthritis in archaeological skeletal series are needed to correct the underrepresentation of these mild forms of dysplasia in past populations.

1. Introduction

Osteoarthritis (OA) is the most common joint pathology in human populations across time and space. It is characterized by marginal proliferation of new bone (marginal osteophytosis or "lipping"), porosity, eburnation and formation of new bone on the joint surface. Radiologically, subchondral sclerosis and cystic degeneration of subchondral bone are observed (Buikstra and Ubelaker, 1994; Ortner, 2003; Waldron, 2009).

According to a number of studies from both clinical and bio-archaeological spheres, age-related factors are the drivers of most of the changes associated with OA (e.g. Calce et al., 2018; Plomp et al., 2015; Sowers, 2001). However, the aetiology of OA is complex, with a number of factors including sex, hormones, nutrition, genetics,

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https://doi.org/10.1016/j.ijpp.2020.04.004
Received 29 May 2019; Received in revised form 6 April 2020; Accepted 10 April 2020
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anatomy, muscle weakness, obesity, and physical activity affecting both disease onset and progression (Burt et al., 2013; Busija et al., 2010; Calce et al., 2018; Domett et al., 2017; Plomp et al., 2015; Sowers, 2001; Waldron, 2009; Weiss and Jurmain, 2007). The prevalence of moderate or severe OA associated with bone changes at large appendicular joints is, however, low before the 5th decade of life (Jordan et al., 2009; Ortner, 2003).

Severe OA occurring earlier in life is thus usually linked with pre-existing abnormalities in affected joints. Premature (secondary) OA may occur locally (monoarticular or pauciarticular OA) in joints affected by factors such as fractures, osteomyelitis, Paget's disease or Legg-Calvé-Perthes (LCP) disease (Ortner, 2003; Waldron, 2009), or as a result of abnormal mechanical stress caused by repetitive activities (Busija et al., 2010; Weiss and Jurmain, 2007). The polyarticular form of premature OA is a rarer occurrence. It is most commonly observed as a secondary feature of systemic conditions affecting a higher number of joints such as erosive arthropathies or metabolic disorders (Ortner, 2003; Solomon, 2001; Waldron, 2009). In addition, gene mutations reducing the functional levels of either collagen fibers or collagen matrix protein, or altering key interactions in their assembly and function, can compromise the biomechanics of joint cartilage and thus lead to development of generalized early-onset OA (Briggs and Chapman, 2002; Chapman et al., 2003; Hildebrand, 2015; Jurgens et al., 2015; Kannu et al., 2010). There are examples of polyarticular hereditary premature OA affecting whole families (Kannu et al., 2010; Mier et al., 2001), but also large portions of geographically- or culturally-isolated populations representing genetically-inbred groups of people are known from clinical practice (Solomon, 2001).

Although Ortner (2003) stressed the significance of hereditary early-onset OA in bioarchaeological studies, cases of polyarticular premature OA are extremely rare in the paleopathological literature. To the best of our knowledge, all reports published so far concern isolated cases, where OA is often linked to other more prominent skeletal pathologies (Domett et al., 2017; Kozieradzka-Ogunmakin, 2011). Typically, generalized OA (regardless of the age of the individual) is uncommon in skeletal assemblages (Waldron, 2009).

In the early medieval skeletal series from the burial site of Milady Horákové Street in Prague, severe osteoarthritic changes non-accordant with age-at-death were observed in multiple joints of four adults, whose skeletal ages ranged from 18–24 to 30–49 years. Through the use of differential diagnosis for these four cases, we aim to address rare systemic conditions that may cause polyarticular premature OA, and call attention to skeletal traits that are linked to these conditions. We additionally discuss the possible consequences of these severe disabling conditions by reconstructing diet of the affected individuals, thus uncovering one of the most important indicators of the quality of everyday life.

2. The Milady Horákové site and the skeletal material

Between 2011 and 2013, an early medieval burial site was uncovered in Prague – Milady Horákové Street, a site close to Prague castle. The area of the burial site was almost fully excavated, revealing 84 graves, organized in regular rows, and containing the remains of 89 individuals (Fig. 1). According to characteristics of the jewelry and ceramics, the burial site was in use from the end of the 9th until the first half of the 10th centuries (Tomková, 2013). Anthropological examination revealed some specific features of the population (see Methods below). First, the majority of remains were those of subadult individuals (n = 51). Within the adults, six were estimated to be males, while 25 were classified as females (in seven cases, sex could not be estimated). A high prevalence of pathological conditions and dental anomalies was found in this population (Stránská, 2012a). This demographic and palaeopathological profile suggests that most of the males of productive age and in good physical condition were buried elsewhere. This might result from some specific function of adult males in relation the nearby Prague castle, while only those who were too young, ill, or weak remained to be buried at Milady Horákové (Kaupová et al., 2019). An analogous situation has been observed at other early medieval burial sites in Bohemia, but rarely so marked as in this case (Frolík, 2013; Stránská, 2012b).

The graves of the four individuals reported in this paper were scattered across the whole burial site (Fig. 1) and did not show any deviation from the usual burial rite. They all showed a west-east orientation. With the exception of G.29, who was buried in a plain pit of relatively straight dimensions, the other three graves probably had internal construction of wood and/or stones. Only G.74 contained simple grave goods represented by a knife and a ceramic pot (Tomková, 2013). Three of the skeletons were found intact, while in the case of G.29, later terrain modification had resulted in the loss of bones of both legs and feet. With this exception, skeletal material was satisfactorily preserved (Fig. 2). However, substantial fragmentation affected the facial parts of skulls, vertebrae, and ribs, which limited the collection of osteometric data.

3. Methods

Sex was estimated on the basis of morphologic and morphometric evaluation of the innominate (Brůžek, 2002; Brůžek et al., 2017). Age-at-death estimation was based on assessment of skeletal maturation (Belcastro et al., 2019; Ferembach et al., 1980; Scheuer and Black, 2000), as well as morphological changes of the pubic symphysis and auricular surface (Schmitt, 2005, 2008). However, as there is a substantial lack of systematic research on the potential effect of skeletal pathologies on osseous age-at-death indicators, these methods should be employed with caution in palaeopathological research (Bertrand et al., 2016). For this reason, the TCA (Tooth Cementum Annulation) method, based on the counting of annual increments of acellular cementum on the roots of monoradicular teeth (Colard et al., 2018; Wittwer-Backofen et al., 2004), was applied as an independent tool for age estimation. The first upper incisor was used in G.29 and G.74, while an upper canine was sampled in G.47 and G.56. An error range of ± 5 years was assumed for the final age estimation when using the TCA method (Rebay-Salisbury et al., 2018).

Skeletons were subject to a detailed macroscopic observation for pathological lesions with the help of a magnifying glass. The location of each bone change was recorded using skeletal diagrams, and its morphology was described, relying on standard paleopathological terminology.

X-ray examination was performed using computed radiography (CR) at the Department of Anthropology, National Museum, Prague. Limb bones were captured in anteroposterior and lateral views, while vertebrae were additionally captured in axial view. Bones were placed directly on the CR cassette, so the geometric magnification of the resulting images is negligible. The standard distance between focal point and CR cassette was 1.5 m, with tube voltages ranging between 48–55 kw and beam intensity between 8 and 20 mA.

Histological sections were prepared from two bones per individual (left capitate and left patella for G.29, right capitate and left patella for G.47, right lunate and left patella for G.56, and right capitate and right patella for G.74). Three samples were taken from each bone, with 10–12 slices per sample. Histological preparation and analysis were carried out at the Department of Histology and Embryology, Medical Faculty in Pilsen, Charles University. The collected tissue samples were fixed, then embedded in Epon blocks. The semithin sections were cut on an ultramicrotome using glass knives. Sections cut at 2–3 μm were floated in water and transferred to a drop of water on a clean microscope slide, and the slide with floating sections was then placed on a hot-plate pre-warmed to 60 °C to adhere to the slide. The dried sections were then stained by dropping filtered toluidine blue onto the sections. After staining, slides were carefully rinsed with distilled water, dried and mounted in solacrylate. The semi-thin sections were examined

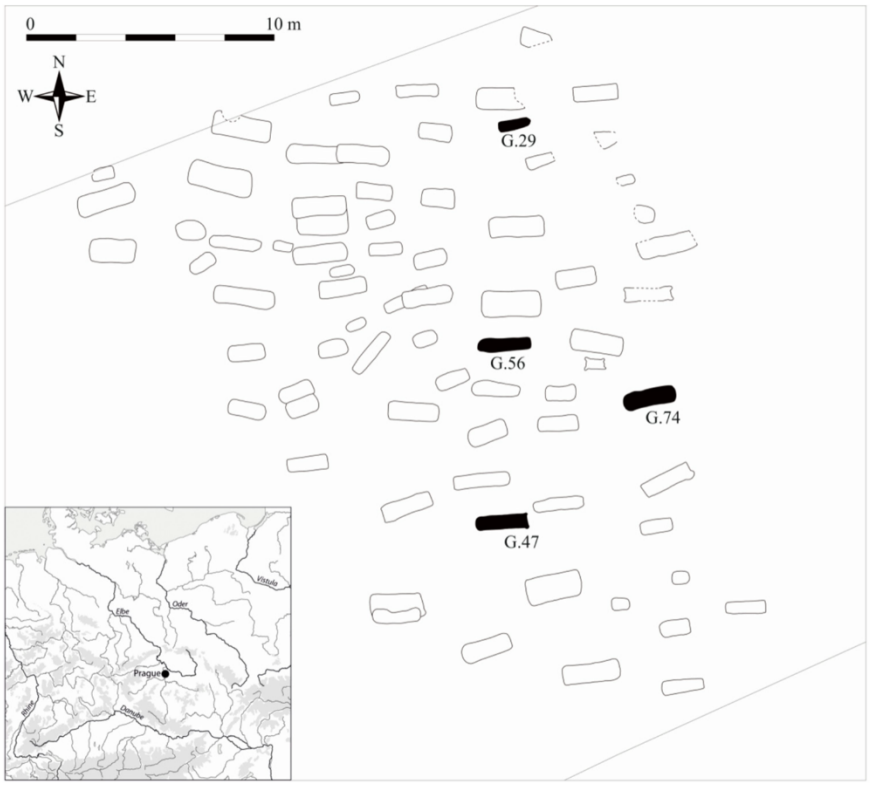


Fig. 1. The geographical location of Prague in the area of Central Europe and the position of the graves of affected individuals within the cemetery area.

under an Olympus BX 51 light microscope (Olympus, Tokyo, Japan).

Postcranial measurements were taken to estimate stature and body proportion. We used standard measurements as defined by Martin and Saller (1957) and Bräuer (1988). Maximal lengths of humerus (H1), radius (R1), ulna (U1), femur (F1) and tibia (T1a) were taken and their ratios (F1/H1, T1a/R1, R1/H1 and T1a/F1) were calculated to describe potential disproportionality of the limbs and their parts. Next, F9, F10, T8a and T9a were measured, and the platymeric (F10/F9) and platycnemic (T9a/T8a) indexes were calculated. For the spine, metrical analysis of vertebral shape was performed to uncover potential platyspondyly (online appendix; Schiess and Haeusler, 2013).

Non-pathological individuals from the Milady Horákové burial site, as well as from other early medieval Prague (Střešovice, Lumbe Garden, Motol) and Czech burial sites (Žalov – Na Panenské, Libice, Lahovice), were measured to evaluate stature and body proportions of the four cases in the context of the contemporary population (n = 119). For each individual, percentile rank of each measurement within the whole dataset was calculated. Stature was estimated using the length of the femur according to the formula proposed by Sjøvold (1990).

The diet of the affected individuals was reconstructed with the help of stable isotope analysis of carbon and nitrogen as a part of a wider study focused on the dietary behavior of the population of early medieval Bohemia. For technical details on the stable isotope analysis see Kaupová et al. (2019).

4. Results

4.1. Macroscopic evaluation

Upon visual examination, all four individuals shared several common features. Notably, multiple severe degenerative lesions of the postcranial skeletons were observed, affecting the spine (zygapophyseal OA, marginal lipping of the vertebral bodies and endplate irregularities), large limb joints as well as small joints of the hand and foot, with the appendicular lesions being symmetrically distributed (Fig. 2). In contrast, the temporomandibular joints (TMJs) were free of lesions, and the costae and clavicles were either not affected or showed only slight remodeling. None of the cranial and postcranial bones exhibited gross abnormalities in shape and/or proportions, and no abnormal scoliotic curvature of the spine was observed.

The individual uncovered in the grave 29 was assessed to be male, based at the morphology of the surviving parts of both innominates (Fig. 2). He was aged 30–39 years at death, based on the complete maturation of the skeleton and state of the pubic symphysis, while the TCA method provided an age-at-death estimation of 41.8 ± 5 years (Table 1). The most severe osteoarthritic changes affected both shoulders, which in the other individuals were either non-pathological or only mildly affected. The distal humeral epiphyses appear deformed, and the articular surfaces are irregular (Fig. 3 and 4). The medial condyle of the right femur shows slight but apparent flattening (Fig. 5).

The male (according to the metrics of both innominates) buried in grave 47 was aged 30–49 years at death based on the maturation score of both pubic symphysis and auricular surface. The recently-fused sternal end of the clavicle with a still-visible epiphyseal line was noted as supplementary evidence of relatively young age (Belcastro et al., 2019), which is also supported by the TCA method (42.5 ± 5 years). This individual is visually the one for whom the pathological process appeared to be the most advanced among the four concerned individuals. Apart from diffuse osteoarthritic changes, deformities were observed in several joints: both elbows were severely deformed (Fig. 3 and 4), the morphology of both hip joints corresponded with bilateral hip dysplasia (Fig. 6), and the lateral femoral condyles were flattened bilaterally (Fig. 5). Additional bone changes observed on the skeleton include a small lytic lesion on the major tubercle of the right humerus

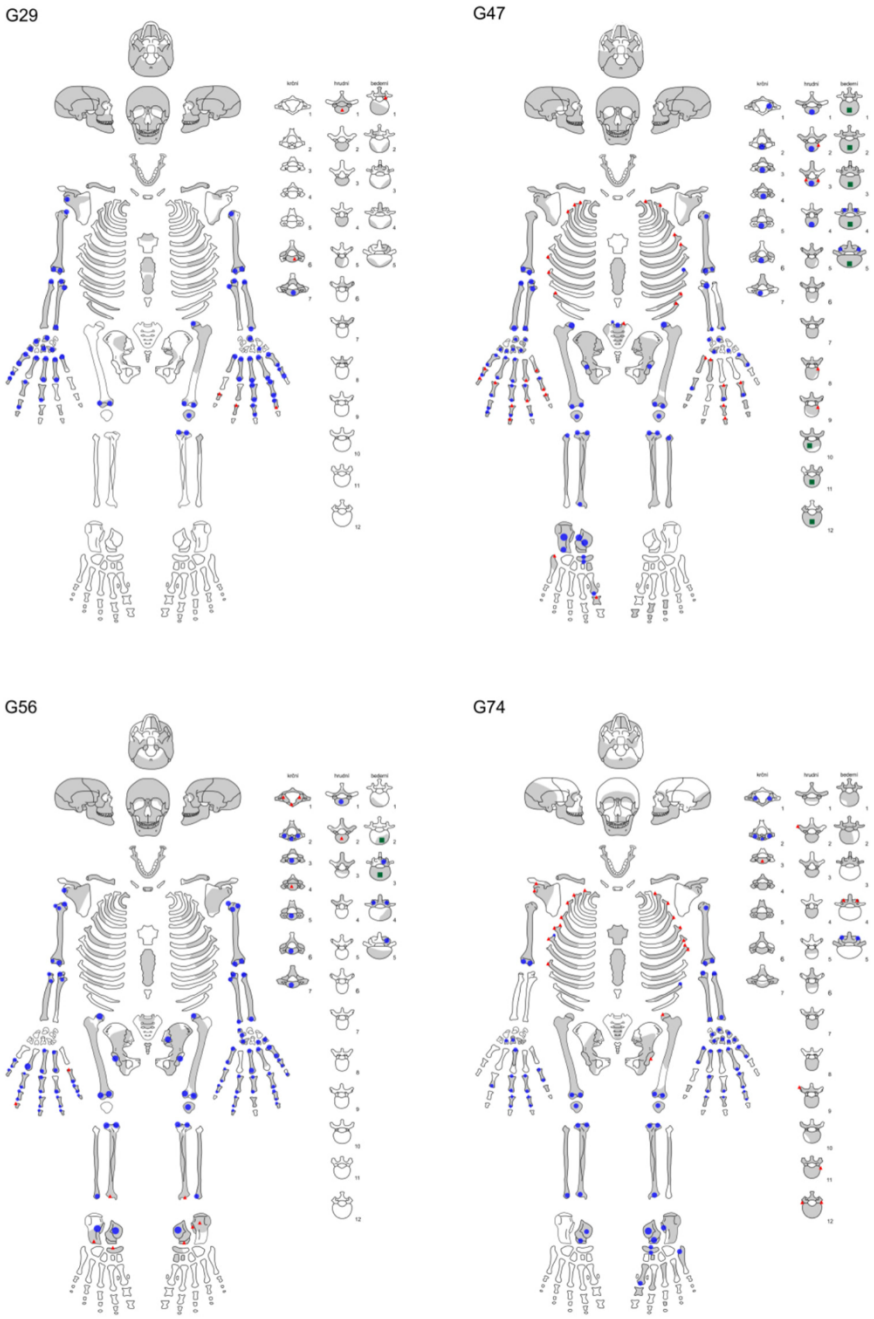


Fig. 2. Skeletal distribution of severe (blue circles) and moderate (red triangles) degenerative lesions; at vertebrae, endplate irregularities are marked with green squares; preserved bones of each individual are marked in grey (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Age-at-death estimations of the four individuals from the Milady Horákové site, applying Tooth Cementum Annulation (TCA) technique; average age of tooth eruption according AlQahtani et al. (2010) for combined sexes was used.

Individual	Tooth	TCA counts in 5 sections	SD	Average tooth eruption	Estimated TCA age in years	SD
G.29	First upper incisor	34.5	4.0	7.3	41.8	5.0
G.47	Upper canine	31.2	3.1	11.3	42.5	5.0
G.56	Upper canine	17.8	1.3	11.3	29.1	5.0
G.74	First upper incisor	24.0	1.9	7.3	31.3	5.0

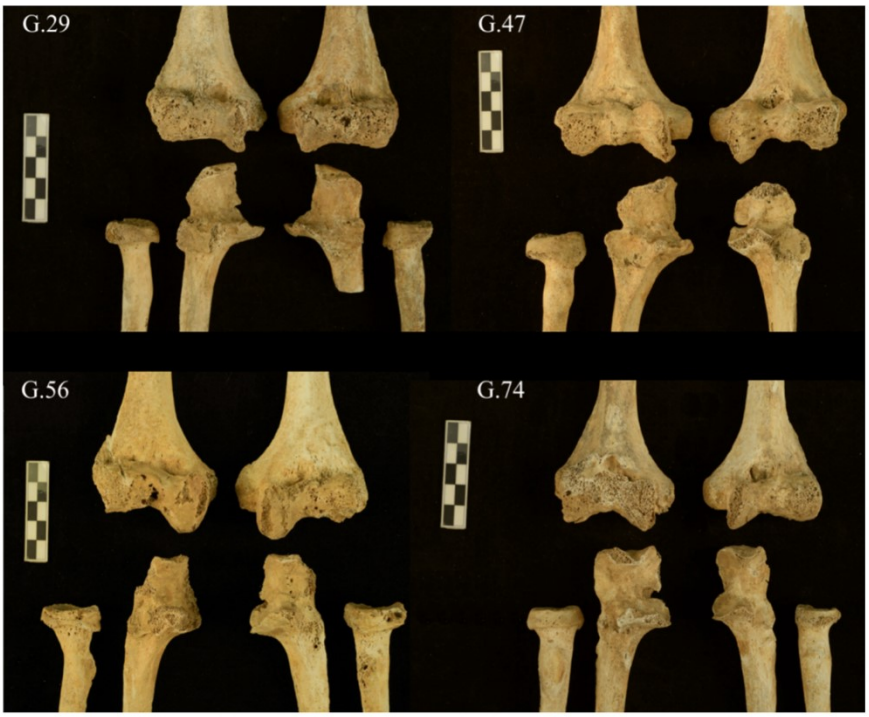


Fig. 3. Anterior view of the elbows of all four individuals; Scale: 5 cm; Photo: M. Jantač.

(Fig. 7), endplate irregularities in some of the thoracic and all the lumbar vertebrae (Fig. 8), *spondylolysis* at L5, and defects suggesting the presence of *osteochondritis dissecans* at the inferior articular surfaces of both tibiae and at the corresponding tali.

The individual buried in grave 56 was assessed to be male according to the metric evaluation of both innominates. He was the youngest of all the individuals, with an estimated age at death of 18–24 years based on the degree of skeletal maturation (unfused sternal end of the clavicle and iliac crests, and only partially fused humeral heads). The TCA method provided a slightly higher estimation of 29.1 ± 5 years, but both intervals are close to each other. As was the case for the above-described individual, both elbows were severely deformed (Fig. 3 and 4). Additional pathological changes include *spondyloschisis* on the vertebral arch of the sixth cervical vertebra, endplate irregularities in two lumbar vertebrae (Fig. 8), osteolytic lesions on both humeral tubercles (Fig. 7), and *osteochondritis dissecans* at the inferior articular surface of the right tibia. G.56 was the only one of the concerned individuals to exhibit a small (approx. 1 × 1 cm) erosive lesion of the auricular surface of the left ilium, but no other signs suggestive of sacroiliitis were present.

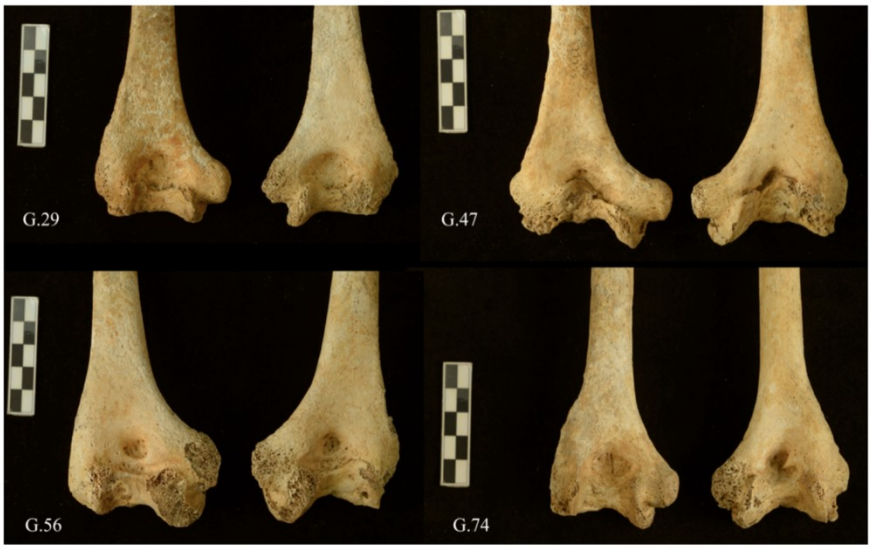


Fig. 4. Posterior view of the humeri of all four individuals; note the substantial joint deformation; Scale: 5 cm; Photo: M. Jantač.



Fig. 5. Posterior view of the preserved distal femoral epiphyses of all four individuals; note the flattening of the femoral condyles in G.29, G.47 and G.74 and the irregular articular surface of G.56; Scale: 5 cm; Photo: M. Jantač.

The last individual, buried in grave 74, was a female according to the morphology of the surviving parts of both innominates and the metric analysis of the better-preserved left-side bone. She was estimated to be 30–39 years old at death based on the complete maturation of the skeleton and the state of the auricular surface and pubic symphysis. The TCA method provided an estimate at the lower end of this interval (31.3 ± 5 years). In general, in this individual, osteoarthritic changes were less advanced than in the others. Both elbows showed substantial deformation (Fig. 3 and 4) and the femoral condyles were visibly flattened (Fig. 5). Additional bone changes include *spina bifida occulta*, entheseal changes at the lesser tubercle of the left humerus (Fig. 7), and *osteochondritis dissecans* at the inferior articular surface of the left tibia.

From the description above, it is evident that all four individuals suffered from polyarticular OA non-accordant with their relatively young age. Elbows (Fig. 3 and 4), knees (Fig. 5 and 9), and the small joints of the hand were affected in all cases. A flattening of the epiphyses of the knee (Fig. 5), severe deformation in the elbows (Fig. 3 and 4), lytic lesions on the humeral tubercles (Fig. 7) and aplastic defects of the neural arch were repeatedly observed.

4.2. Radiographic and histological appearance

X-ray images showed a typical picture of degenerative changes with some subchondral cysts (Figure S1). Osteopenia was not observed. Histological images showed changes in subchondral bone, with lacunae of the cells that eroded the surface of the spongy trabeculae being apparent in the thin sections. The bone trabeculae underlying the traumatized eburnated surface showed cystic degeneration (Fig. S2). In the patella of individual G.29, intra-trabecular resorption cavities filled with vascular connective tissue and with the appearance of granulation tissue (Fig. S2) were found. The granulation tissue was originally composed of fibroblasts, vessels and round cell infiltration (Stevens et al., 2002).

4.3. Osteometric features

The stature of three of the four individuals appeared to be below average when compared to other early medieval Czech individuals of the same sex (Table 2). In G.29, maximum lengths of all the measurable long bones were the lowest among the entire dataset. The stature calculation produced the value of 154 cm. The difference in the lowest measurement value compared to non-pathologic individuals varied between 10 (H1, U1) and 19 (F1) mm, which gives an approximately 5 cm difference in stature estimates (based on F1). In G.47, all the long bone lengths except T1 were the second lowest in the dataset (stature estimate = 156 cm). G.29 and G.47 also showed the shortest stature compared with the slightly younger dataset of 88 males from 11th to 14th-century Bohemia (Kaupová et al., 2013). In female G.74, stature was not as reduced, with all the maximum lengths (again with the exception of T1-percentile 44) varying between percentiles 10 and 20 (stature estimate = 159 cm). In contrast, G.56 showed above average stature, with all the measurable long bone lengths varying at or above the 80th percentile and a stature estimate of 176 cm.

The ratios that could be computed suggest that the affected individuals had relatively short femurs and long tibiae. Their values were, however, within or close to the normal population variability and in G.47, it could be at least partially affected by the dysplastic femoral heads. This also suggests that the above-mentioned statures may be slightly underestimated.

Interestingly, platymeric and platycnemic indexes of the affected individuals showed an unusual flattening of femurs in the antero-posterior axis and of tibiae in the medio-lateral axis.

Analysis of the shape of the vertebral bodies was limited due to the substantial fragmentation of vertebrae of the concerned individuals, as well as of the comparative dataset. However, the measurable dimensions of the four individuals did not suggest the presence of platyspondyly. For a detailed description of the vertebral metrics, see the online appendix.

4.4. Diet reconstruction

Isotopic analysis of the four individuals revealed a diet characterized by a low intake of animal products. This component of the diet differed in comparison with the majority of the Milady Horákové individuals (exact Mann-Whitney test; $p = 0.007$ for nitrogen isotopic values), as well as with the rest of the population of early medieval Bohemia (Kaupová et al., 2019; Fig. 10).

5. Differential diagnosis

Differential diagnosis focused on two main groups of skeletal disorders: inflammatory arthropathies and skeletal dysplasias resulting in premature OA (Table 3). Metabolic causes of premature OA such as gout, alkaptonuria, mucopolysaccharidoses or Paget's disease can be

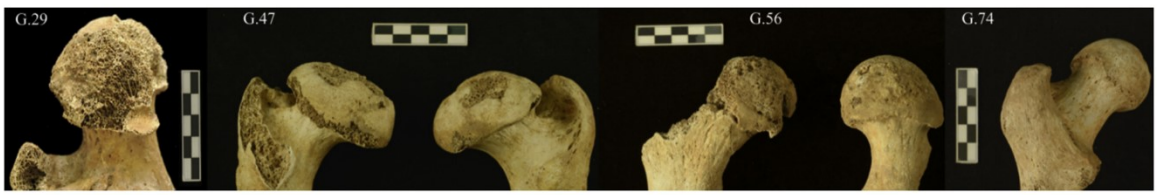


Fig. 6. Views of the preserved femoral heads of all four individuals, highlighting the severe femoral head deformation in G.47; left femoral head of G.29 shows severe postmortem damage; Scale: 5 cm; Photo: M. Jantač.



Fig. 7. Views of the preserved humeral heads of G.47, G.56 and G.74; lytic lesions on the humeral tubercles marked with arrows; Scale: 5 cm; Photo: M. Jantač.

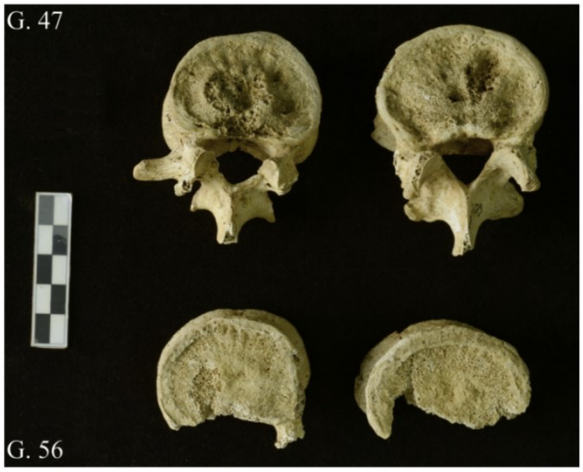


Fig. 8. Example of the vertebral endplate irregularities of G.47 (Th12 and L1) and G.56 (L2 and L3); Scale: 5 cm; Photo: M. Jantač.



Fig. 9. Superior view of the tibiae of G.47, G.56 and G.74; only small fragments of the left tibia were preserved in G.29; Scale: 5 cm; Photo: M. Jantač.

ruled out due to the absence of characteristic skeletal symptoms of these conditions (e.g. *humerus varus* deformity, *dysostosis multiplex*, scooped-out defects, fusion of vertebral bodies), normal X-ray images, as well as age-at-death of the affected individuals (Kacki et al., 2013; Ortner, 2003; Waldron, 2009; White, 2011).

Amongst inflammatory polyarthropathies, rheumatoid arthritis (RA) can be readily dismissed based on the presence of significant bone proliferation in all the affected individuals, location of erosive lesions in central parts of the joint surfaces, and significant involvement of the spine including thoracic and lumbar vertebrae (Ortner, 2003; Waldron, 2009).

Juvenile chronic arthritis (juvenile idiopatic arthritis; JIA) could also be considered, as at least one-third of the patients exhibited ongoing active disease into their adult years and others show sequelae of previous inflammation (Foster et al., 2003). However, diagnosis of JIA is improbable in the current cases, as JIA resembles the clinical picture of adult type RA, and characteristic features of JIA were absent, including bony ankyloses of carpals and tarsals and diarthrodial joints of the spine, involvement of the TMJ, and general underdevelopment of the mandible (Burt et al., 2013; Ortner, 2003; Twilt et al., 2004; Weiss and Ilowite, 2007).

From the group of spondyloarthropathies (SpAs), psoriatic arthritis, inflammatory bowel disease-related arthritis and undifferentiated SpAs could be considered due to their polyarticular involvement including that of the large appendicular joints. As with RA, juvenile forms of SpAs have been described (Rothschild et al., 1997), so the young age of the individuals (especially G.56) does not preclude this diagnosis. Entheseal changes at fibrocartilaginous attachments, which are typical for SpAs (Benjamin and McGonagle, 2001; Villotte and Kacki, 2009), were observed in three individuals (G.47, G.56 and G.74) at the insertion of *m. supraspinatus*, *m. infraspinatus* and *m. subscapularis*. However, osteolytic lesions at the rotator cuff are also relatively common in individuals without SpA (Villotte and Kacki, 2009), thus such observations could not be used as exclusive criteria for SpA. Moreover, sacroiliitis, which is typical of SpA, was not present. Further, asymmetry of lesions is an important diagnostic criterion for SpAs. From other characteristic traits of SpAs, the TMJ was spared, neither ankyloses of the spine nor the typical cup-and-pencil morphology of interphalangeal joints was observed, and distal interphalangeal joints did not show greater severity of lesions than other joints (Ortner, 2003; Samsel et al., 2014; Waldron, 2009).

Certain conditions from the wide spectrum of skeletal dysplasias must be considered as potential causes of bone changes observed in the four individuals. In both spondylo-epiphyseal dysplasia (SED) and multiple epiphyseal dysplasia (MED), a number of genes may be involved causing, for example, defects of type II or IX collagen fibers, collagen matrix protein (COMP), proteins involved in intracellular transport, or defects of growth factors controlling expression of collagen (e.g. Chapman et al., 2003; Garcia Segarra et al., 2012; Terhal et al., 2015). These mutations result in defects in cartilage formation and maintenance, leading to premature OA.

Table 2
Comparison of postcranial metric data of the four documented cases with those of other individuals of the same sex from early medieval Bohemia; n = number of individuals; %ile = percentile; percentiles under 10 and above 90 in bold font; n, median and range are calculated including the affected individuals; preferentially, left-side bones were measured, when absent or fragmented, measurement was taken from the right side.

Measurement	Males									Females				
	n	Median	Range	G.29		G.47		G.56		n	Median	Range	G.74	
				Measurement	%ile	Measurement	%ile	Measurement	%ile				Measurement	%ile
H1	38	330	290 – 369	290	**0**	305	5	360	**95**	36	302.1	275 – 340	292	20
R1	34	247.5	219 – 275	219	**0**	225	**3**	262.5	79	36	228	199 – 260	212	14
U1	16	270	240 – 297	240	**0**	250	**7**	284	80	22	246.6	221 – 275	230.5	**10**
F1	52	457.5	400 – 517	400	**0**	405	**2**	482	84	62	418.5	370 – 465	403	16
F9	56	34	29 – 40	33	29	30	**2**	38	**91**	67	31	26 – 36	32	65
F10	56	26.5	19 – 32	22	**2**	19	**0**	23	**7**	67	23	19 – 29	21.2	18
T1	36	372	335 – 425			350	14			46	345	298 – 397	342	44
T8a	52	34.5	27 – 42			29	**2**	30.5	**6**	63	31	26 – 37	27.5	13
T9a	51	24	21 – 28			23	24	22.5	22	63	22	18 – 25	24	89
F1/H1	34	0.71	0.68 – 0.75	0.73	67	0.75	**97**	0.75	**91**	32	0.72	0.70 – 0.78	0.72	68
T1/R1	23	0.66	0.62 – 0.69			0.64	**9**			26	0.65	0.62 – 0.70	0.62	**0**
R1/H1	28	0.75	0.72 – 0.81	0.76	56	0.74	19	0.73	**7**	31	0.75	0.70 – 0.78	0.73	23
T1/F1	34	0.81	0.76 – 0.86			0.86	**100**			41	0.82	0.77 – 0.89	0.85	82.5
Platymeric index	55	0.77	0.61 – 0.97	0.67	11	0.63	**3**	0.61	**0**	67	0.74	0.66 – 0.93	0.66	**2**
Platycnemic index	51	0.69	0.56 – 0.84			0.79	**92**	0.73	78	62	0.71	0.57 – 0.87	0.87	**100**

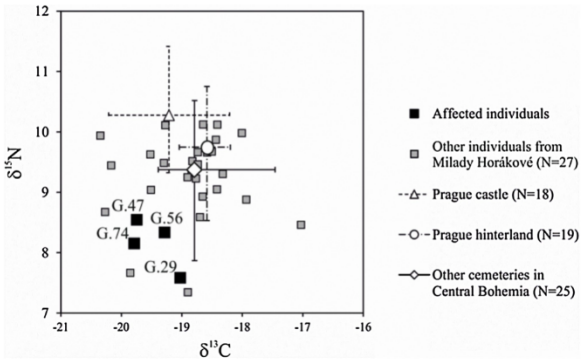


Fig. 10. Carbon and nitrogen isotopic values of the four affected individuals compared to the Milady Horákové population and other early medieval burial sites from Central Bohemia (mean, percentile 2.5 and 97.5); higher nitrogen isotopic value indicates higher consumption of animal products, higher carbon isotopic value indicates higher consumption of millet; modified according to Kaupová et al. (2019).

For SED, major involvement of the spine is typical, resulting in disproportion between the trunk and limbs. The more severe congenital forms (SEDC) may be excluded, as typical clinical findings include severely reduced stature (with a height below –7 SD, in general below 150 cm), with marked platyspondyly and a posterior wedging of the vertebrae. In adults, the anteroposterior diameter of the vertebrae is severely reduced. Furthermore, odontoid hypoplasia, coxa vara with flattened femoral heads, flattened femoral condyles, genua vara/valga, cleft palate or clubfoot, and broad thorax are commonly found in SEDC patients (Amirfeyz et al., 2005; Terhal et al., 2015). With the exception of flattened distal femurs and dysplastic femoral heads, none of these traits was found in any of the studied individuals.

The other forms of SED, which manifest in later childhood (SEDtarda; SEDT) and thus show milder degrees of dysplasia with less affected stature and body proportions, may be considered for some individuals. They can be excluded, however, for the female individual G.74, due to their X-linked recessive inheritance (Amirfeyz et al., 2005; Bannerman et al., 1971; Garcia Segarra et al., 2012). Some of the characteristic features of SEDT were present in the other individuals, namely vertebral endplate irregularities, dysplastic hips, *osteochondritis dissecans* and other changes related to premature OA. On the other hand, platyspondyly, anterior wedging of the vertebrae, brachydactyly, or pelvic deformities with small ilia compensated by long ischia and pubis (Schantz et al., 1988) were not observed, making this diagnosis unlikely. A specific type of SEDT with progressive arthropathy (SEDT-PA) is also improbable due to the absence of characteristic traits such as platyspondyly, brachydactyly with enlarged metaphyses and epiphyses of MCP and phalanges, hypoplastic or fused carpals and/or tarsals, osteopenia and periarticular calcifications (Garcia Segarra et al., 2012; Kocyigit et al., 2000; Wynne-Davis, 1982; Yoleri et al., 2011).

Considering the absence of platyspondyly and short-trunk stature, MED seems to be a more probable diagnosis. The condition, which has in most cases an autosomal-dominant inheritance, is characterized by predominant involvement of the epiphyses, where an irregular epiphyseal growth affects a pair or many joints symmetrically, forming flattened and dysplastic articular surfaces. Stature of the affected individuals is normal or moderately short (about 150 cm in adults), but cases with above-average height have also been described (Ballhausen et al., 2003; Chapman et al., 2003; Jakkula et al., 2004; Unger et al., 2008; Wynne-Davies and Gormley, 1985). In addition, body proportions are usually normal, or in some cases mild shortening of the limbs may occur (Haga et al., 1998). The hips and knees are prominently affected in MED patients but the involvement of hands, feet, ankles and shoulders have been noted (Jakkula et al., 2004). For the spine, endplate irregularities are typical, but mild platyspondyly may also occur, as well as genu varum or valgum. Hip dysplasia (observed in G.47) is typical. In clinical practice, when cases of bilateral hip dysplasia (and LCP disease) are diagnosed, further testing for MED is strongly advised (Unger et al., 2008). Importantly, the histological finding of granulation tissue observed in several histological sections of G.29 is typical of MED (Stevens et al., 2002), further supporting this diagnosis.

Much less common autosomal recessive forms of MED are improbable, as several characteristic traits (prominent thumbs, brachydactyly, accelerated ossification of carpals resulting in reduced carpal width, club foot or so called “double-layered patella”) were not present in any of the studied individuals (Ballhausen et al., 2003; Huber et al., 2001; Mäkitie et al., 2003; Unger et al., 2008).

A final differential diagnosis, at the mildest end of the spectrum of skeletal dysplasias, is the group of type II collagenopathies. Premature OA is a leading symptom of these autosomal inherited disorders. Symmetrical lesions of virtually all large and small joints of the postcranial skeleton, without substantial changes in stature and/or body proportions, are typical. The onset of disease is relatively late in

Table 3
Distinctive features of degenerative joint disease (DJD), rheumatoid arthritis (RA), spondyloarthropathies (SpAs), spondylo-epiphyseal dysplasia (SED), multiple epiphyseal dysplasia (MED) and type II collagenopathies, and their presence in the skeletons under study; (+) presence; (+/−) possible but not common presence; (−) absence; (?) not described in the medical literature.

	G.29	G.47	G.56	G.74	DJD	RA	JIA	SpAs	SED	MED	Type II collagenopathies
Sex/Sex predilection	Male	Male	Male	Female	None	Females	Females	None	None (exc. X-linked form)	None	None
Stature	Moderately reduced	Moderately reduced	Above-average	Below-average	Normal	Normal	Normal	Normal	Short-trunk dwarfism	Normal or moderately reduced	Normal
Lesions symmetry	(+)	(+)	(+)	(+)	(−)	(+)	(+)	(−)	(+)	(+)	(+)
Bone proliferation	(+)	(+)	(+)	(+)	(+)	(−)	(−)	(+)	(+)	(+)	(+)
Bone erosion	(+)	(+)	(+)	(+)	(−)	(+) mainly marginal	(+) mainly marginal	(+)	(+)	(+)	(+)
Joint ankylosis	(−)	(−)	(−)	(−)	(−)	(+/−)	(+)	(+)	(−)	(−)	(−)
Osteopenia	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(−)	(−)	(−)	(−)
Entheseal changes (erosive lesions)	(−)	(+)	(+)	(+)	(+); rotator cuff disease	(+/−)	(+/−)	(+)	(?)	(?)	(?)
TMJ involvement	(−)	(−)	(−)	(−)	(−)	(+/−)	(+)	(+)	(−)	(−)	(−)
Spine involvement	(+)	(+)	(+)	(+)	(+)	(+/−; cervical spine)	(+; cervical spine)	(+)	(+)	(+)	(+)
Vertebral endplate irregularities	Non-evaluable	(+)	(+)	(−)	(+)	(−)	(−)	(−)	(+)	(+)	(+)
Sacroiliitis	(−)	(−)	(−)	(−)	(−)	(+/−) may be found but uncommon	(−)	(+)	(?)	(?)	(?)
Distal humerus dysplasia	(−/+)	(+)	(+)	(+)	(−)	(−)	(−)	(−)	(+)	(+)	(+)
Flattened epiphyses of the knee	(+)	(+)	(−)	(+)	(−)	(−)	(−)	(−)	(+)	(+)	(+)
Hip dysplasia	(−)	(+)	(−)	(−)	(−)	(+/−) may be found but uncommon	(−)	(−)	(+)	(+)	(+)
Fibrovascular granulation tissue in the histological image	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(?)	(+)	(?)

comparison to other skeletal dysplasias and varies widely between late childhood and early adulthood (Ala-Kokko et al., 1990; Hildebrand, 2015; Hoornaert et al., 2006; 2007; Husar-Memmer et al., 2013; Jurgens et al., 2015; Kannu et al., 2010). Vertebral endplate irregularities are common, while scoliosis and mild platyspondyly may occur but without substantial impact on stature or body proportions (Ala-Kokko et al., 1990). *Osteochondritis dissecans,* flattened epiphyses of the knee joint and hip dysplasia imitating LCP disease are also common (Hoornaert et al., 2006; Husar-Memmer et al., 2013; Kannu et al., 2010).

6. Discussion

6.1. A picture of mild skeletal dysplasia

All four individuals from the Milady Horákové site show skeletal changes consistent with the diagnosis of skeletal dysplasia as described in the medical literature, including symmetrical osteoarthritic lesions of the postcranial skeleton, dysplasia of the joints (i.e. dysplastic hips imitating LCP disease, severe deformation of the distal humerus, and visibly flattened femoral condyles), endplate irregularities of the vertebrae, and *osteochondritis dissecans*. The absence of severe stature reduction (moderate stature reduction was observed in G.29 and G.47) and the absence of substantial changes in body proportion suggest that the causative condition(s) is/are from the mild end of the dysplasia spectrum, with a moderate form of MED or a type II collagenopathy being the most probable diagnoses. A definitive diagnosis cannot be made, however, as there is a considerable variation in the clinical manifestations of these conditions, making it impossible to distinguish between the conditions on the basis of skeletal lesions alone. Even in clinical practice, genetic testing is needed to determine the exact type of dysplasia (Jurgens et al., 2015; Terhal et al., 2015).

Since the prevalence of skeletal dysplasia is extremely low (Ikegawa, 2006), the co-occurrence of four cases within the Milady Horákové cemetery, which was used for a brief duration, suggests that all four individuals may have suffered from the same skeletal dysplasia. This would be well in accord with the autosomal dominant inheritance of both MED and type II collagenopathy. However, in the absence of genetic analysis of the skeletons, it is not possible to assess the kinship of the individuals and to confirm this hypothesis. The four individuals may alternatively have suffered from different forms of skeletal dysplasia, co-occurring in the same population. It is noteworthy, however, that the slightly different distribution of skeletal lesions among the four individuals cannot be viewed as evidence for differing diagnoses, as a huge range of variation in clinical manifestation can be observed within one family (Kannu et al., 2010).

The involvement of the elbows in all of the individuals would perhaps make a type II collagenopathy a more probable diagnosis, as severe involvement of the elbows is not described in MED patients. However, it should be mentioned that OA of the elbow is much more common in archaeological populations than in modern populations (Domett et al., 2017; Ortner, 2003). The reasons for this are not understood, but differing workloads is considered a likely cause (Felson, 1994).

6.2. Skeletal features unreported in the medical literature

Alongside classic bone changes linked to skeletal dysplasia caused by MED and type II collagenopathy, the four skeletons from the Milady Horákové cemetery exhibit bone changes that are not described in the medical literature. First, the involvement of entheses is not described as a feature of either MED or type II collagenopathies. However, tendons and entheses include, to a great extent, the same components as cartilage (e.g. collagen types II and IX, cartilage oligomeric matrix protein). Defects of genes encoding these proteins are among the most common causes of MED or premature OA (Briggs and Chapman, 2002; Chapman et al., 2003; Hildebrand, 2015; Jurgens et al., 2015; Kannu et al., 2010). Experimentally, dramatic changes in the biomechanical properties of tendons have been found in individuals with COMP mutation related to MED (Piróg et al., 2010). Thus, the involvement of muscular insertions of the rotator cuff observed in G.47, G.56 and G.74 (Fig. 7) may hypothetically be caused by MED and type II collagenopathy. Moreover, the rotator cuff region is a common site of microtrauma resulting from excessive load (Benjamin and McGonagle, 2001). Both micro and macrotrauma might occur when locomotion in individuals is compromised by severely-affected hips and knees. Finally, pitting may be found at insertions of rotator cuff muscles together with osteoarthritic changes of the gleno-humeral and acromio-clavicular joints in a degenerative condition called rotator cuff disease (Hardy et al., 1986; Waldron, 2009).

Aplastic defects of the vertebral neural arch (observed in G.49, G.56 and G.74) are also not reported in association with MED and type II collagenopathy, but have been linked to other skeletal dysplasias (Schiess and Haeusler, 2013). Developing from improper endochondral ossification, isolated defects of the neural arch have been found to be caused by mutations in the same genes as cause MED (Cai et al., 2015). The absence of reports of these defects in clinical records may be due to their clinical unimportance. However, the co-occurrence of these defects in at least three of the four individuals (the spine of the fourth individual, G.29 fragmentary) clearly deserves our attention as a potential trait of mild skeletal dysplasia observable in the archaeological record, but should not be overinterpreted since *spina bifida occulta* (G.74) is a relatively common condition (Ferembach, 1963). Similarly, spondylolysis (G.47) may result from trauma, especially in individuals with chronic overload (hyperextension) of the lumbar spine (in today's populations e.g. in gymnasts and basketball players) and occurs in hereditary disorders of connective tissues (Marfan syndrome, Ehlers-Danlos syndrome) and generalized inflammatory diseases (rheumatoid arthritis) (Hailer and Hailer, 2018; Horn et al., 2018; Selhorst et al., 2019). Alternatively, the co-occurrence of these developmental anomalies may also reflect kinship of the concerned individuals rather than their pathology (Barnes, 2012; Pietrusewsky and Douglas, 1992).

As the degree of platymery and platycnemy are traditionally linked to physical load (Ruff et al., 2006), the unusual shape of the shafts of the femur and tibia could also be caused by unusual loading in order to preserve mobility. Lastly, the effect of nutrition cannot be excluded (Angel, 1984), as well as a genetic component (Lovejoy et al., 2003), as the affected individuals were possibly biologically related.

6.3. Social effects of skeletal dysplasia

Although in the context of skeletal dysplasias, the presence of MED and type II collagenopathy are relatively mild, they would still have impacted the quality of life. Skeletal dysplasias are associated with pain and reduced mobility. In present-day populations, most affected individuals undergo surgery during puberty or early adulthood. Skeletal dysplasias may also be associated with ocular and hearing problems, especially when the mutation of genes encoding type II collagen is involved (Terhal et al., 2015). All of these symptoms might lead to dependence of the affected individual on his or her family or wider society.

It is interesting that the diet of the four individuals differed from that of the majority of the Milady Horákové population, as well as from the rest of the population of early medieval Bohemia (Kaupová et al., 2019), showing a characteristically low dietary input of animal products. Digestive or metabolic problems, which could cause the observed isotopic pattern, have not been described in skeletal dysplasias. Other reasons for their somewhat unique diet, such as migration, cannot be excluded (note that the dietary pattern was not exclusive to the affected individuals). The present data suggest that the disability of these people may have limited their access to animal products. Also, we cannot exclude the possibility that the mild stature reduction observed in G.29

and G.47 resulted from improper diet during the period of growth, rather than from the skeletal dysplasia itself, or that both direct and indirect effects of the disease acted in synergy. However, it should be noted here that isotopic analysis of bone collagen reconstructs diet from the last decades of individual's life. To describe the childhood diet, a different sampling strategy using tooth dentine is needed (Balasse et al., 1999).

7. Conclusion

The four skeletons from the Milady Horákové site exhibit bone changes that are consistent with a set of milder forms of skeletal dysplasia, such as multiple epiphyseal dysplasia or type II collagenopathy. Only a few isolated cases of most severe forms of the conditions, showing notable change in stature and/or body proportion, have been described in the palaeopathological literature (Arcini and Frölund, 1996; Kozieradzka-Ogunmakin, 2011; Wakely, 2000), and these do not include isolated cases of premature OA (Domett et al., 2017). Although the prevalence of skeletal dysplasias causing premature OA is usually very low (Amirfeyz et al., 2005; Unger et al., 2008), it is possible that this condition might be under reported in skeletal series, as well.

Based on clinical literature, we recommend considering a diagnosis of MED and/or type II collagenopathy in cases of bilateral hip dysplasia. Our study calls attention to other skeletal lesions that may potentially be associated with mild skeletal dysplasia such as defects of the vertebral neural arch and erosive lesions at entheses. We advocate further study of the potential links between skeletal dysplasias and these less reported conditions. Also, further study focusing on premature OA in skeletal populations are needed to identify mild forms of dysplasia in the archaeological record and to better assess their impact upon everyday life of affected individuals in past populations.

Funding

This work was supported by the Czech Science Foundation (Grant number: 14-36938G) and the Ministry of Culture of the Czech Republic (Grant number: DKRVO 2019-2023/7.II.a, 00023272), National Museum; funding sources had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Declaration of Competing Interest

None.

Acknowledgements

We would like to thank the following institutions for financial support: Czech Science Foundation (Grant number: 14-36938G), Ministry of Culture of the Czech Republic (Grant number: DKRVO 2019-2023/7.II.a, 00023272), National Museum. We thank International Science Editing for the English language editing and Marek Jantač for the photographic documentation. Finally, we appreciate the insightful editor and reviewer comments on a first version of this manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijpp.2020.04.004.

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Received: 16 February 2021 | Revised: 7 June 2021 | Accepted: 29 June 2021

DOI: 10.1002/oa.3022

RESEARCH ARTICLE

The impact of pathological conditions on carbon and nitrogen isotopic values in the bone collagen of individuals with known biographic data and medical history

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Funding information
Ministry of Culture of the Czech Republic, Grant/Award Numbers: DKRVO 2019-2023/7.II.c, 00023272; Charles University Grant Agency, Grant/Award Number: GAUK 1276217; Czech Science Foundation, Grant/Award Number: 19-13265S

Abstract

This study aimed to refine our understanding of the extent to which chronic diseases with and without skeletal manifestation affect the isotopic values in bone collagen. Carbon and nitrogen isotopic values were measured in the ribs and femurs of 65 individuals from a documented skeletal collection (19th–21st centuries, Czech Republic). Statistically significant differences in both carbon and nitrogen isotopic values were found between particular families. There was a notable effect of the year of death on nitrogen isotopic values in ribs. Neither the presence of chronic disease in general nor the presence of carcinoma, age-related physical debility, or tuberculosis had a significant impact on isotopic values. Cases of cancer with skeletal metastases however showed an increase of 1‰–1.5‰ in nitrogen isotopic values in (i) metastasis tissue, (ii) the healthy areas of bone affected by the metastasis, and (iii) the healthy rib bone. The results demonstrate that distinct dietary habits in particular families are the main driving force affecting the isotopic values in the sampled individuals. A minor but notable temporal shift in the nitrogen isotopic values of ribs may be linked to environmental and/or societal changes during the studied period. The observed increase in the nitrogen isotopic values of individuals with bone cancer metastases is in accordance with the state of cachexia and negative nitrogen balance. Despite the low number of cases, this result urges caution, as isotopic changes to the observed degree may significantly affect the dietary interpretation of the isotopic data.

KEYWORDS
cancer, dietary reconstruction, documented skeletal collection, pathophysiology, stable isotopes

1 | INTRODUCTION

Stable isotope analysis is, in terms of direct geochemical approaches, the most common way of reconstructing the diets of past populations. It is based on the basic principle that the isotopic signal from the diet consumed remains preserved in the consumer's tissues (Lee-Thorp, 2008). It is however well known at an experimental level that pathophysiological processes may affect stable isotope fractionation and distribution in the body (Reitsema, 2013). A number of serious health conditions are linked to wasting and altered protein metabolism and may thus influence the isotopic values of proteins synthetized over the course of the disease. The resulting impact on the human metabolism (and thus on isotopic values) results from a complex interaction between multiple factors influenced by the disease, such as nutrition, physical activity, cytokine levels, and medication (Welle, 1999).

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Nitrogen balance is the most common (though not the only) source of isotopic variation in such cases and has been explored in the contexts of eating disorders and pregnancy among humans, as well as in a number of controlled-feeding experiments on different animal species (for review, see, e.g., Reitsema, 2013). A negative nitrogen balance results from efforts by the organism to compensate for protein insufficiency, which could arise from either improper diet or pathology. In these circumstances, body tissues are catabolized and recycled, repeating the fractionating processes of transamination and deamination. As a result, the affected individual's newly formed tissues are more enriched in ^{15}N (Reitsema, 2013). However, there are some exceptions from the general pattern, suggesting the existence of a threshold level of stress below which $\delta^{15}N$ values remain unchanged (Hatch, 2012). Interestingly, the increased bioavailability of protein (due to an excess of protein in the diet or a physiological cause) has a similar impact on $\delta^{15}N$. This is due to deamination resulting from the organism's efforts to discharge the protein surplus (Sponheimer et al., 2003).

Carbon isotopic values have generally been found to be affected by decreased protein bioavailability in the opposite direction. As a result of the altered ratio between routed dietary and endogenously synthesized amino acids and the mobilization of fat stores, the $\delta^{13}C$ of affected individuals may be decreased (Beaumont et al., 2018; Mekota et al., 2006; Neuberger et al., 2013; Schwarcz, 2002). The typical isotopic "stress pattern" is thus reflected by the opposing covariance of $\delta^{15}N$ and $\delta^{13}C$ (Beaumont et al., 2018). However, as suggested by Salesse et al. (2019b), the threshold effect of stress may be unique for each isotopic proxy, and the internal metabolic pools of carbon and nitrogen may be affected in different ways and following different schedules during episodes of stress. Their data suggest that the carbon balance may become negative more rapidly than that of nitrogen, and carbon isotopic values may therefore be a better indicator of milder forms of nutritional/physiological stress.

The dietary reconstruction of past populations is based on the assumption that human tissues broadly reflect the isotopic signals of consumed foods, plus an isotopic offset reflecting the fractionation occurring during metabolism and the incorporation of ingested atoms into tissues. The routinely used diet-collagen offset is 4.8 ± 0.5 for $\delta^{13}C$ (Fernandes, Nadeau, & Groote, 2012). For $\delta^{15}N$, the magnitude of the trophic shift has been estimated to vary between 3‰ and 5‰, based on controlled feeding experiments with different animal species (Hedges & Reynard, 2007), while direct estimates for humans (Fernandes, Rinne, et al., 2012; O'Connell et al., 2012) suggest higher values of ∼5.5‰–6‰. Taking into account that isotopic shifts of around 1.5‰, that is, one half to one quarter of the estimated trophic shift, are commonly observed as a result of pathophysiological stress (D'Ortenzio et al., 2015; Mekota et al., 2006; Neuberger et al., 2013; Reitsema, 2013), it is evident that its undisclosed presence has the potential to significantly blur the results of dietary reconstruction.

Though the impact of physiological factors on isotopic values has been demonstrated repeatedly (for review see, e.g., Reitsema, 2013), the majority of studies have reported isotopic data recovered from tissues with a rapid rate of biosynthesis, such as urine, blood, or hair. Our knowledge of how the aforementioned processes impact the isotopic composition of human bone (which turns over on the order of years or even decades) is still limited (Dent, 2017; Katzenberg & Lovell, 1999; Olsen et al., 2014; Richards & Montgomery, 2012). The issue here is not only whether the individual survived long enough to incorporate the altered isotopic signal into such slowly remodeling tissue; we should keep in mind that the later effects of certain conditions may differ from their early effects (Welle, 1999).

Isotopic studies focusing on modern skeletal materials with known medical history are still very rare. A pioneering work was published by Katzenberg and Lovell (1999) with the aim of examining the $\delta^{13}C$ and $\delta^{15}N$ values in the bone collagen for four medically documented pathological and traumatic cases (atrophy, fracture, periostitis, and osteomyelitis). More recently, Salesse et al. (2019b) have found statistically significant differences in isotopic values between syphilitics versus nonpathological controls from a 19th century pathological-anatomical reference collection.

As an alternative approach, a limited number of studies have focused on isotopic analysis in paleopathological cases from archaeological contexts. In these cases too, however, the number of individuals has generally been very limited (Dent, 2017; Olsen et al., 2014). Moreover, additional drawbacks cannot be avoided in this type of study: the exploration is limited only to pathologies with skeletal manifestation, and the paleopathological diagnosis often remains unspecific. Finally, it is difficult to estimate the isotopic variability of non-pathological subjects in this type of study, because the absence of a pathological skeletal manifestation does not necessarily imply the absence of a pathological condition (DeWitte & Stojanowski, 2015; Wood et al., 1992).

This study presents isotopic data from a relatively large sample of human skeletal remains in a modern skeletal collection with known osteobiographic data, including diagnoses based on medical records. The main aim of the study was to refine our understanding of the extent to which chronic conditions (both with and without skeletal manifestations) impact the isotopic values in bone collagen and thus increase the testimonial power of isotopic analysis in the dietary reconstruction of past populations.

At the same time, knowledge of other biographic details from the individual lives helped to eliminate the potential impact of other factors, which might affect human isotopic values and thus blur the impact of disease. Therefore, the relationships between isotopic values and the following factors were explored:

Year of the death: the dating of the studied skeletal collection ranges from the mid-19th to the beginning of the 21st centuries and thus covers a period of rapid environmental and societal changes affecting both human behavior and the isotopic values at the base of the food chain (Bateman & Kelly, 2007; Marino & McElroy, 1991; Rychlík, 2020; Snyder, 2012).

Sex: although there is no physiological basis for isotopic differences between males and females (DeNiro & Schoeniger, 1983; Lovell et al., 1986), culturally determined differences in food preferences or in the access to certain foodstuffs between sexes are present in a number of past (for the isotopic evidence, see,

e.g., Kaupová et al., 2018; Reitsema & Vercellotti, 2012) and modern (Adams, 2015; Rothgerber, 2013) populations and cannot be ruled out in this sample, especially in its 19th century part. At that time, society was still highly patriarchal, and a hierarchic division of food within the family, with father and sons making their food choices before mother and daughters, was a common feature. This could lead to the undernutrition of women and young girls even in middle class families (Lenderová et al., 2017).

Age at death: organismal senescence is accompanied by aging-related disease and multi-morbidity (Calimport et al., 2019) with the general atrophy of the organism commonly found in elderly people.

In females, the potential impact of parity status and interval from last birth: changed nitrogen balance during pregnancy has been found to affect isotopic values in modern humans (D'Ortenzio et al., 2015; Fuller et al., 2004). Moreover, isotopic shifts associated with lactation have been observed in primates (Reitsema et al., 2016).

And finally, the presence of isotopic differences between distinct families was tested and interpreted in the context of occupation and social status. For the main part of the studied period, socioeconomic status was an important determinant of diet. In particular, there was a strong difference in meat consumption. Although in the upper and middle classes of both urban and rural society, meat, together with meaty stock, was a common part of the daily menu; the diet of the lower classes was based mostly on cereals and potatoes. In comparison with some other parts of Europe, animal breeding played much more minor role in Czech agriculture, which led to a relative scarcity of animal products in the diet of the poor. Caloric intake was also highly variable, with both the urban and rural middle classes having a daily input of about 3000–3500 kcal, while the poor were often endangered by undernutrition, consuming only 600–700 kcal. (Lenderová et al., 2017). The socioeconomic differences in diet diminished after the communist coup d'état in 1948 (Merhaut, 2015), but this concerns only a minority of the studied individuals.

This study undertook intraindividual sampling with samples taken from skeletal elements with distinct turnover rates. It is well known that bone undergoes constant turnover, and subsequently, its isotopic values reflect the average diet for some period prior to death. The length of this period is variable and affected by a number of factors such as sex, nutritional status, physical activity, and above all the age of the individual (Hedges et al., 2007; Martin & Armelagos, 1985; Seibel, 2003). Not only does bone turnover rate differ between individuals, but there is high intraindividual variation between different bones, distinct parts of the same bone, and between cortical and trabeluar bone of the same skeletal element (for a review, see, e.g., Fahy et al., 2017). In this study, we have intentionally applied the sampling strategy commonly used in an archaeological context with samples taken from the cortical bone of rib and femur. Due to the higher turnover rate in the rib area, it was expected that rib samples would reflect the pathophysiological changes occurring during fatal illness to a greater degree, while femur samples would reflect a long-term dietary average. It is difficult to quantify this difference in bone turnover rate but the estimates for ribs mostly refer to the last decade of life, while for the femoral midshaft, the isotopic signal covers a much longer period. It has been suggested that even in middle aged and elderly individuals, a substantial portion of femoral collagen has been synthesized during adolescence (Cox & Sealy, 1997; Fahy et al., 2017; Hedges et al., 2007; Sealy et al., 1995). Originally, it was assumed that the more rapid turnover in ribs is mainly due to the higher proportion of trabecular bone, but the osteon population density in the cortical area suggests the higher turnover rate of the compact bone probably as a result from the continuous load arising from respiration (Fahy et al., 2017; Skedros et al., 2013).

2 | MATERIAL

The genealogically documented sample includes the skeletal remains of 64 adult individuals (38 males and 26 females, aged 20 to 91 years) and one juvenile (male 9 years old), who died between 1857 and 2006. They came from 13 family tombs and four family graves from 10 Roman Catholic cemeteries in the central part of the Czech Republic located within 50 km of Prague. For the map showing the exact location of the tombs, see supporting information file no. 1. Permission for research was granted by the family members responsible for the graves/tombs. Exhumations were conducted on the occasion of necessary repair to the family tombs or the required relocation of skeletal remains.

Biographical data were known for each individual. These came from the private archives of the families, regional registry offices, hospitals, and some institutions, mainly the State Regional Archives in Prague, Třeboň, and Litoměřice, the Prague City Archive, the Archive of the Prague Archbishopric, and the Vatican Secret Archives. These sources provided vital records including death certificates containing official causes of death based on medical examination, church data on the anointing of the sick, autopsy protocols, genealogical data, files concerning the socioeconomic status of the families concerned including some basic information on their diet (land and marriage contracts), inheritance files including diagnostically relevant information (bills for treatment, funerals, etc.), correspondence, diaries, and photographs of the individuals. Identification of the individuals was based on documents from the relevant families and cemeteries and on the positions of the bodies in the tombs. For each tomb, there was a list of buried individuals with complete information (Cvrček et al., 2018; Cvrček et al., 2020).

The social status of the particular families has been reconstructed based primarily on the education and occupation of their male members. In the case of landowner families, a role in village administration (mayoralty) or in professional societies was considered a mark of higher social standing, as was the presence of affiliated ownership (such as a pub in the case of Family 3). Further information from family archives (such as contracts or personal diaries) was also taken into account. A detailed description of all the families, their socioeconomic status, and mutual relationships including family trees is provided in supporting information file no. 2.

The health status of each individual was reconstructed using the diagnosis on their death certificate and the supporting information

from other resources (autopsy protocol, date of the final anointing, etc.) where available. All the individuals were divided into two groups: those with chronic disease and those who died suddenly. In the cases of diseases prevalent enough in the sample, the potential impact of a particular condition on human isotopic values was explored. These include cancer (although carcinomas of different types were included in one group), age-related physical debility, and tuberculosis (TBC). The category of chronic heart failure was not included in this analysis: although this diagnosis is commonly associated with wasting (i.e., cardiac cachexia; Welle, 1999) and is present in a substantial number of individuals, this condition was mostly linked to severe co-morbidities of distinct origin. The "sudden death" group comprises individuals who died of cerebrovascular accident (CVA), violent death, strangulated hernia, acute bronchitis, scarlet fever, acute kidney failure, and gastro-intestinal catarrh. In these cases, there was no indication of long-term disability, other disease, or any paleopathological finding. Details on health status categorization and limits of diagnostic information are provided in supporting information file no. 3.

Where possible, two samples of cortical bone were taken from each individual. The first sample was taken from a femoral midshaft of all 65 individuals. As ribs were completely absent in 17 individuals (Nos. 4, 12, 14, 18, 26, 27, 32, 33, 39, 40, 41, 49, 51, 52, 66, 71, and 72), cortical bone of was sampled from ribs in 48 cases. Although according to Fahy et al. (2017) occipital bone would be even more suitable than femoral for ascertaining the long-term dietary average, femurs were used in order to avoid destruction of the skulls. In addition, for seven individuals with skeletal pathologies (Nos. 2 and 11—osteomyelitis, No. 17—rheumatoid arthritis, and Nos. 19, 22, 28, and 39—various metastatic carcinomas; details in supporting information file no. 4), samples of pathological bone were taken and compared to healthy bone tissue from the same bone (if possible) or from the healthy counterpart.

After completion of the analyses, the skeletal remains were reburied. All of the data are stored in the archive of the Department of Anthropology of the National Museum in Prague.

3 | METHODS

3.1 | Bone collagen preparation and isotope measurements

Collagen samples were prepared in the laboratory of the Department of Anthropology, National Museum, Prague. In accordance with common practice in bioarchaeological research, cortical bone was separated from trabecular bone as much as possible; excellent preservation made this efficient even for the ribs. No attempt was made to analyze healthy trabecular bone. Prior to collagen extraction, bone samples were defatted in a methanol-chloroform mixture (2:1, v/v) (Kates, 2010; Liden et al., 1995). The powdered bone samples were put into 10 ml of the mixture and were left overnight at room temperature. Subsequently, several 10-min rinses in an ultrasonic bath followed. After each rinse, the supernatant containing lipids was discarded and the solution renewed; this step was repeated until there was complete elimination of fatty acids and their derivatives. The samples were then thoroughly rinsed in deionized water and oven-dried at 65°C for 7 h. Bone collagen was extracted using the method of Longin (1971) as modified by Bocherens (1992). Elemental analysis-isotope ratio mass spectrometry (EA-IMRS) was performed at Iso-Analytical, Crewe (UK). The uncertainty of the isotopic measurements calculated on the different standard replicates was less than 0.2‰ (1 SD) for nitrogen and 0.1‰ for carbon. In the data presented, the mean 1 SD for duplicates was even better than 0.1‰. Routine analytical error for %C and %N was 2% in relative standard deviation (RSD) terms.

3.2 | Statistical analysis

The potential impacts of the following predictors were measured: year of death, age at death, sex, family membership, and the information on health status coded as a factor in two different ways: (i) presence of chronic disease versus sudden death and (ii) three types of chronic diagnoses frequent enough to give a reasonable power of statistical test versus sudden death. In female subsample, the potential impact of parity status and time since the last birth was assessed. All the statistical operations were effectuated for (i) $\delta^{13}C$ in femur, (ii) $\delta^{13}C$ in rib, (iii) $\delta^{15}N$ in femur, and (iv) $\delta^{15}N$ in rib. In cases where the impact of health status was tested, rib–femur isotopic offsets were also used.

The statistical analysis proceeded in three stages. First, univariate statistical tests were performed. To test possible relationships between isotopic values and continuous explicative variables (year of death, age at death, parity status, and time since the last birth), Spearman's rank correlation coefficient was calculated. For categorical explicative variables (sex, family membership, presence of chronic disease, and presence of a particular disease) the Mann–Whitney or Kruskal–Wallis test were used. If necessary, variants of these tests for small datasets—the exact MW test and Monte Carlo resampling procedure—were applied. In several cases where the data met the condition of normal distribution, a *t* test was used.

Second, linear models of the effects of the predictors were calculated, and the best model was chosen by forward selection using the Akaike information criterion (AIC). At this stage, the information on parity status was not included due to the excessive shrinkage of the studied dataset.

Finally, linear mixed effect models with family as random effect factors were used to test the effect of diagnosis and year of death on isotopic values, when adjusting for the impact of family membership without substantial decrease of the number of degrees of freedom. Such models were tested by likelihood-ratio test (using a chi-square distribution). Both corrected and uncorrected multiple comparisons gave the same results.

All the statistical operations were carried out using the statistical program R (R Core Team, 2018).

4 | RESULTS

In the studied sample, collagen yield was between 35.5 and 203.3 mg/g, with a median of 189.7 mg/g. Carbon content was between 35.3% and 46.7% with a median of 42.9%. Nitrogen content varied between 13.1% and 17.3% with a median of 16.0% and C:N ratio varied between 3.05 and 3.19 with a median of 3.10. This means that all samples met the criteria for good collagen preservation (DeNiro, 1985; van Klinken, 1999; supporting information file no. 5).

Carbon isotopic values in femur samples ranged between −20.3‰ and −19.3‰ with a median of −19.8‰. In rib samples, $\delta^{13}C$ reached slightly higher maxima, ranging between −20.3‰ and −18.9‰ with the same median as femur samples. Nitrogen isotopic values in femur samples ranged between 9.0‰ and 13.44‰ with a median of 11.6‰. The rib values were slightly higher, ranging from 10.5‰ to 13.7‰ with a median of 12.0‰. Carbon isotopic offset between rib and femur sample ($\Delta^{13}C_{rib\text{-}femur}$) was between −0.3‰ and 0.8‰ with a median of 0.0 ‰, while $\Delta^{15}N_{rib\text{-}femur}$ was between −0.4‰ and 1.33‰ with a median of 0.3‰. This difference between rib and femur samples was statistically significant (paired *t* test; $p < 0.001$).

The carbon isotopic values in nine samples of pathological bone ranged from −20.1‰ to −18.6‰ with a median of −19.7‰, while nitrogen isotopic values in these samples varied between 10.5‰ and 13.1‰, with a median of 12.5‰. Complete isotopic data together with explicative variables are given in supporting information file no. 5.

Spearman's rank correlation revealed no significant relationship between the year of death and $\delta^{13}C$ in either femur or rib samples (Table 1). Nitrogen isotopic values on the other hand correlated with the year of death (Figure 1a), but only in rib samples, with younger samples (mainly individuals who died after 1940) showing lower $\delta^{15}N$. This trend remained statistically significant even after excluding both of the youngest individuals, who died around 2000 and showed one of the lowest $\delta^{15}N$. Age at death had no significant impact on carbon and nitrogen isotopic values in either femur or rib samples. The statistical comparison of isotopic values between both sexes revealed a small but significant difference in femoral $\delta^{13}C$, with male $\delta^{13}C$ being slightly higher than female (Table 2). In ribs, the pattern was the same, but the result was not statistically significant, probably as a result of the lower sample size. Nitrogen isotopic values of both sexes were almost identical. There was no statistically significant correlation between isotopic values and parity status, or time since the last birth in females (Table 1). The impact of family membership was highly significant, affecting both $\delta^{13}C$ and

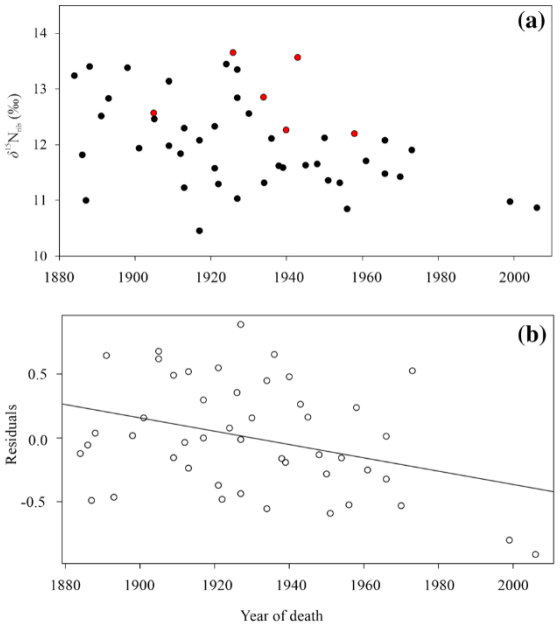


FIGURE 1 Rib nitrogen isotopic values of the sampled individuals with respect to year of death (a); individuals affected by metastatic cancer marked in red) and residuals of the linear mixed effect models with family as the random effect (b) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Relationships between continuous explicative variables (year of death, age at death, parity status, and time since the last birth) and the carbon and nitrogen isotopic values of individuals: Results of Spearman's test of correlation

		$\delta^{13}C$		$\delta^{15}N$	
		Rho	***p***	**Rho**	***p***
Year of death	Femur	0.080	0.529	0.029	0.820
	Rib	−0.008	0.957	**−0.356 (−0.305)**	**0.016 (0.041)**[a]
Age at death[b]	Femur	0.047	0.715	0.025	0.845
	Rib	−0.024	0.877	−0.067	0.660
Parity status	Femur	−0.187	0.382	−0.002	0.994
	Rib	0.045	0.865	−0.119	0.650
Time since the last birth	Femur	−0.305	0.178	−0.047	0.838
	Rib	−0.296	0.284	−0.356	0.193

Note: *p* values significant at the 0.05 level of significance are in bold font.
[a]Result of alternative testing when the two individuals who died after 1990 were excluded from the analysis.
[b]A subadult individual (No. 50) was excluded from the analysis.

TABLE 2 Relationships between binomial explicative variables (sex and presence of chronic disease) and the carbon and nitrogen isotopic values of the studied individuals: Basic descriptive statistics and results of statistical operations

Sex						
	Males			Females		
	N		Median (Q1–Q3)	N	Median (Q1–Q3)	*p* value[a]
Femur	38	$\delta^{13}C$	−19.7 (−19.9 to −19.6)	26	−19.9 (−20.1 to −19.7)	**0.014**[t]
		$\delta^{15}N$	11.6 (11.2 to 12.1)		11.6 (11.3 to 12.1)	0.741[t]
Rib	29	$\delta^{13}C$	−19.7 (−19.9 to −19.6)	18	−19.9 (−20.0 to −19.7)	0.515[t]
		$\delta^{15}N$	12.1 (11.5 to 12.8)		12.0 (11.5 to 12.6)	0.277[t]
Presence of chronic disease						
	Presence of chronic disease			Sudden death		
	N		Median (Q1–Q3)	N	Median (Q1–Q3)	*p* value
Femur	41	$\delta^{13}C$	−19.7 (−20.0 to −19.6)	13	−19.8 (−19.9 to −19.7)	0.898[t]
		$\delta^{15}N$	11.6 (11.2 to 12.1)		11.8 (11.3 to 12.0)	0.846[t]
Rib	33	$\delta^{13}C$	−19.8 (−19.9 to −19.7)	9	−19.7 (−19.9 to −19.6)	0.462[M–W]
		$\delta^{15}N$	11.8 (11.3 to 12.3)		12.1 (11.6 to 13.2)	0.168[M–W]
Rib–femur offset	33	$\delta^{13}C$	−0.03 (−0.16 to 0.12)	8	0.22 (−0.05 to 0.26)	0.148[M–W]
		$\delta^{15}N$	0.24 (−0.05 to 0.48)		0.36 (0.20 to 0.88)	0.340[M–W]

Note: *p* value significant at level of significance 0.05 is in bold font.
[a]*t* = independent sample *t* test; M–W = exact Mann–Whitney test.

TABLE 3 Relationships between categorical explicative variables (family membership and diagnosis) and the carbon and nitrogen isotopic values of the studied individuals: Results of Kruskal–Wallis test

		$\delta^{13}C$	$\delta^{15}N$
Family membership	Femur	**0.008**	**<0.001**
	Rib	**0.004**	**<0.001**
Diagnosis	Femur	0.729	0.368
	Rib	0.751	**0.038**
	Rib–femur offset	0.400	0.943

Note: *p* values significant at the 0.05 level of significance are in bold font; a descriptive statistic of the studied subsamples is shown in supporting information file no. 6.

$\delta^{15}N$ in femur and rib samples (Table 3 and Figure 2). The presence of chronic disease in general or a particular diagnosis had no significant impact on isotopic values (Tables 2–4) or rib–femur isotopic offset (Figure 3), with the exception of the relationship between diagnosis and rib $\delta^{15}N$. This was mainly due to the lower $\delta^{15}N$ observed in TBC cases.

The role of family membership as a major force affecting the isotopic values was confirmed by the linear modeling. For the $\delta^{13}C$ in femurs, based on AIC, isotopic values were best explained by a model containing family membership, and sex (AIC = −18.9), with family membership being highly significant (Table 4) and explaining 72.7% of data variation, while the impact of sex being insignificant and explaining only 0.2% of data variation.

For rib $\delta^{13}C$, the impact of family is again dominant explaining 46.5% of data variation, while sex and year of death have only marginal impacts explaining 3% and 2% of data variation respectively (AIC = 1.8). Nitrogen isotopic values in femurs were best explained by a model containing family membership, together with age and diagnosis (AIC = 47.6). The effect of family on $\delta^{15}N$ (explaining 83.1% of variability) was highly significant, but the effects of age (0.6% of data variability) and diagnosis (1.3% of data variability) were not significant. The conditions of the model were not met by rib $\delta^{15}N$, and thus, it is necessary to rely on the results of univariate statistical tests. In case of rib–femur isotopic offsets, there was no statistically significant factor (Table 4), although for $\Delta^{15}N_{rib\text{-}femur}$ (AIC = 43.1), age at death approaches statistical significance, explaining 10% of data variability. The value of $\Delta^{15}N_{rib\text{-}femur}$ declines by 0.0085‰ with each year of life.

As the final step of the analysis, linear mixed effect models with family as random effect factors were applied to uncover the potentially more subtle impacts of diagnosis and year of death. These variables were chosen with respect to the significant result of univariate statistical testing and the main research topic of this paper. For year of death (Table 5), the result of univariate statistical testing was confirmed showing a significant impact on rib $\delta^{15}N$ (likelihood-ratio test, $\chi^2 = 8.374$; $p = 0.004$, Figure 1b). The result remains significant even if the *p* value is multiplied by 6 in order to avoid the multiple testing problem. The decrease of $\delta^{15}N$ is however relatively subtle: with each year $\delta^{15}N$ decline by 0.008‰. The statistical significance disappears when the two individuals who died after 1999 are excluded from the analysis ($\chi^2 = 1.932$; $p = 0.165$). The impact of diagnosis was insignificant even in the ribs (Table 6; Figure 4).

A deeper investigation of isotopic differences among particular families was effectuated by Tukey post hoc testing. The results are depicted in Figure 2.

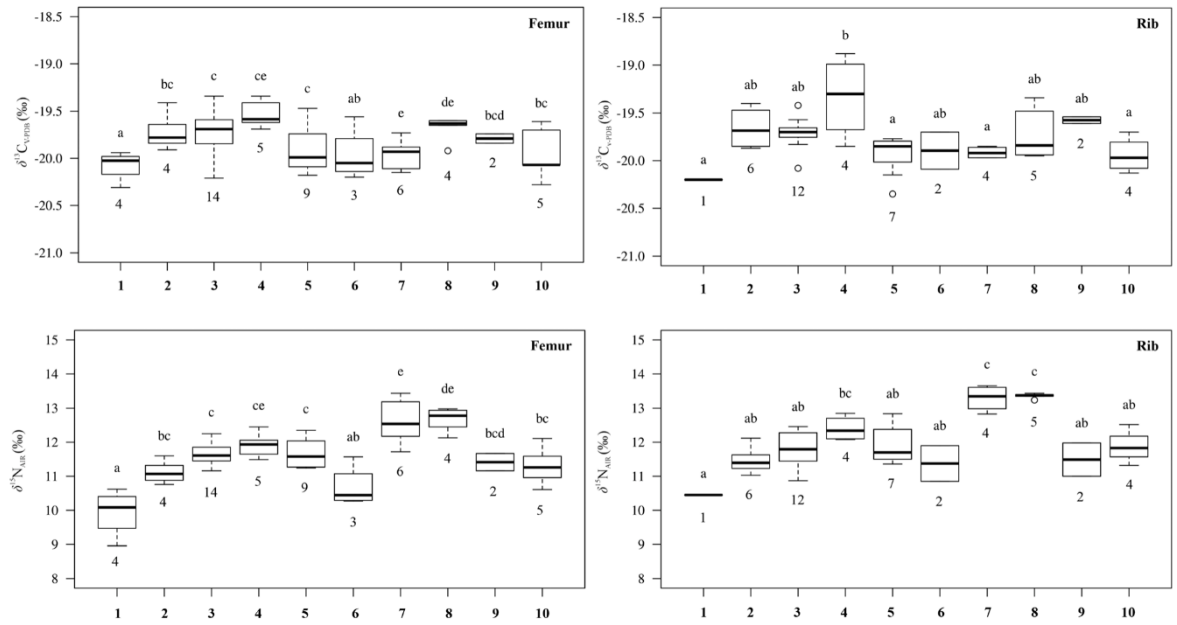


FIGURE 2 Boxplots of the isotopic values of particular families. Letters depict the results of the Tukey post hoc test: Families sharing the same letter do not differ from each other at the 0.05 significance level. Numbers depict the number of individuals in particular families. The boxes represent the interquartile range, and the whiskers the complete data range. The continuous lines illustrate median values

4.1 | Isotopic values in individuals with rare diagnoses

For the individuals with rare diagnoses, who could not be included in the statistical analysis, three criteria were considered to imply the impact of biological stress: First, carbon or nitrogen isotopic values in the rib and/or femur outside the range observed in cases of sudden death and deviant in terms of the stress pattern. Second, a rib–femur offset higher than 0.4‰ for $\delta^{15}N$ and −0.2‰ for $\delta^{13}C$. And finally, a deviation of $\delta^{15}N$ or $\delta^{13}C$ from the family median outside the range observed in sudden death cases and deviant in the sense of the stress pattern (supporting information file no. 7). The individuals meeting at least two of these three criteria are discussed below.

No. 7 died from CVA, which however occurred as a consequence of chronic disease linked to hypertension and showed highest rib $\delta^{15}N$ within the framework of his family, being 0.7‰ above the family median.

No.15, who died from complications linked to non-insulin-dependent *diabetes mellitus*, showed one of the highest $\Delta^{15}N_{rib\text{-}femur}$ values (0.7‰), and his rib $\delta^{15}N$ was exceptional within the framework of his family, being 0.5‰ above the family median.

No. 35, who died from emphysema, showed exceptionally high $\delta^{15}N$ in the rib sample, even within the framework of his family (1.1‰ higher than the family median).

No. 48 showed one of the highest rib $\delta^{15}N$ values in the whole dataset (13.6‰). According to the death certificate, he died from heart failure, but the paleopathological examination revealed formations suggesting metastases in his vertebral bodies and thus the presence of an un-diagnosed carcinoma.

Finally, No. 69, who died from kidney failure, showed one of the lowest $\delta^{13}C$ values (−20.1‰ in rib sample) together with relatively high $\delta^{15}N$ in both femur and rib samples and her rib $\delta^{15}N$ values were exceptional within the framework of her family, being 0.7‰ above the family median. In this regard, mention needs to be made of another case of kidney failure, individual No. 47, who showed the highest $\Delta^{15}N_{rib\text{-}femur}$ value (1.1‰), although within the framework of her family, her $\delta^{15}N$ value was among the lower, being 0.6‰ below the family median. A third individual (No. 40), who had a kidney failure of chronic progress, lacked a preserved rib, and the value of her evidence is thus limited; her femoral carbon isotopic value was relatively low (−20.3‰).

4.2 | Isotopic values in pathological bone

At the first glance, isotopic values in the pathological bone samples are within the variation observed in healthy rib and femur samples. However, taking into account the dominant effect of family membership on isotopic values, our interpretation has to depend mainly on the isotopic offset between the pathological samples and femurs ($\Delta_{patho\text{-}femur}$), the offset between pathological tissues and the comparative samples of healthy tissue ($\Delta_{patho\text{-}healthy}$), and comparison with the rib values of members of the families concerned ($\Delta_{patho\text{-}family}$).

TABLE 4 The effect of the predictors as modeled by the linear model after the choice of the best model by forward selection using the Akaike information criterion (AIC)

FEMUR						
	$\delta^{13}C$			$\delta^{15}N$		
	DF	F value	p-value	DF	F value	p-value
Year of death	Not included into the model			Not included into the model		
Age-at-death	Not included into the model			1	1.1	0.300
Sex	1	0.4	0.520	Not included into the model		
Family membership	**9**	**15.8**	**<0.001**	**9**	**16.6**	**<0.001**
Presence of chronic disease	Not included into the model			Not included into the model		
Diagnosis	Not included into the model			3	0.8	0.524
RIB						
	$\delta^{13}C$			$\delta^{15}N$		
	DF	F value	p-value	DF	F value	p-value
Year of death	1	1.7	0.196	Conditions of the model not met		
Age-at-death	Not included into the model					
Sex	1	2.2	0.143			
Family membership	**9**	**3.7**	**0.002**			
Presence of chronic disease	Not included into the model					
Diagnosis	Not included into the model					
RIB-FEMUR OFFSET						
	$\Delta^{13}C$			$\Delta^{15}N$		
	DF	t- value	p-value	DF	F value	p-value
Year of death	Not included into the model			Not included into the model		
Age-at-death	Not included into the model			1	2.0	0.101
Sex	-	-1.6	0.110	Not included into the model		
Family membership	Not included into the model			9	4.2	0.056
Presence of chronic disease	Not included into the model			Not included into the model		
Diagnosis	Not included into the model			3	0.4	0.778

Note: *p* values significant at the 0.05 level of significance are in bold font.

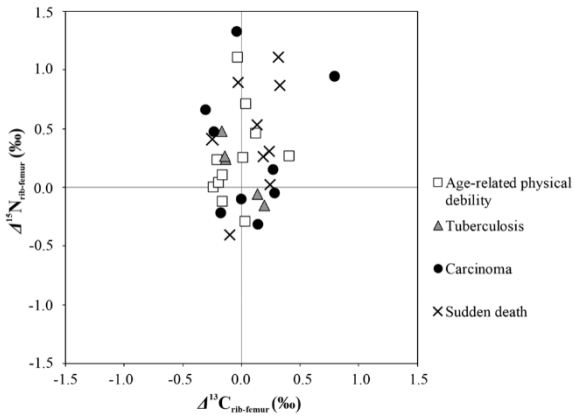


FIGURE 3 Rib–femur isotopic offsets according to particular diagnoses (age-related physical debility; tuberculosis; and carcinoma) compared to sudden death cases

Two cases of osteomyelitic bone (Nos. 2 and 11; supporting information file no. 8) show only minimal isotopic deviations from the comparative samples of femur, rib, and in the case of No. 11 a healthy sample from the counterpart acetabulum. For No.2, $\delta^{15}N$ is 0.3‰ lower than the family median, and $\delta^{13}C$ is close to the median. In No. 11, $\delta^{15}N$ is 0.5‰ lower than the family median and $\delta^{13}C$ is close to the median. In case of rheumatoid arthritis (No. 17; supporting information file no. 8), the $\Delta^{15}N_{patho\text{-}femur}$ is slightly positive, in a case of erosive lesion of the humerus above the 0.4 cutoff value. However, similar values were observed in comparative sample of the healthy tissue of the same humerus and in the counterpart healthy acetabulum. Carbon isotopic values were also slightly increased in comparison to the femur but were similar in both pathological and healthy comparative samples. Nitrogen isotopic values are above the median of the concerned Family 3 but only by 0.3‰.

All four cases of metastatic carcinoma, on the other hand, show increased $\delta^{15}N$ (Figures 5 and 6a). In No. 19 (lung carcinoma), highly

TABLE 5 The impact of year of death after adjustment for the influence of family: Likelihood-ratio test of mixed-effect models on $\delta^{15}N/\delta^{13}C$ in the femur/rib and the rib–femur isotopic offset

Femur													
	$\delta^{13}C$							$\delta^{15}N$					
	Df	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**	**Df**	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**
Effect of family only	3	−1.8	3.9	−7.8				3	115.8	−54.9	109.8			
Effect of family and year of death	4	0.2	3.9	−7.8	0.038	1	0.845	4	117.8	−54.9	109.8	<0.001	1	0.989
Rib													
	$\delta^{13}C$							$\delta^{15}N$					
	Df	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	**p-value**	**Df**	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	**p-value**
Effect of family only	3	10.8	−2.4	4.8				3	94.7	−44.4	88.7			
Effect of family and year of death	4	12.3	−2.2	4.3	0.505	1	0.477	4	88.4	−40.2	80.4	**8.374**	**1**	**0.004**
Rib–femur offset													
	$\Delta^{13}C$							$\Delta^{15}N$					
	Df	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	**p-value**	**Df**	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	**p-value**
Effect of family only	3	−5.5	5.7	−11.5				3	57.8	−25.9	51.8			
Effect of family and year of death	4	−4.3	6.1	−12.3	0.782	1	0.376	4	52.6	−22.3	44.6	**7.263**	**1**	**0.007**

Note: *p* values significant at the 0.05 level of significance are in bold font.

increased $\delta^{15}N$ was observed not only in the metastasis affecting the right scapula ($\Delta^{15}N_{patho\text{-}femur} = 1.3‰$) but also in the fracture callus on the left tibia ($\Delta^{15}N_{patho\text{-}femur} = 1.1‰$). The $\Delta^{13}C_{patho\text{-}femur}$ of these samples was slightly but significantly negative (between 0.3 to 0.8‰). Samples of healthy rib tissue and the compact bone surrounding the fracture callus show isotopic shift in the same direction but of much lesser extent ($\Delta^{15}N_{sample\text{-}femur} = 0.5‰$), while healthy tissues of the right scapula and the right tibia show isotopic values close to the value observed in the femur.

In No.22 (prostate cancer), a $\Delta^{15}N_{patho\text{-}femur}$ of around 0.9‰ was observed in both the metastasis and a recently healed fracture of the rib. Similar values were also shown by the comparative samples of healthy rib tissue. The compact bone surrounding the metastasis showed a slightly but not significantly lower value.

In No. 28 (stomach cancer), a high $\Delta^{15}N_{patho\text{-}femur}$ of 1.4‰ was observed in the metastasis affecting the occipital. The healthy rib value was almost identical, and the healthy occipital fragment showed only slightly lower $\delta^{15}N$ ($\Delta^{15}N_{patho\text{-}healthy} = 0.3‰$). The carbon isotopic values of all three samples were almost identical to that of the femur.

In No. 39 (officially cause of death was age-related physical debility, although metastatic cancer was revealed by paleopathological examination), the metastasis affecting the lumbar vertebra showed the highest $\Delta^{15}N_{patho\text{-}femur}$ of 1.6‰, with the healthy part of the same vertebra showing $\delta^{15}N$ only 0.3‰ lower. The carbon isotopic values were again similar to those of the femur.

Comparison of pathological samples with the values observed in the ribs of the concerned families (Figure 6b) revealed that the $\delta^{15}N$ of the former ($N = 3$) are 0.4–1.3‰ higher than the family median. An analogous pattern can also be observed in the healthy rib tissue of these individuals ($N = 4$), with $\delta^{15}N$ being 0.5‰–0.9‰ above the family median. In cases of carcinoma without skeletal metastases, deviations from the family median in rib samples are much lower and are comparable to individuals who died suddenly. For carbon isotopic values, the pattern is not so obvious: deviations in both the metastases and ribs of metastatic cases are higher than in cases without skeletal metastases, but mostly within the variation observed in individuals who died suddenly. In femoral samples, deviations from the family median observed in cases of metastatic carcinoma did not differ from other groups.

5 | DISCUSSION

The isotopic values of the studied dataset are typical for a C3 based diet with a relatively large proportion of animal products. This accords

TABLE 6 The impact of diagnosis after adjustment for the influence of family membership: Likelihood-ratio test of mixed-effect models on $\delta^{15}N/\delta^{13}C$ in the femur/rib and the rib–femur isotopic offset

	$\delta^{13}C$							$\delta^{15}N$						
Femur	**Df**	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**	**Df**	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**
Effect of family only	3	0.7	2.7	−5.3				3	69.7	−31.9	63.7			
Effect of family and diagnosis	6	5.1	3.5	−6.9	1.598	3	0.660	6	74.8	−31.4	62.8	0.887	3	0.829
Rib	$\delta^{13}C$							$\delta^{15}N$						
	Df	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**	**Df**	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**
Effect of family only	3	17.0	−5.5	11.0				3	66.8	−30.4	60.8			
Effect of family and diagnosis	6	19.6	−3.8	7.6	3.371	3	0.338	6	68.7	−28.4	56.7	4.109	3	0.250
Rib–femur offset	$\Delta^{13}C$							$\Delta^{15}N$						
	Df	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**	**Df**	**AIC**	**logLik**	**Deviance**	**Chi-square**	**Df**	***p* value**
Effect of family only	3	4.5	0.7	−1.5				3	43.5	−18.7	37.5			
Effect of family and diagnosis	6	6.5	2.7	−5.5	4.023	3	0.259	6	47.9	−18.0	35.9	1.563	3	0.668

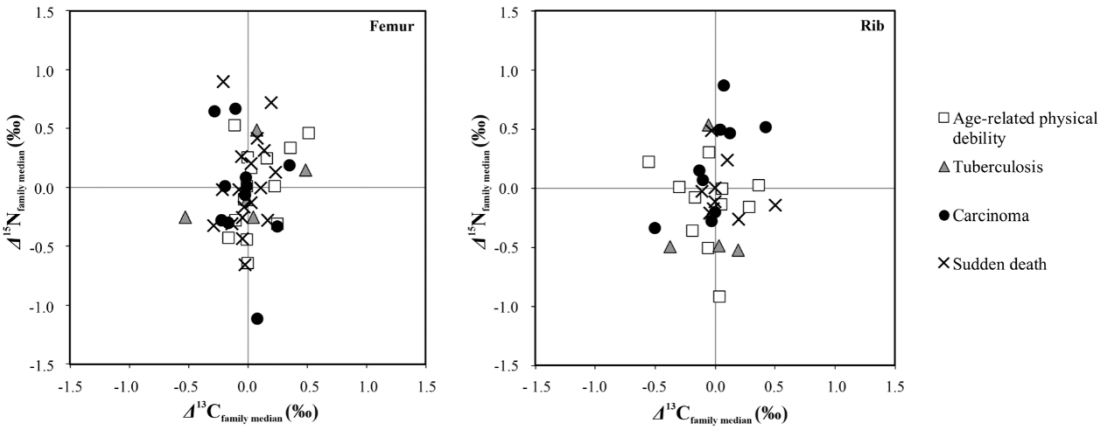


FIGURE 4 Isotopic offsets against the family median according to particular diagnoses (age-related physical debility; tuberculosis; and carcinoma) when compared to sudden death cases

with historical sources from the 19th and the beginning of the 20th centuries, describing the dominant role of C3 cereals (mainly rye and wheat), which represented the dietary base of all socioeconomic strata. Among the lower socioeconomic classes in particular, these were accompanied by potatoes. Meat was relatively scarce, with an average consumption of 7.5 kg/year in the 1850s. There was however a strong socioeconomic gradient in meat consumption. Milk was an important source of animal protein, which could contribute to the observed isotopic pattern, consumed mainly in its natural form while dairy products were relatively scarce in comparison with some other European countries (Lenderová et al., 2017). Similarly high $\delta^{15}N$ and a similar range of nitrogen isotopic values are commonly found in other datasets dated to the 19th century (Beaumont et al., 2013; Jørkov & Gröcke, 2017; Nitsch et al., 2010; Sarkic et al., 2019; supporting

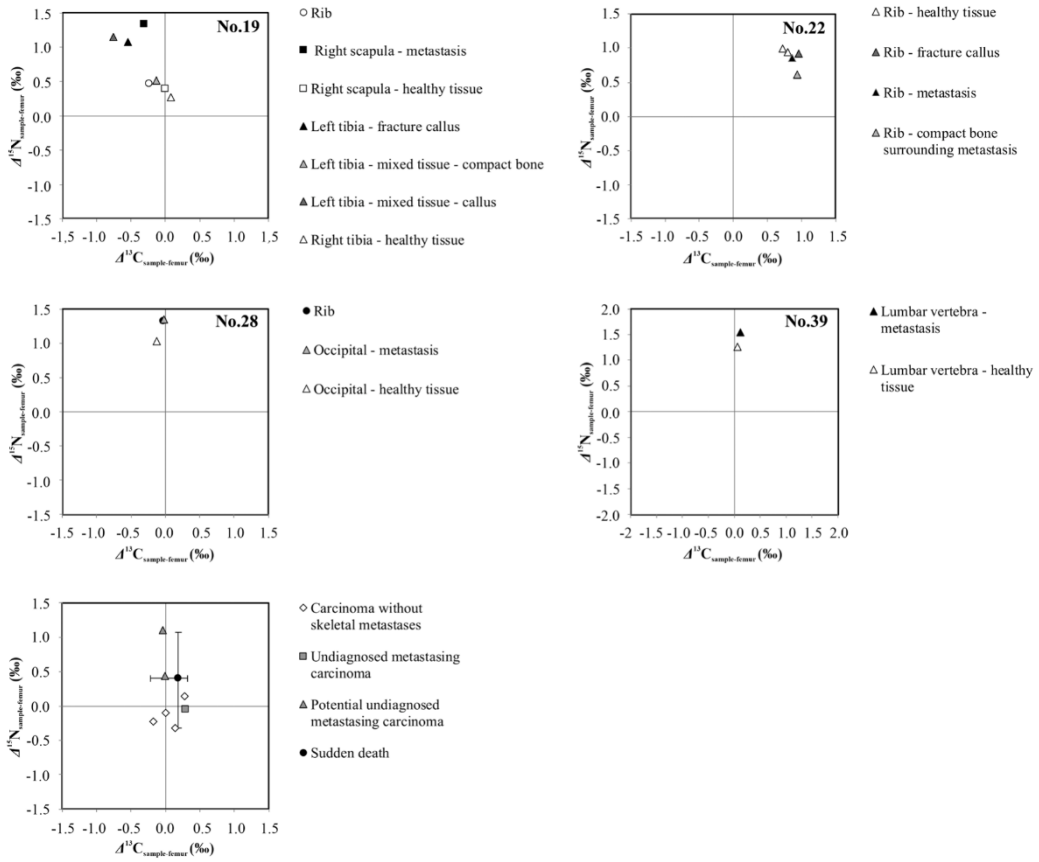


FIGURE 5 Isotopic offsets between the particular bone segment and healthy femoral tissue in cancer cases with skeletal metastases. For comparison, values of carcinoma cases without skeletal metastases and sudden death cases (median, percentile 2.5 and 97.5) are shown

information file no. 9). A study by Salesse et al. (2013) shows that a similar dietary pattern was already common in early modern Prague. On the other hand, lower $\delta^{15}N$ are typical for the contemporary population of central Europe (Hülsemann et al., 2015). This is in accordance with the lower $\delta^{15}N$ observed in the two most recent individuals in our dataset (died 1999 and 2006). Carbon isotopic values are also similar to those observed by Salesse et al. (2013), showing a narrow range typical for a terrestrial C3 plant environment. This is in accordance with the evidence from other sources suggesting that millet, popular during the Middle Ages, lost its significance in central European agriculture at the beginning of the Modern era in favor of potatoes, which are better suited to local environmental conditions. Corn, which became increasingly popular in southwestern parts of the Europe during the 19th century, never gained such importance in this geographical area (Lenderová et al., 2017; Winklerová, 2011).

Before discussing the impact of the particular factors, it is necessary to define significant isotopic offset: as analytical uncertainties in the isotopic measurements are below 0.2‰ for $\delta^{13}C$ and 0.4‰ for $\delta^{15}N$ at 2 standard deviations, these have been chosen as the cutoff values to define biologically significant isotopic offset. This criterion is used mainly when interpreting the individual cases but also as a complementary argument when interpreting the differences between different subsamples. Katzenberg and Lovell (1999) observed the nitrogen isotopic variation within a single non-pathological bone of three individuals, who died from stroke, heart attack, and car accident, as ranging from 0.3‰ to 0.4‰. For carbon, this variation is higher than our cutoff value, ranging from 0.2‰ to 0.7‰, but their sample came from an environment with more isotopically variable foods (i.e., marine fish and C4 plants).

5.1 | Impact of year of death, sex, age, and parity status

During the period under study, the isotopic values of the individuals may have changed systematically in two ways. First, isotopic shifts at the base of the food chain can be supposed for both ^{13}C and $\delta^{15}N$ due to the unprecedented anthropogenic impact that followed the industrial revolution. These include the overall changes that have been observed in the isotopic composition of atmospheric CO_2, due to increased emissions of isotopically light fossil fuel (Marino & McElroy, 1991). For nitrogen, the fertilization of fields with industrial

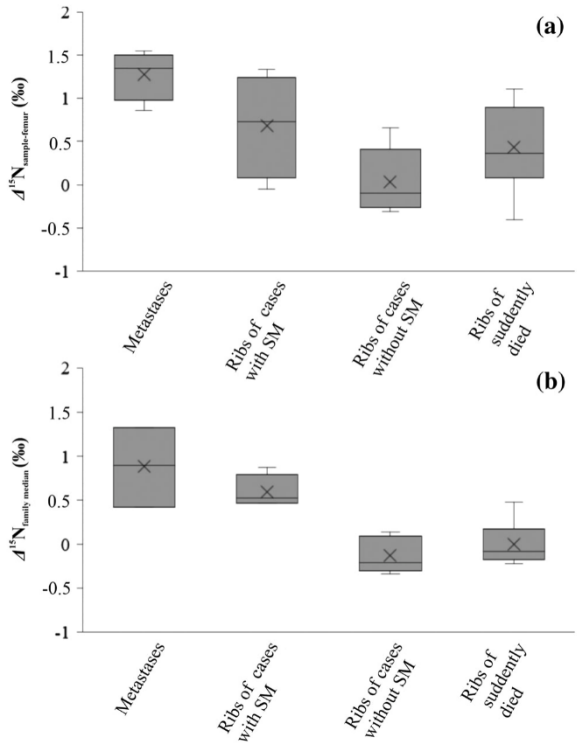


FIGURE 6 Nitrogen sample-femur offsets (a) and offsets against the rib family median (b) in samples from metastatic carcinomas when compared with carcinomas without skeletal metastases (SM) and sudden death cases. The boxes represent the interquartile range and the whiskers the complete data range. The continuous lines illustrate median values and the crosses illustrate mean values

manures, which were manufactured in the Czech Lands from 1872, could have decreased the $\delta^{15}N$ of crops in comparison to those where traditional organic manure was used (Bateman & Kelly, 2007). Second, human isotopic values may well have been changed by challenging dietary practices driven by huge societal changes. Before the 1940s, the diet of the Czech population was relatively stable, based mainly on local production of C3 plants and animal products, with the only disruption caused by the general food shortage during WW1 (Lenderová et al., 2015). WW2 too brought severe economic worries to the Czech Lands, with even the families of landowners being affected by substantial requisitions for the needs of the army. With the country not yet recovered from the hardship of war, the Czechoslovak communist coup d'état significantly changed the living conditions of the individuals under study. The nationalization of agricultural production, as well as the collective entrance of women into employment, forced a transition from locally and more traditionally grown foods to mass production under socialized agriculture. The following period brought a general decline of the Czech culinary tradition, with diet often based on starchy foods, sugar, and animal fats (Merhaut, 2015). For most of the individuals under study, who came from the families of landowners, the new regime meant the loss of the vast majority of their property, and a subsequent worsening of their social status (Rychlík, 2020; Snyder, 2012). After the regime change of 1989, diet in general became more variable with more vegetable and dairy products. The production area of the foods consumed was again enlarged significantly with the introduction of the open market (Merhaut, 2015). This change, however, could have impacted only the diet of the two most recent individuals (who died in 1999 and 2006). Although it is difficult to suggest the exact impact of the complex changes in the dietary regimen after 1948 and 1989 on carbon and nitrogen isotopic values, a notable decrease in $\delta^{13}C$ and $\delta^{15}N$ within the study period is hypothesized on the basis of the aforementioned isotopic shifts at the base of the food chain, and the significant worsening of social status beginning in the 1950s.

The absence of a significant impact of year of death on carbon isotopic values was thus quite surprising, taking into account that according to Ferrio et al. (2005, 2006) the average $\delta^{13}C$ of atmospheric CO_2 decreased by approximately 1.6‰ between 1857 and 2006, that is, the years of death of the earliest and most recent samples in our dataset. The observed pattern may at least partially be caused by the uneven distribution of the dates of death in the dataset. When excluding the two individuals who died after 1999, the isotopic shift suggested by the data of Ferrio et al. for the interval 1857–1974 is only 0.8‰. It is necessary to consider that in many parts of Europe, the industrialization process was quite advanced in the second half of the 19th century, with Bohemia playing a pioneering role in the industrialization of the Austro-Hungarian monarchy (Jakubec & Jindra, 2007). The local isotopic values of atmospheric CO_2 may thus already have been lower than those in the study by Ferrio et al., which was based on observations of Antarctic ice cores. It is however true that neither of the two individuals who died after 1999 deviate from the rest of the dataset. Analogically, Sarkic et al. (2019) did not observe systemic temporal shifts in $\delta^{13}C$ from a 16th–20th century community of nuns in Spain.

For nitrogen, a notable impact of the year of death on isotopic values was observed in ribs, following the hypothesized pattern of decreasing $\delta^{15}N$ through time, which could be explained in several ways. The interpretation is however complicated by the absence of an analogous pattern in femur samples. It is also true that the results of statistical operations are close to the border of statistical significance and the result of the linear mixed effect model actually loses its statistical significance when the two most recently born individuals are excluded. The observed discrepancy between the two sampled bones could however be partially due to the slower turnover of the femoral midshaft (Hedges et al., 2007); its isotopic signal covers a long period, varying substantially with the age of the individual, which may blur the expected correlation. It is apparent that the main decrease in rib $\delta^{15}N$ occurred around the time of WW2—$\delta^{15}N$ did not exceed 12.5‰ in any of the individuals who died after 1943. When cases of metastatic carcinoma (see below on their impact) are excluded, the trend appears even more pronounced, with the last individuals with rib $\delta^{15}N$ above 12.5‰ dying during the 1930s (Figure 1). The observed pattern thus accords with war-induced food shortage, followed by a

worsening of social status and the massive introduction of artificial fertilizers after 1948 (Merhaut, 2015; Rychlík, 2020; Snyder, 2012).

However, neither does this theory fully explain the observed shift, as the rib $\delta^{15}N$ of individuals born later do not fall below the level observed in femurs but rather approach it from higher values. It is therefore necessary to take into account the potential impact of the family and property organization of landowner families before WW2. Most of the individuals died not as active farmers but living within or close to the families of their adult children, to whom they gave over their farms. To ensure good living standards, they made contracts with their children, stating minimum quantities of crops required from the new farmer's household. For example, in a contract made in Family B between parents Nos. 1 and 2 and their son No. 3, the parents require 300 kg of wheat, 800 kg of rye, 100 kg of oat, 1000 kg of potatoes, 15 kg of poppy seed, 25 kg of salt, 25 kg of butter, one goose and one pig (at least 70 kg) annually, and 2 L of milk daily. From this example, it is evident that retired landowners had access to large amounts of food, which might have been of even higher quality than that of active farmers. This organization would surely have been disrupted after 1948 with former landowners being reliant on state pensions, which were usually very low for this unfavored group of declared "enemies of the state" (Rychlík, 2020).

Concerning the other tested factors, the difference in carbon isotopic values between femur samples of both sexes was significant when using univariate statistical testing. The actual difference between the male and female medians is only 0.2‰, which is within the analytical precision of the instrument; this difference is therefore probably not biologically significant. As this result was not confirmed by linear modeling, the observed pattern may well be caused, for example, by the different rates of males and females in particular families. Alternatively, this could reflect a slight difference in dietary behavior—for example, the diet of females could have included slightly more C4 plants; alternatively, males may consume slightly more fats, which are isotopically depleted (Schwarcz, 2002). However, quantitative dietary estimates such as FRUITS models (Fernandes et al., 2014) attest that an isotopic difference of such a small extent reflects a negligible dietary difference when the basic isotopically distinct food groups are considered. As the actual isotopic difference was of the same extent in both ribs and femurs (though in ribs, this result was not statistically significant), it is unlikely that this difference was caused by physiological shifts linked to the worsening health during the final years of life. It was expected that the isotopic stress pattern would be observed more often in individuals over 80 years, due to age-related physical debility. This diagnosis is assigned almost implicitly in modern clinical praxis to all individuals over 80 years and includes a number of senescent changes, including asthenia, cachexia, and general atrophy (World Health Organization, 2004). Age at death, however, had no impact on isotopic values. Parity status and time since the last birth also showed no significant correlation with isotopic values. This is however not surprising when the age composition of the dataset is considered, as the average age of the sampled females was 65.5 (±17) years and the average interval since the last birth 35 (±16) years. Previous research (Nitsch et al., 2010) has found some minor isotopic changes attributable to pregnancy only in the ribs of females who gave birth less than 5 years before death. Theoretically, the isotopic signal from the period of pregnancy could be preserved in the femoral tissue of elderly females due to its slow turnover, but this was not found in this study. It is possible that pregnancies—though often repeated several times—represented too brief a period in the lives of the sampled females to change their femoral isotopic values significantly. Moreover, isotopic shifts may change in opposite directions during a single pregnancy depending on female weight gain (or loss) and nitrogen balance (Fuller et al., 2004, 2005). Due to the age composition of the sample, the absence of a significant correlation between parity status and isotopic values cannot be taken as evidence that pregnancy does not influence bone isotopic values in general.

5.2 | Power of the family

Univariate tests and linear modeling both showed that family membership is the main factor affecting the isotopic values in both the femur and rib (Figure 2). To the best of the authors' knowledge, there is no study that would suggest linking genetically based variation in human metabolic processes to observable shifts in isotopic values. Moreover, several features observed in the dataset further suggest that the differences observed could be attributed rather to the distinct dietary habits of particular families. First, the isotopic differences among biologically related families (Nos. 1–4, 7, and 8) are higher than in the group of unrelated families (5, 6, 9, and 10). Second, of the 22 married couples, 10 are isotopically indistinguishable; that is, the differences between their femoral carbon and nitrogen isotopic values are lower than the analytical precision; in another five couples, there was a notable difference in only one isotope. Finally, nitrogen isotopic values, which are linked to the dietary proportion of animal protein (Lee-Thorp, 2008), correspond to the social status of the families. Though Family Nos. 1–3 and 7–9 were all families of landowners, and mostly biologically related (supporting information file no. 2), notable differences existed in their education, social status, and corresponding standard of living. Family No. 1 had the lowest social status. Although individual No. 41 married his second cousin in an effort to strengthen the importance of the family, all four of their descendants suffered from mental and physical disorders, and only one son and daughter survived to adulthood, living in complete poverty and subsisting mainly on vegetables. As is evident from Figure 2, the $\delta^{15}N$ values in the femurs of this family are the lowest in the studied dataset and differ significantly from all but Family No. 6. The same was true for carbon isotopic values. In contrast, Family Nos. 7, 8 and 3 had a high position in village society. Male members mostly received a good education and repeatedly served as village or district mayors and/or held functions in professional associations. At the same time, Family Nos. 7 and 8 showed the highest femoral $\delta^{15}N$, significantly different from most of the other families. The femoral nitrogen isotopic values of Family No. 3 are also high, though not to the same extent.

Of the families who were not direct agricultural producers (Nos. 4–6 and 10), Family No. 4 had the highest social status, with male members being well-educated officials employed by the state or aristocratic families. This family shows high femoral $\delta^{15}N$ with no difference between them and the aforementioned Family Nos. 7 and 8. Family No. 5 also included officials and also traders, foresters, and teachers. No. 10 is the family of a craftsman–carpenter. The social status of the Family No. 6, who were wage laborers, mostly working in the fields of the families mentioned above, was the lowest. Their femoral nitrogen isotopic values are the second lowest in the dataset, close to Family No. 1 and significantly differing from Family Nos. 3, 4, 5, 7, and 8. In the ribs the isotopic pattern was the same, although statistically significant differences were observed less frequently. This could be due to the higher impact of other factors, or—at least partially—to the smaller sample size.

All these features suggest that dietary differences linked with social status are the main force driving the observed isotopic variation.

5.3 | Impact of pathological conditions

Before discussing the impact of pathological conditions on the isotopic values in the dataset, it is necessary to describe the potential extent of the pathologically conditioned isotopic changes. Most of the current knowledge on the impact of cachexia comes from the context of restricted dietary intake. Concerning $\delta^{15}N$, Fuller et al. (2005) observed an increase of 0.3‰–1.0‰ in the hair of eight pregnant females suffering from episodes of morning sickness. In the hair of severely starved anorectic patients the nitrogen isotopic offset was between 0.6‰ and 2.2‰, but in most cases between 1.1‰ and 1.5‰ (Mekota et al., 2006). In an analogous study, however, Hatch et al. (2006) observed lower isotopic shifts, comparable to those from Fuller et al. (2005). In the hair of two patients with cancer, isotopic increases of 1.6‰ and 1.9‰ were observed by D'Ortenzio et al. (2015). Concerning dentine collagen data, Beaumont and Montgomery (2016) found the isotopic manifestation of nutritional stress in an approximately 1‰ rise in $\delta^{15}N$ in victims of the Great Irish Famine. In the context of documented skeletal collections, a still unique study by Katzenberg and Lovell (1999) reported nitrogen isotopic offset of 1.6‰ between non-pathological versus pathological sections of the osteomyelitic bone of an individual with AIDS accompanied with general wasting. Though the number of observations in all the studies mentioned is very low, based on their results, one might reasonably expect physiological stress to manifest in a shift in $\delta^{15}N$ between 0.4 (the upper range of analytical precision) and 1.5‰. This isotopic shift should be displayed to the full extent in the pathological bone samples, which were substantially remodeled during the time of the disease; these numbers however are expected to be rather at the lower boundary of the suggested interval in the non-pathological rib samples, which remodel at a much lower rate.

In the case of carbon, research to date has not found an accord on the direction or extent of isotopic shifts linked to starvation. Mekota et al. (2006) observed negative shifts in $\delta^{13}C$, which were higher than in $\delta^{15}N$. Fuller et al. (2005), Katzenberg and Lovell (1999), and D'Ortenzio et al. (2015) found no change in $\delta^{13}C$, while Neuberger et al. (2013) observed even cases with $\delta^{13}C$ increasing along with $\delta^{15}N$. This discrepancy has not yet been seriously discussed, but the stage of malnutrition together with intraindividual differences in the amount of body fat may well have played a role. In the early phases of malnutrition, carbon from energy sources may build up in the skeletons of endogenously synthesized nonessential amino acids, causing their depletion in ^{13}C, but later, the effect of catabolized body proteins may be prevalent (King et al., 2018; Salesse et al., 2019b).

The data from this study do not show a systemic impact of the presence of chronic disease on carbon and nitrogen isotopic values in the femur and/or rib. It must however be taken into account that the "chronic disease" group includes a number of diagnoses of different origins and pathophysiological mechanisms. Though immobility, impaired nutrition, and subsequent wasting are common in the terminal stages of the included diseases, their extent and duration may vary substantially. Moreover, other mechanisms influencing protein metabolism (e.g., nitrogen retention) may play a role in particular diseases (Reitsema, 2013; Welle, 1999).

Though wasting and cachexia are typical of all three diagnoses tested separately (cancer, age-related physical debility, and TBC), a substantial impact of the diagnosis on isotopic values was not observed, even when they were adjusted for the major impact of family membership (Figures 3 and 4). The only notable pattern, although different than that expected for cases of cachexia, was observed in the TBC cases, which show relatively low rib $\delta^{15}N$ (supporting information file no. 6), about 0.5‰ lower than the family median, in three of the four individuals. The linear mixed effect model, however, did not confirm the statistical significance of this pattern. This may be due to individual No. 9, who diverges from this trend to show a value above the family median. In this case, however, the interpretation is complicated by the severe comorbidity of liver cirrhosis. As the liver is the essential site for the synthesis of a majority of nonessential amino acids as well as for the removal of nitrogen via urea production, the loss of its function results in a severely altered protein metabolism. The presence of cirrhosis does not, however, explain the distinct isotopic pattern of No. 9. Though malnutrition and muscle wasting is common in these patients, Petzke et al. (2006) observed significantly lower $\delta^{15}N$ in the hair of cirrhotic patients than in healthy controls, potentially as a result of nitrogen retention. Given that the rib–femur isotopic offset is within the analytical precision, the evidence for an altered nitrogen balance in this individual is low. Besides the potential effect of competing factors, an alternative explanation may be found in the autopsy protocol, according to which No. 9 showed a good nutritional status at the time of death despite the severe diagnoses he suffered from. This may suggest the relatively rapid progress of both diseases, with death occurring before any notable decay of the organism could take place.

Interesting information yielded by the autopsy protocol for No. 73, which describes advanced cachexia (*cachexia gravis*), atrophy of the muscle tissue and general asthenia. Despite the

unexceptionable presence of cachexia, there is no indication of a stress isotopic pattern: the rib nitrogen isotopic value of this individual ($\delta^{15}N = 10.6‰$) is below the family median ($\delta^{15}N = 11.4‰$; although only two individuals had preserved ribs) and close to the femur family median ($\delta^{15}N = 10.3‰$). The rib–femur isotopic offset is within the analytical precision for both carbon and nitrogen.

In individual 43, it was not possible to compare rib isotopic values to the rest of the family as most of the individuals from Family 1 have no preserved ribs. Thus, the only possible comparison with typical family values could be done through contrasting his rib isotopic values ($\delta^{13}C = -20.2‰$; $\delta^{15}N = 10.5‰$) to the femur family median ($\delta^{13}C = -20.0‰$; $\delta^{15}N = 10.1‰$). This, together with the femur rib isotopic offset ($\Delta^{13}C = -0.2‰$; $\Delta^{15}N = 0.5‰$), indicates a notable, albeit not extremely strong, isotopic stress pattern. This could be due to the combination of two factors: first, individual 43 suffered from miliary TBC, where the *Mycobacterium tuberculosis* spreads into the whole body including the intestinal tract (Sharma et al., 2005), which was severely altered according to the autopsy protocol. Second, the individual was one of the youngest in our dataset (20 years) and thus showed a higher bone turnover rate.

At the first glance, the presence of carcinoma does not significantly affect the isotopic values in the rib and femur. This was quite surprising as first, the presence of a tumor increases whole-body protein turnover, and second, cachexia is common in cancer patients, resulting from the interaction of anorexia and malnutrition, together with increased levels of cytokines and other catabolic hormones, the impact of surgery (post-surgical proteolysis; Ward et al., 1985) and the protein metabolism of the tumor (Welle, 1999). In the most recently dated individuals (especially No. 37, died 1970), the effect of chemotherapy, which stimulates a negative nitrogen balance, cannot be excluded (Oktábec & Jampílek, 2013). The observed pattern changes however when separating a group of carcinomas with skeletal metastases (Figures 5 and 6). Concerning the tissue of the metastasis itself, all the samples ($N = 4$; No. 36 cannot be sampled due to the bad state of preservation) show $\Delta^{15}N_{patho\text{-}femur}$ similar in extent to the isotopic changes observed by Mekota et al. (2006), Katzenberg and Lovell (1999), and D'Ortenzio et al. (2015), corresponding to a prolonged state of cachexia and negative nitrogen balance. These values are also exceptional in the framework of the families. Carbon isotopic offsets are much more variable (in both directions) and thus confirm previous findings that the response of the organism to physiological stress may differ between individuals. The only individual (No. 19) showing an isotopic shift toward more negative values, which are typical of a stress reaction, was according to the autopsy protocol still obese at the time of death and thus may well have mobilized his fat stores to synthetize the missing proteins. Regrettably, the other relevant individuals did not undergo autopsies so it is not possible to compare the carbon isotopic values to their habitus at the time of death.

Interestingly, $\delta^{15}N$ levels in the healthy rib tissue of these cases ($N = 4$; in No. 39, no rib was present) are also notably increased when compared to femurs (with the exception of No. 36), and also high within the family. A notable rib–femur offset is also present in both individuals with the probable presence of metastases of un-diagnosed carcinoma (Nos. 46 and 48). The healthy parts of the bones affected by metastases showed increased $\delta^{15}N$ in all cases with the exception of No. 19. The increased isotopic values observed in the fracture calluses of Nos. 19 and 22 may be explained by the timing of their injuries. In No.19, the fracture of the tibia occurred about 1 year before death, so development and remodeling of the fracture callus may well have taken place during the development of the cancer. It is however impossible to separate the impact of the cancer from the impact of the injury itself (Olsen et al., 2014; Salesse et al., 2019a). In No. 22, a rib fracture took place shortly before death as a direct outcome of the attenuation of the rib by the numerous metastases and the patient's severe cough.

Although the number of individuals with metastatic carcinoma is low ($N = 5$), the present data suggest the impact of negative nitrogen balance during cachexia not only on the isotopic values of bone metastases and the other areas where new bone was formed during the disease, such as fracture calluses, but most probably also on macroscopically healthy skeletal elements with a higher turnover rate—that is, ribs and the healthy parts of the affected bones. The extent of these changes is surprising, as in the study by D'Ortenzio et al. (2015), isotopic shifts were notable as late as at 3 and 4 months before death in the hair of two cancer patients. Although there has been some indication of pathologically determined isotopic shifts in the macroscopically healthy bone areas close to the lesion site in the case of osteomyelitis, these were of a much lesser extent (Olsen et al., 2014). In this light, it is important to mention that in the two cases where the healthy parts of the affected bones showed the highest increase ($\Delta^{15}N_{sample\text{-}femur} > 1‰$), these come from bones with a high proportions of trabecular bone, which shows a higher turnover rate (Hill & Orth, 1998). Although trabecular bone was mostly separated during sample preparation, this may not have been complete in more cancellous bones such as the vertebrae and occipital.

There is a striking difference between rib isotopic values in individuals with bone metastases and without them. The explanation may lie in the combination of several factors: first, for tumors, an increase in the number of cells (hyperplasia) as well as an increase in nuclear size due to the multiplication of DNA and nucleoproteins is typical. This vastly increases the need for nitrogen, to the extent that tumors may be called "nitrogen traps." On the other hand, the decay of the tumor cells is also markedly increased, which results in the precipitated turnover of nitrogen within the body (Kodama & Nakayama, 2020; Kurmi & Haigis, 2020; Medina et al., 1992). Although Welle (1999) suggests that the direct impact of increased protein turnover in tumor tissue on the whole-body protein metabolism is rather minor due to the relatively small proportion of tumor mass to body weight, this impact may be strongly enhanced along with an increasing number of secondary sockets—metastases.

Second, bone tissue may be invaded by the tumor cells even in areas where the bone still appears healthy at a macroscopic level. This may well explain the increase in $\delta^{15}N$ in non-pathological parts of bones affected by metastases. This may also contribute to the high nitrogen isotopic offset between macroscopically healthy ribs and

femurs, as the attack by tumor cells on the ribs is much more prevalent than on the long bones of the limbs (Qiu et al., 2015; Quint et al., 1995; Riihimäki et al., 2016; Wen et al., 2019).

As there is likely a genetic component affecting individual susceptibility to cachexia (Tan et al., 2008, 2011), it is important to describe the mutual relationships between the affected individuals, who come from the four biologically related families (Families 1 and 3–5). However, actual biological kinship was present only between Individuals 19 and 39 (fourth degree), 19 and 22 (second degree), and 28 and 36 (third degree) (see supporting information file no. 2). Isotopic shifts suggesting cachexia were thus observed in three biologically independent lines.

It must also be taken into account that the observed isotopic shifts may well have been driven not solely by tissue catabolism but might have been amended by the attempts of caregivers to invert (or prevent) anorexia and wasting by the employment of a special dietary regime. Even in modern patients, easily consumed, digestible and protein rich foods, such as milk (when diarrhea is not present), eggs and stock comprise the basis of the dietary recommendations for cancer patients (Wilhelm, 2004). Similar dietary remedies could also have been applied in the past, with systematic medical recommendations concerning diet in use from the beginning of the 20th century (note that 1903 was the year of death for the oldest case of metastatic cancer in this study). One of the earliest expert recommendations for the diet of patients suffering from wasting diseases (H. Martin, 1906) focused on Nestlé milk biscuit flour (based on condensed milk, sugar, and wheat flour) as the foundation of the patients menu, accompanied by meaty stock thickened with hulled grains and jellied meat. Later, too (Fischl, 1936), the role of meaty stock was stressed as part of the diet for patients with cancer of the gastrointestinal tract.

5.3.1 | Rare diagnoses with and without skeletal manifestation and their impact on isotopic values

Although osteomyelitis—as a chronic infectious disease—has been found to be linked to increased protein turnover and catabolism (Welle, 1999), there were no notable isotopic shifts in the two present cases (supporting information file no. 8). This result differs from that of Olsen et al. (2014), who observed a strong indication of negative nitrogen balance in ostemyelitic bones. Salesse et al. (2019a), on the other hand, observed no systemic change of carbon or nitrogen isotopic values, suggesting that isotopic values in osteomyelitic bone may change along with the phase of the disease and the subsequent remodeling.

In this instance, no compelling evidence was found of the impact of rheumatoid arthritis on the isotopic values of No. 17 (supporting information file no. 8), although depletion of muscle protein and the protein catabolic state occur in such cases as a result of inactivity and the changed hormonal milieu (Welle, 1999). Today, these changes may be diminished in patients through strength training. However, in the family archive, a letter dated 1909 (8 years before death) was found in which the individual's husband asked the local council to approve the employment of a maid for the pub he managed, because of the illness and disability of his wife. Another indication comes from the family chronicle, which mentions that the woman "only sat during her last years."

The possibilities for explaining the isotopic values of No. 7 in the framework of his health status are limited due to an ambiguous diagnosis. According to the death certificate, he died from CVA, but the paleopathological examination revealed osteological changes at the inner surface of the cranial vault resulting from severe chronic hypertension. This can be linked with systemic changes in the human metabolism including acidosis (Adeva & Souto, 2011). Acidosis increases the turnover of body protein and the increased excretion of NH_4^+ during acidosis contributes to negative nitrogen balance (Welle, 1999). However, without knowing more about the comorbidities of No. 7, the mechanisms influencing the protein metabolism remain uncertain.

For non-insulin-dependent diabetes, there is no clear evidence of changed protein turnover or significant loss in body protein (Welle, 1999). Most research, however, has been conducted on subjects undergoing modern pharmacological treatment, which was not available to the individual concerned (No. 15, died 1913). Marchesini et al. (1982) found increased proteolysis in subjects with poor control of hyperglycemia. Regrettably, the age at onset of the disease is not known for this individual. It is however evident from his photo portraits that he lost weight dramatically during the last years of his life, which may suggest the presence of cachexia. In his testament, written 3 years before death, there are also some remarks that suggest notable health troubles were already occurring at that time.

In emphysema (No. 35) patients, hypoventilation may lead to acidosis with the consequences described above (Cohn et al., 1954). The episodes of acidosis may well have repeated during the recurrent attacks of the pulmonary disease which are described in his medical history.

Chronic kidney failure (Nos. 47 and 69) is associated with wasting. Anorexia is common in patients, together with hormonal abnormalities, for example, elevated levels of cytokines and cortisol. Acidosis is a common complication, with the effects described above (Garibotto et al., 2010; Welle, 1999). Further research is needed into this issue with the respect to the fact that the impact on protein excretion differs with the stage of the disease; at first, a significant amount of protein is lost in urine, while in the terminal stage polypeptide retention occurs.

In general, the isotopic results of the rare cases mentioned above suggest that the list of conditions causing long-term changes in nitrogen balance and subsequent isotopic shifts may be much longer than previously imagined. More work needs to be done to explore the outlined issues on much larger datasets.

6 | CONCLUSION

This study clearly shows that the dietary habits of particular families, linked to their social status, are the main force driving the carbon and

nitrogen isotopic values of the individuals concerned. The impact of other factors, such as sex, age at death, and parity status in the case of females, was not significant. Surprisingly, there was no relationship between carbon isotopic values and the year of death, which could theoretically be explained by the high degree of industrialization at the beginning of the studied period. For nitrogen, there was a trend of decreasing $\delta^{15}N$ in ribs, as was hypothesized based on the combination of agricultural and societal changes; this interpretation is however complicated by the absence of an analogical pattern in femur samples.

The data show the notable impact of metastastic carcinomas on the nitrogen isotopic values of (i) metastatic tissue, (ii) the healthy parts of bones affected by metastases, and (iii) healthy rib tissue. Carbon isotopic values were variable and did not show any clear pattern. Although more cases are needed to confirm this finding, the nitrogen isotopic shifts of 1‰–1.5‰ are high enough to significantly change the results of dietary reconstruction in the sense of overestimating the dietary proportion of animal protein. Prevention may lie in thorough X-ray examination of axial skeletons, which however is not a common tool in large skeletal samples. Alternatively, it is possible to diminish the chance of result distortion by avoiding the sampling of bones from the axial skeleton. Ribs especially are a very popular sampling site, as they are often fragmented and their utilization in osteological analysis is relatively low. To avoid the destruction of the long bones of the limbs, the sampling of the smaller long bones of the hands or feet may be a suitable solution, as these are rarely affected by metastases (Qiu et al., 2015; Quint et al., 1995; Riihimäki et al., 2016; Wen et al., 2019); their turnover rate is however also relatively rapid (Fahy et al., 2017). Further research is therefore needed into the sampling of other skeletal elements, as well as into individuals suffering from distinct types of cancer. The variation in carbon isotopic values confirms previous findings suggesting that the response of the organism to physiological stress may differ between individuals.

The isotopic data of several other individuals suffering from distinct diseases also suggest the potential impact of pathological processes. These include miliary TBC, non-insulin-dependent diabetes, emphysema, and chronic kidney failure. However, these cases are mostly unique in this dataset, and isotopic shifts are not so obvious as in case of metastatic carcinoma.

No indication was found of age-related physical debility, carcinoma without skeletal metastases, or lung tuberculosis significantly affecting bone isotopic values. This is true even for a case of severe cachexia documented by the autopsy protocol. However, these results cannot be generalized, as the age composition of the dataset may well have played a role: the average age at death was 77 years. In younger individuals, a higher bone turnover rate as well as distinct disease behavior (e.g., Chen et al., 2016; Saito et al., 2006) may cause isotopic shifts, which are not visible in this dataset. Familial susceptibility or resistance to developing cachexia (Tan et al., 2008, 2011) also requires further analyses to confirm these findings on biologically uninterlinked individuals.

ACKNOWLEDGMENTS

We would like to thank the following institutions for their financial support: the Czech Science Foundation (Grant number: 19-13265S), the Charles University Grant Agency (Grant Number: GAUK 1276217), and the Ministry of Culture of the Czech Republic (Grant number: DKRVO 2019-2023/7.II.c, 00023272). Alastair Millar helped to revise the English. We would especially like to thank all the families who provided the remains of their ancestors for this research and provided us with their private archives, namely, F. Andres Sr. and his family, P. Drba and his family, E. Palmová, P. Pauček, H. Kleczková and her family, F. Kopp, P. Eret, L. Cvrček, M., K. & T. Cvrček, and P. Čašek and his family. We also thank the late spouses K. Cvrček and J. Cvrčková and Z. Rezek, because this work was also created with their support.

CONFLICT OF INTERESTS

The authors declare no potential conflict of interests.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article (Online supporting information file No. 5).

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How to cite this article: Drtikolová Kaupová, S., Cvrček, J., Grossová, I., Hadrava, J., Půtová, L., & Velemínský, P. (2021). The impact of pathological conditions on carbon and nitrogen isotopic values in the bone collagen of individuals with known biographic data and medical history. *International Journal of Osteoarchaeology*, 1–20. https://doi.org/10.1002/oa.3022