Univerzita Karlova

1. lékařská fakulta

Studijní program: Postgraduální doktorské studium biomedicíny

Studijní obor: Fyziologie a patofyziologie člověka

MUDr. Alena Krajčoviechová

Genetické a molekulární mechanizmy hypertenze ve vztahu k zánětu, oxidačnímu stresu a chronickému renálnímu onemocnění

Dizertační práce

Vedoucí závěrečné práce/školitel: Prof. MUDr. Renata Cífková, CSc.

Praha, 2017

Charles University in Prague

First Faculty of Medicine

Study program: Doctoral study programs in biomedicine

Human Physiology and Pathophysiology

MUDr. Alena Krajčoviechová

Genetic and molecular mechanisms of arterial hypertension in relation to chronic inflammation, oxidative stress, and chronic kidney disease

Doctoral dissertation

Supervisor: Prof. MUDr. Renata Cífková, CSc.

Prague, 2017

Declaration:

I, Alena Krajčoviechová, hereby declare that this doctoral dissertation entitled "Genetic and molecular mechanisms of arterial hypertension in relation to chronic inflammation, oxidative stress, and chronic kidney disease" has been compiled by me under the supervision of Prof. MUDr. Renata Cífková, CSc. This dissertation contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. I also undertake that any quotation or paraphrase from published or unpublished work of another person has been duly acknowledged.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem řádně uvedla a citovala všechny použité prameny a literaturu. Současně prohlašuji, že práce nebyla využita k získání jiného nebo stejného titulu.

Souhlasím s trvalým uložením elektronické verze mé práce v databázi systému meziuniverzitního projektu Theses.cz za účelem soustavné kontroly podobnosti kvalifikačních prací.

V Praze dne 5.6.2017 ……

Alena Krajčoviechová

Podpis doktoranda

Identifikační záznam (Referencing):

KRAJČOVIECHOVÁ, Alena. *Genetické a molekulární mechanizmy hypertenze ve vztahu k zánětu, oxidačnímu stresu a chronickému renálnímu onemocnění. [Genetic and molecular mechanisms of arterial hypertension in relation to chronic inflammation, oxidative stress, and chronic kidney disease]*. Praha, 2017. Počet stran 80 , počet příloh 4. Dizertační práce. Univerzita Karlova, 1. lékarská fakulta, Centrum kardiovaskulární prevence 1. LF UK a TN. Školitel Prof. MUDr. Renata Cífková, CSc.

Abstract

This thesis provides an appraisal of the structure of clustering of metabolic phenotypes and evaluates the pathophysiological mechanisms underlying the relationship between urinary uric acid and albumin excretion. Two population-based studies were involved. In the first part, we used data obtained in a large representative cross-sectional survey in the Czech Republic (Czech post-MONICA study). We showed that the urinary albumin/creatinine ratio (uACR) was an independent factor for an increase in serum uric acid (SUA) levels in adults without manifest metabolic syndrome (MetS), but with 1–2 MetS component(s). Furthermore, SUA levels increased by the synergistic interaction of uACR with visceral adiposity and blood pressure, which may suggest obesity-related hypertension with altered renal hemodynamics as the primary mechanism. In the second part, we analyzed data captured in a representative population sample of French Canadians (CARTaGENE study) with more detailed urine biochemical analyses available. This study yielded two novel observations. First, we showed that the rs13129697 major T allele, which has been associated with increased SUA levels in our analysis as well as in prior publications, was associated with a paradoxical decrease in uACR. The reason for this discrepant finding is the interaction between rs13129697 genotype and the current rate of FEUA, suggesting tubular uric acid/albumin exchange. Second, using the mediation analysis approach, we propose that decreased urinary uromodulin and sodium excretion explain 70% of the relationship between decreased FEUA and increased uACR, which further supports the role of altered blood pressure regulation in this relationship. Finally, we identified two correlated but still unique principal components of the MetS and derived the principal component scores. With slight disparities, the first and second principal component scores exhibited comparable loading patterns between the studies. In the CARTaGENE study, urinary uromodulin explained 9% of the correlation between the principal component scores.

In conclusion, urinary uromodulin may not only be in a causal pathway between uric acid and albumin excretion but, also, to a much lesser extent, between the principal components of the MetS.

Key words: renal pathophysiology; metabolic syndrome; albumin/creatinine ratio; fractional excretion of uric acid; uromodulin

Abstrakt

Tato dizertační práce se zabývá metabolickým syndromem a patofyziologickými mechanizmy, které vysvětlují vztah mezi poklesem vylučování kyseliny močové ledvinami a albuminurií. Zpracovali jsme data z celkem dvou populačních studií. V první části jsme použili data z průřezového šetření reprezentativního vzorku české populace (studie Czech post-MONICA). Ukázali jsme, že u osob bez manifestního metabolického syndromu (MetS), ale s 1−2 kritérii MetS, je poměr albumin/kreatinin v moči (uACR) spojen se zvýšenou hladinou kyseliny močové v séru. Zároveň se hladina kyseliny močové v séru zvýšila synergickou interakcí mezi uACR s indexem viscerální adiposity a krevním tlakem. Ve druhé části jsme analyzovali data z reprezentativního vzorku populace Kanaďanů francouzského původu (studie CARTaGENE) s dostupnou detailnější analýzou moče. Tato studie přinesla dvě nová zjištění. Za prvé, ukázali jsme, že T alela rs13129697 polymorfizmu, která byla v dosud publikovaných pracích spojena se zvýšenou hladinou kyseliny močové v séru, je spojena s paradoxním poklesem uACR. Příčinou této diskrepance je interakce mezi rs13129697 genotypem a aktuální hodnotou FEUA, což podporuje hypotézu o tubulární výměně kyseliny močové za albumin. Za druhé, použitím mediační analýzy jsme prokázali, že pokles močové exkrece uromodulinu a sodíku vysvětluje až 70 % vztahu mezi poklesem FEUA a albuminurií, což dále zdůrazňuje roli porušené regulace krevního tlaku v tomto vztahu. Konečně, přidáním uACR společně s hladinou kyseliny močové v séru anebo FEUA k zavedeným složkám MetS jsme v obou populacích identifikovali dvě vzájemně související, ale jedinečné hlavní komponenty syndromu. S nepatrnými rozdíly vykazovaly první a druhá hlavní komponenta MetS srovnatelnou strukturu v obou studiích. Ve studii CARTaGENE vysvětlila močová exkrece uromodulinu 9 % korelace mezi hlavními komponentami MetS.

Lze tedy uzavřít, že pokles močové exkrece uromodulinu může být jedním z patofyziologických mechanizmů, který vysvětluje nejen pokles FEUA v přítomnosti albuminurie, ale i vztah mezi hlavními komponentami MetS.

Klíčová slova: renální patofyziologie; metabolický syndrom; poměr albumin/kreatinin v moči; frakční exkrece kyseliny močové; uromodulin

Acknowledgements

I would like to express my sincere gratitude to my mentor Prof. MUDr. Renata Cífková, CSc, for her patient guidance and strong academic support throughout the years.

I would also like to give special thanks to Prof. Pavel Hamet, MD, PhD, and his wife Prof. Johanne Tremblay, PhD, for the opportunity to undertake a significant part of the research at the Montréal University Research Center, Quebec, Canada. Thank you for your valuable advices and insightful suggestions.

My thanks also go to Prof. Philip Awadalla, PhD, for his assistance and help with acquiring the CARTaGENE study data and to Prof. Francois Madore, MD from the Sacré-Coeur Hospital Research Centre in Montréal, Quebec, Canada for providing us with the renal data.

I would also like to recognize my co-workers MUDr. Peter Wohlfahrt, PhD, and MUDr. Jan Bruthans, CSc, from the Center for Cardiovascular Prevention at Charles University in Prague and Thomayer Hospital, as well as Carole Long, Pierre Dumas, PhD, Francois−Christophe Marois−Blanchet, Francois Harvey, and Mounsif Haloui, PhD, from the Montréal University Research Center, Quebec, Canada – you all have contributed to the progress I have made.

Above all, my deepest expression of appreciation belongs to my partner, Ondřej, and my whole family for all the support, patience, and encouragement during my research.

Contents

List of Tables

List of Figures

Abbreviations

β	Beta coefficient
BP	Blood pressure
CI	Confidence interval
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eGFR	Estimated glomerular filtration rate
FEUA	Fractional excretion of uric acid
FENa	Fractional excretion of sodium
GFR	Glomerular filtration rate
HDL	High-density lipoprotein
Ln	Natural logarithm
MAF	Major allele frequency
MAP	Mean arterial pressure
MetS	Metabolic syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
OR	Odds ratio
PCA	Principal component analysis
RAS	Renin-angiotensin system
Sβ	Standardized beta
SBP	Systolic blood pressure
SD	Standard deviation
SNP	Single-nucleotide polymorphism
SLC2A9	Solute carrier family 2, member 9
SUA	Serum uric acid
TAL	Thick ascending limb of the loop of Henle
uACR	Urinary albumin/creatinine ratio
UAE	Urinary albumin excretion
uUCR	Urinary uromodulin/creatinine ratio
VAI	Visceral adiposity index

1 Introduction and literature review

1.1 Frame of the thesis

Arterial hypertension is a multifactorial disease characterized by elevated blood pressure (BP) and increased risk of cardiovascular events. Chronic kidney disease (CKD) represents abnormalities of kidney structure or function defined by decreased glomerular filtration rate (GFR), increased urinary albumin excretion (UAE) or histologic evidence of renal parenchymal injury. Arterial hypertension is tightly related to CKD with a deleterious feedback effect. The decrease in kidney function may be accompanied by an increase in BP, and uncontrolled hypertension accelerates kidney disease progression toward toward the end stage increasing also the risk of cardiovascular events *(Judd & Calhoun, 2015)*.

To date, the pathophysiological link between hypertension and CKD has remained only partially resolved; however, evidence suggests that it is not straightforward but, rather, reflects a complex cardiovascular disease (CVD) risk *(Dzau et al., 2006)*. Increased BP tends to cluster with visceral obesity, hypertriglyceridemia, and abnormal glucose metabolism, forming together a constellation of cardiovascular risk factors recognized as metabolic syndrome (MetS) *(Kahn et al., 2005)*. However, there are doubts about the construct of the MetS itself as the criteria are ambiguous, categorical thresholds are misleading and insulin resistance as the single underlying etiology remains questioned. In order to update the MetS construct, specific lines of investigation have been urged: (i) use of a multivariate score system, (ii) search for additional defining phenotypes, and (iii) search for the mechanism explaining the clustering *(Kahn et al., 2005)*.

Metabolic syndrome often co-occurs with increased serum uric acid (SUA) levels. Furthermore, uric acid becomes increasingly recognized as an emerging causal factor in the development of arterial hypertension, MetS *(Kanbay et al., 2016)*, and CVD *(Borghi et al., 2015)*. All uric acid-related adverse effects are supposed to be facilitated by reactive oxygen species (ROS) and oxidative stress with subsequent endothelial dysfunction *(Kanbay et al., 2013)*, which in turn is a major pathophysiological mechanism leading toward cardiovascular events. The SUA levels are predominantly determined by uric acid renal excretion, which is controlled by several urate transporters

(Lipkowitz, 2012). The solute carrier protein 2 family member 9 (SLC2A9) represents the major urate transporter identified to date *(Caulfield et al., 2008)*.

Increased UAE is an important risk factor in hypertensive patients further increasing the risk of cardiovascular events independently of estimated glomerular filtration rate (eGFR) *(Weir, 2007)*. In fact, increased UAE reflects renal structural changes detectable earlier than any substantial decline in eGFR *(Matsushita et al., 2015)*. Similar to increased UAE, it has been well documented that high SUA levels predict cardiovascular outcomes *(Borghi et al., 2015)*. However, the predictive power of SUA levels may decrease by adjusting for increased UAE, and vice versa, which suggests partially interrelated mechanisms between these risk factors *(Scheven et al., 2014)*. It is also of interest that hyperuricemia and increased UAE occur together in individuals with hypertension *(Viazzi et al., 2005)*, type 2 diabetes *(Resl et al., 2012)*, and *MetS (Rodilla et al., 2009)*.

The exact pathophysiological mechanism explaining the relationship between uric acid and UAE remains elusive. In one way, the link between SUA levels and UAE has been explained by assuming that hyperuricemia may induce oxidative stress and endothelial dysfunction resulting in increased UAE. The other way round, Scheven et al. have recently shown that albuminuria is associated with increased tubular uric acid reabsorption and increase in SUA levels *(Scheven et al., 2014)*. The authors suggested tubular uric acid-albumin exchange and speculated that either the urate transporters could be up/down-regulated in the presence of albuminuria, or the positive association of UAE with uric acid reabsorption is mediated by sodium transport. In line with Scheven et al., large body of evidence indirectly support the hypothesis that the effect of urinary uric acid excretion on UAE may be mediated by urinary uromodulin through modulation of sodium reabsorption in the thick ascending limb of the loop of Henle (TAL) *(Padmanabhan et al., 2014)*. Better clarification of the underlying pathophysiology may help to further elucidate the role of uric acid in hypertension, MetS, and CVD, and to develop targeted therapeutic approaches.

1.2 Arterial hypertension

1.2.1 Definition and epidemiology

Arterial hypertension is a complex disease with heterogeneous etiology. About 90−95% of hypertension cases are primary (so called polygenic hypertension) due to the interaction between genetic and lifestyle factors, such as high-salt diet, obesity, smoking, and excessive alcohol intake. The remaining 5−10% of hypertension cases are due to secondary causes such as endocrine disease, CKD, sleep apnoe syndrome, aortic coarctation, and rare monogenic disorders *(Poulter et al., 2015)*. Even though the relationship of BP values with cardiovascular and renal outcomes is continuous, the definition of arterial hypertension in everyday practice is based on arbitrary cut-off values of ≥140 mmHg for systolic blood pressure (SBP) and ≥ 90 mmHg for diastolic blood pressure (DBP) *(Mancia et al., 2013)*.

Arterial hypertension is the second leading cardiovascular risk factor, to which about 17.8% of the global annual deaths are attributed, and thus represents a major global health problem *(Murray et al., 2012)*. In 2010, almost one third of the world´s adults were hypertensive *(Mills et al., 2016)*. Even though the prevalence of hypertension appears to be stable or slightly decreasing in high-income countries, it remains alarmingly high in many developed European countries, mainly due to the aging of the population and increasing obesity *(Cifkova et al., 2016)*. In the Czech Republic, the longitudinal trends in the prevalence, awareness, and treatment of major cardiovascular risk factors have been monitored since 1985. To date, six cross-sectional population surveys with randomly selected adults aged 25−64 years residing in six districts of the Czech Republic have been conducted, with the latest one conducted in 2007/2008. Over the period of 22 to 23 years, there was a significant decrease in the prevalence of hypertension in women (from 42.5% to 37.2%; $p<0.001$) whereas the prevalence of hypertension in men remained unchanged (51.9% vs. 50.2%) *(Cifkova et al., 2010[a])*. Of note, the prevalence of hypertension is tightly associated with the prevalence of CKD. In the Chronic Renal Insufficiency Cohort trial examining the risk factors for kidney disease progression in CKD patients, the prevalence of self-reported hypertension was 86%, which is 3 times higher when compared with the general US population *(Lash et al., 2009)*.

1.2.2 Genetic component of arterial hypertension

The heritability estimates of arterial hypertension range between 30−68%, which implies a moderately to strongly heritable trait *(Hottenga et al., 2005; Miall & Oldham, 1963)*. There are rare monogenic forms of familial hypertension characterized by major gene defects with large effect size of about 10 mmHg for SBP. These rare monogenic genes follow the principles of Mendelian inheritance and involve molecular mechanisms of renal sodium handling or steroid hormone metabolism. Affected individuals develop hypertension at an early age and high BP is often accompanied by electrolyte and hormonal abnormalities *(Lifton et al., 2001)*. Contrarily, the genetic underpinning of polygenic hypertension is poorly understood. A large proportion of genome variance is contained in single-nucleotide polymorphisms (SNPs), which is a DNA sequence variation at a certain genetic position. Of note, more than 95% of the SNPs are located outside the expressed genomic regions. To date, more than 40 independent SNPs with small effect size (about 1 mmHg for SBP) have been identified using the genome-wide approach in large population samples. Even though these SNPs are common, they collectively explain only 1−2% of the BP variance, leaving a vast majority of the BP heritability unexplained (so called missing heritability). Another aspect is limited utility of these SNPs in risk prediction *(Ehret & Caulfield, 2013)*. In addition, most of these SNPs are localized in the regions whose link with BP was not expected, or exert a pleiotropic effect *(Padmanabhan et al., 2012)*. One example of the pleiotropic action are the common correlated SNPs localized near the uromodulin gene associated with hypertension *(Graham et al., 2014)*, and CKD *(Kottgen et al., 2010)*.

On the other hand, difficulties in deciphering the genetic basis of essential hypertension were expectable assuming that it represents a complex trait, and thus pertubations in several pathways and molecular mechanisms are involved in its pathogenesis. In addition, enviromental exposures may considerably modulate the genetic component of the BP level *(Waken et al., 2017)*.

1.3 Chronic kidney disease

1.3.1 Definition

Chronic kidney disease is defined as abnormalities of kidney structure or function present for ≥ 3 months, classified based on GFR and albuminuria category as indicated in Tables 1 and 2 *(CKD Work Group)*. The term decreased GFR refers to categories G3a−G5. Chronic kidney disease refers to categories G3a-G5 or UAE of ≥30 mg/24 hours sustained for ≥ 3 months. Urinary albumin excretion of ≥30 mg/24 hours corresponds to urinary albumin/creatinine ratio (uACR) of ≥3 mg/mmol in a random morning urine sample.

Table 1 GFR categories in CKD

GFR category	GFR (ml/min/1.73 m^2)	Terms
G1	≥90	Normal or high
G2	60−89	Mildy decreased
G3a	45−59	Mildly to moderately decreased
G3b	30−44	Moderately to severely decreased
G4	15−29	Severely decreased
G5	<15	Kidney failure

CKD, chronic kidney disease; GFR, glomerular filtration rate

Adopted from the CKD Work Group

Table 2 Albuminuria categories in CKD

Category	UAE (mg/24 hours)	uACR (mg/mmol)	Terms
A1	<30	<3	Normal
A2	30−300	3−30	Moderately increased (microalbuminuria)
A3	>300	>30	Severly increased (macroalbuminuria)

CKD, chronic kidney disease; UAE, urinary albumin excretion; uACR, urinary albumin/creatinine ratio

Adopted from the CKD Work Group

Risk factors associated with CKD include age, ethnicity, family history of kidney disease, drug use, smoking, socioeconomic status, low birth weight, diabetes mellitus, hypertension, obesity, and MetS *(Kazancioglu, 2013)*. Of note, diabetes mellitus and arterial hypertension account together for more than two thirds of all end-stage renal disease patients in the United States *(2016 USRDS annual data report, 2016)*. Similarly, diabetes mellitus and arterial hypertension were the greatest risk factors for CKD in Poland *(Zdrojewski et al., 2016)*.

1.3.2 Epidemiology of decreased GFR

In 2010, the age-standardized prevalence of CKD categories G3a−G5 in high-income countries in adults aged 20 years and older was 4.3% and 5.7%, respectively *(Mills et al., 2015)*. Contrarily, a considerable variation in CKD prevalence has been reported across European countries, ranging between 1% in central Italy and 5.9% in northeast Germany *(Bruck et al., 2016)*. This variation cannot be completely explained by differences in the prevalence of hypertension, diabetes, and obesity; however, other factors like differencies in socioeconomic status, dietary habits, and genetic susceptibility could potentially contribute to the variation of CKD prevalence in Europe *(Hamet et al., 2017; Pattaro et al., 2016)*.

1.3.3 Epidemiology of albuminuria

Although GFR is accepted as the best general marker of kidney function, UAE reflects renal damage detectable earlier than any substantial decline in GFR. Albuminuria defined as a uACR $\geq$ 3 mg/mmol is broadly accepted as a surrogate marker of cardiovascular risk and a therapeutic target in hypertension and diabetes *(Currie & Delles, 2013)*. In fact, according to the meta-analysis of 24 cohorts in the Chronic Kidney Disease Prognosis Consortium, the uACR outperformed eGFR in cardiovascular risk prediction, which was more evident in hypertensive and diabetic individuals *(Matsushita et al., 2015)*. In addition, UAE in healthy individuals was related to cardiovascular risk also within the normal range *(Arnlov et al., 2005)*.

The prevalence of increased UAE in the apparently healthy individuals ranges between 5 and 7% *(Hillege et al., 2001; Romundstad et al., 2003)*. On the contrary, increased UAE is present in 10% to 40% of hypertensive patients; its prevalence increases with duration and severity of hypertension and is higher among untreated individuals *(Horner et al., 1996; Parving et al., 1974)*.

1.4 Oxidative stress

Oxidative stress refers to increased levels of ROS, so called free radicals (superperoxide, hydrogen peroxide, etc.), and reduced antioxidative capacity of the cell to counteract the ROS effect. Short-term increased production of ROS is favorable for the immune system to annihilate pathogens. However, long-term oxidative stress is involved in the pathogenesis of multiple diseases including arterial hypertension, atherosclerosis, CVD, and kidney disease. Abundant ROS induce lipid peroxidation, oxidation of the nucleobases, and proteins resulting in damage to cell structures. Furthermore, superoxide produced by nicotinamide adenine dinucleotide phosphate (NADPH) or xanthine oxidase reacts with nitric oxide (NO) with subsequent formation of peroxynitrite. This has several consequences. First, the bioavailability of NO is reduced resulting in imbalance between contractile and relaxing factors in the endothelium (endothelial dysfunction), which is one of the major pathophysiological mechanisms leading to cardiovascular events. Second, peroxynitrite is an even more reactive free radical sustaining the cyclic reaction of ROS production *(Munzel et al., 2010)*. Finally, increased ROS production leads to enhanced activation of the renal and intracellular renin-angiotensin system (RAS), which in turn may induce arterial hypertension, activate pro-inflammatory transcription factors, enhance growth factors production, and induce fibrosis *(Fanelli & Zatz, 2011)*.

1.5 The role of the kidney in blood pressure regulation

1.5.1 Kidney function

The physiological role of the kidney is to control extracellular volume, acid base homeostasis, balance of plasma electrolytes, and excretion of metabolites. The

mechanisms of kidney function include filtration of plasma into the glomerulus, tubular reabsorption of water, electrolytes, glucose, proteins, and amino acids, and tubular secretion. Normally, the rate of plasma filtration (indicated as GFR) is approximately 125 ml/min or 180 liters each day with the majority of the ultrafiltrate being reabsorbed resulting in the production of about 1.5−2 liters of urine per day. The amount of solute finally excreted in the urine may be expressed by volume, a ratio to creatinine, or fractional excretion ($Solute_{urine}/Solute_{serum}$ divided by $creatinine_{urine}/creatinine_{serum}$).

1.5.2 Pressure-natriuresis relationship

Blood pressure level is the result of cardiac output and peripheral vascular resistance, which are controlled by a network of pathways of extracellular volume homeostasis, cardiac contractility, and regulation of vascular tone. The kidney plays a key role in BP control through regulation of sodium and water excretion and vascular tone. The process of renal control of sodium and water balance is called pressure natriuresis. Under physiological conditions, increased sodium intake is associated with increased BP and renal perfusion pressure, which inhibits the RAS axis resulting in dilatation of afferent arterioles, increase in GFR and decrease in sodium reabsorption, so that the level of sodium intake matches the sodium excretion *(Wadei & Textor, 2012)*. Thus, by modulating GFR, the pressure-natriuresis relationship maintains the BP and extracellular sodium concentration at steady state. However, a decline in GFR due to parenchymal kidney damage, changes in tubular sodium and chloride handling, or enhanced activation of the RAS may shift the balance between sodium intake and excretion and lead to arterial hypertension *(Guyton, 1990)*.

1.5.3 The renin-angiotensin system

The RAS plays a central role in modulating the renal perfusion pressure. Renal ischemia, increased activity of the sympathetic nervous system, or reduced salt intake may enhance the secretion of renin resulting in increased angiotensin II production. Angiotensin II is directly involved in vasoconstriction of afferent and efferent arterioles, increases sodium reabsorption in proximal tubules and in the TAL, and stimulates

aldosterone secretion from the adrenal cortex, which in turn induces sodium and water retention in the distal tubules *(Simoes & Flynn, 2012)*. Furthermore, the kidney itself is a source of afferent sympathetic signaling.

1.6 Pathophysiological mechanism of kidney damage in hypertension

The pathogenesis of hypertensive nephropathy involves two arbitrarily distinct initial injuries, which is transmission of increased arterial pressure to the glomeruli, and ischemic injury to the glomerular and tubulointerstitial structures. These are, however, largely interrelated due to the interaction of involved molecular mechanisms that are oxidative stress, endothelial dysfunction, and enhanced RAS activation.

Normally, the glomeruli are protected from an acute increase in arterial BP by glomerular afferent arteriole constriction. If the arterial pressure exceeds the range of this autoregulatory mechanism, the pressure increase is transmitted to the glomerular capillaries, which in turn leads to maladaptive structural changes resulting in glomerulosclerosis. Chronic elevation in glomerular perfusion pressure leads to adaptive hypertrophy of the afferent arterioles, which, however, makes the diffusion of oxygen across the smooth muscle wall more difficult and results in glomerular and tubulointerstitial ischemia *(Mennuni et al., 2014)*. In turn, if vasoconstrictors prevail over vasorelaxing factors, renal blood flow is decreased, which further enhances RAS activation *(Ponnuchamy & Khalil, 2009)*. Finally, a decrease in GFR causes that the balance between sodium intake and excretion is only maintained by an increase in BP, and thus progressive renal injury is sustained.

1.7 Origin of albuminuria

Under physiological conditions, the final urine contains no or only a small amount of albumin. The traditional view on the origin of albuminuria is that it primarily results from glomerular damage. The Steno hypothesis suggested that increased UAE is a marker of glomerular damage as well as of a more generalized vascular risk *(Deckert et al., 1989)*. The amount of the filtered albumin is normally very small and may be endocytosed by the high-affinity, low-capacity megalin/cubilin receptor into the

lysosomes of the proximal tubular cells, where it is degraded to amino acids. Later on, it has been suggested that UAE is not only attributable to the amount of albumin filtered by the glomerulus but, also, to dysfunction in the retrieval and degradation pathway in the proximal tubule *(de Zeew et al., 2006)*. Finally, a tubular model of albuminuria proposed by Russo et al. was strongly opposed to the long accepted view. The amount of filtered albumin is deemed to be relatively high, and intact albumin is further retrieved to the peritubular blood supply through transcytosis. Unretrieved filtered albumin undergoes lysosomal degradation in the proximal tubular cells *(Comper et al., 2008; Russo et al., 2009)*. Although the model of tubular origin of albuminuria raised a lot of criticism *(Norden & Unwin, 2012)*, the most recent evidence has further emphasized the importance of the proximal tubule in albumin reabsorption *(Dickson et al., 2014)*.

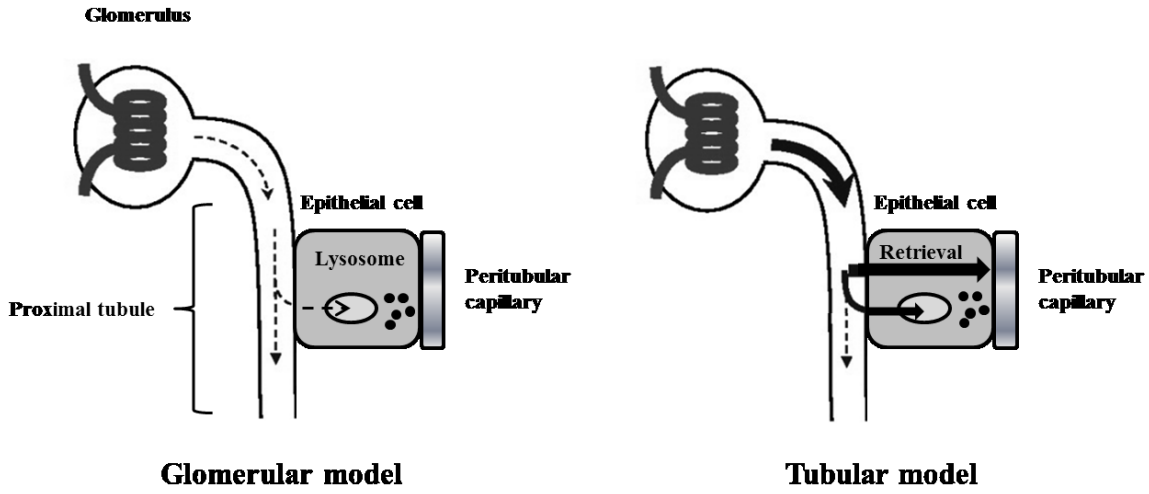


Figure 1. Models of renal albumin processing. In the glomerular model, UAE is related to the function of the glomerular filtration barrier. A small amount of albumin undergoes lysosomal degradation. In the tubular model, a high amount of filtered albumin is retrieved into the blood supply, whereas unretrieved albumin undergoes lysosomal degradation. Adapted from *(Comper et al., 2008)*.

1.8 Metabolic syndrome

Increased BP often co-occurs with visceral obesity, hypertriglyceridemia, and abnormal glucose metabolism, forming together a constellation of cardiovascular risk factors recognized as MetS. To diagnose MetS, at least 3 components out of the following 5 are routinely required: an increase in waist circumference, serum triglycerides, BP and fasting glucose or a decrease in high-density lipoprotein (HDL) cholesterol *(Assmann et al., 2007)*.

Over the past years, concerns about the construct of the MetS have been raised as the criteria are ambiguous, thresholds are misleading, and insulin resistance as the single underlying etiology remains questioned *(Kahn et al., 2005)*. Formerly, the cornerstone of MetS was based on insulin resistance, as it appeared to be related to most of the syndrome features. However, as MetS represents a set of numerous combinations of its components, one may assume a set of variably linked pathophysiological mechanisms underlying its clinical expression. The concept of MetS has also been criticized for representing only an independent clustering of its constituents, as several studies showed that MetS conveys no additional information to its individual components in cardiovascular risk prediction; likewise, treatment of MetS and its defining components is identical *(Yarnell et al., 1998)*. Conversely, an earlier study provided evidence that the level of clustering of metabolic phenotypes exceeds chance associations *(Schmidt et al., 1996)*. Finally, it has been somewhat uncertain which factors comprise the syndrome and thus several definitions have been adopted. While emerging factors like adiponectin *(Yudkin et al., 1999)*, pro-inflammatory *(Festa et al., 2000)* and pro-thrombotic markers *(Festa et al., 1999)* have been omitted, microalbuminuria, which was initially included in the World Health Organization definition of MetS along with its key traits *(Alberti & Zimmet, 1998)*, was withdrawn for two reasons: (i) urine collection was not easily performed in routine practice, and (ii) whether increased urinary UAE is a cardiovascular risk factor independent of the other MetS components was subject to debate. Later on, these concerns had been disproved as it has been demonstrated that increased UAE has an added value to MetS and its individual defining factors in predicting type 2 diabetes and cardiovascular outcomes *(van der Velde et al., 2012)*. Presently, uACR determined from a morning urine sample is easily available and showed a reliable association with the 24-hour UAE rate *(Dyer et al., 2004)*.

1.9 Uric acid

1.9.1 Uric acid metabolism and excretion

Hyperuricemia represents a complex phenotype resulting from the interplay between genetic and environmental factors regulating uric acid production and excretion. Uric acid is the end product of dietary and endogenous purine (adenine, guanine, hypoxanthine, xanthine, caffeine, theobromine etc.) breakdown, with the use of several enzymes, e.g., nucleotidases, deaminases, and xanthine oxidase *(Maiuolo et al., 2016)*. Increased dietary and endogenous purine leads to increased urate production in the liver and muscles, resulting in an increased urate load to the kidney. Serum uric acid levels increase with male gender, age, purine- and fructose-rich food consumption, alcohol intake, and use of thiazide diuretics, whereas they decrease with use of allopurinol, losartan, and fenofibrate.

In humans, SUA levels are primarily determined by renal clearance, with 90% of hyperuricemia cases resulting from decreased renal uric acid excretion. Renal clearance accounts for approximately two thirds of uric acid excretion, whereas one third is intestinal. In the kidney, uric acid is freely filtered into the proximal tubule, with subsequent reabsorption, secretion, and post-secretory reabsorption. This process, which is mediated by urate transporters, results in 90−95% of urate retrieval *(Lipkowitz, 2012)*. Several urate transporters are involved in urate flux across the apical and basolateral membranes of epithelial cells in the proximal tubules. The solute carrier protein 2 family member 12 (SLC22A12), is the target of uricosuric drugs and has long been considered a major player responsible for 50% of urate reabsorption *(Graessler et al., 2006)*. Later on, functional experiments showed that the SLC2A9, which had been originally thought to serve only in a glucose and, to lesser extent, fructose transport, is a high-capacity urate transporter *(Caulfield et al., 2008)*. The SLC2A9 gene is located on chromosome 4, contains 13 coding and one non-coding exons, and has two transcript variants. The long isoform is expressed mostly in basolateral membranes of renal proximal tubular cells, in the liver, and placenta, while the short isoform is expressed only in the apical membranes of proximal tubular cells *(Augustin et al., 2004)*. The role of several other molecules involved in urate reabsorption and secretion has also been characterized, including the

multiple organic anion transporters (OATs 1-4), the urate transporter (UAT), and a voltage dependent organic anion transporter (OATv1).

1.9.2 Genetic component of serum uric acid levels

The regulation of SUA levels is strongly determined by genetics with the heritability estimates ranging between 20% and 70% *(Rao et al., 1982; Whitfield & Martin, 1983)*. However, about 30 independent genomic loci that have been identified in a number of genome-wide association studies explain together only 3−7% of the variance in SUA levels. Several of the loci map to the genes involved in uric acid transport across membranes of proximal tubular cells (SLC2A9, SLC17A1, SLC17A3, SLC22A11, SLC22A12, ABCG2, PDZK1) *(Kolz et al., 2009; Li et al., 2007; Yang et al., 2010)*, whereas others appear to be involved in glucose metabolism and inhibin/activin signaling pathways (GCKR, TRIM46, IGF1R, NFAT5) *(Kottgen et al., 2013)*. The most significant SNPs located within the SLC2A9 gene have been replicated in different Caucasian cohorts *(Wallace et al., 2008)* as well as in African Americans *(Charles et al., 2011)*, Mexican Americans *(Voruganti et al., 2013)*, and American Indians *(Voruganti et al., 2014)*, with the minor alleles of most of the SNPs being associated with lower SUA concentrations. The SLC2A9 variants decreasing SUA levels have also been associated with increased fractional excretion of uric acid (FEUA), which is the proportion of uric acid filtered by the glomeruli that is finally eliminated in the urine with physiological values of around 5% *(Caulfield et al., 2008; Kottgen et al., 2013; Vitart et al., 2008)*.

1.10 The role of uric acid in arterial hypertension and metabolic syndrome

1.10.1 Epidemiological evidence

Whether uric acid is causally involved in the pathogenesis of cardiometabolic diseases or only represents a secondary phenomenon to these pathologies as well as the direction of the associations, becomes increasingly debated. The strongest and most consistent evidence exists on the pivotal involvement of uric acid in hypertension *(Johnson et al., 2005)*. In prospective studies, increased SUA levels were dose-dependent and one of the most reproducible predictors of essential hypertension *(Wang et al., 2014)*.

However, the role of hyperuricemia has also been implicated in obesity, MetS *(Krajcoviechova et al., 2016)*, type 2 diabetes *(Kanbay et al., 2016)*, and CVD *(Borghi et al., 2015)*. Besides an association with current and future MetS and all its defining components *(Babio et al., 2015; Facchini et al., 1991; Lu et al., 2012; Peng et al., 2015)*, increased SUA levels precede new-onset diabetes *(Lv et al., 2013)* and new-onset albuminuria *(Jalal et al., 2010)*.

1.10.2 Evidence from functional studies

Although uric acid may serve as an antioxidant in extracellular settings, intracelullar uric acid has a direct adverse effect on endothelial cells *(Yu et al., 2010)*, vascular smooth muscle cells *(Kang et al., 2005)*, and adipocytes *(Sautin et al., 2007; Zhang et al., 2015)*, all mediated by oxidative stress. Reactive oxygen species are generated through uric acid production with xanthine oxidase as well as by intracellular uric acid *per se* through stimulation of NADPH oxidases *(Kanbay et al., 2016)*. Soluble uric acid enters the cells via a specific urate transporter, where it upregulates the RAS, activates vasonstrictive substances (such as thromboxane, endothelin−1 and angiotensin II), initiates release of growth factors, reduces NO bioavailability, and increases production of inflammatory markers. Thus, by generating intracellular oxidative stress, uric acid may lead to proliferation of vascular smooth muscle cells, dysfunction of vascular endothelial cells, and inflammatory changes and lipid peroxidation in adipocytes *(Kanbay et al., 2016)*. Furthermore, uric acid has been shown to stimulate gluconeogenesis *(Cicerchi et al., 2014)* and induce insulin resistance *(Zhi et al., 2016)*. Finally, in pancreatic islet cells, uric acid reduces insulin secretion *(Scott et al., 1981)* whereas, in liver cells, it triggers triglyceride accummulation by generating mitochondrial oxidative stress *(Lanaspa et al., 2012)*.

In the kidney, uric acid initiates subtle renal injury, with changes similar to those also found in salt-sensitive hypertension. Rat models describe a two-stage mechanism of hyperuricemic hypertension. The first uric acid-dependent and sodium-independent stage is characterized by intermittent episodes of renal vasoconstriction and cortical ischemia. As a result, pre-glomerular arteriolopathy and tubulointerstitial inflammation develop, leading eventually to chronic sodium-dependent and uric acid-independent rise in

systemic BP *(Johnson et al., 2005; Mazzali et al., 2001)*. Of note, rats with experimentally induced hyperuricemia develop two stages of hypertension with all hemodynamic and histological changes observed in essential hypertension in humans *(Johnson et al., 2002)*. Initially, these changes could be either prevented or reverted by allopurinol (a xanthine oxidase inhibitor), an angiotensin-converting-enzyme inhibitor or L-arginine (stimulates endothelial NO production) *(Mazzali et al., 2001; Mazzali et al., 2002)*. In addition, fructose-fed rats develop hyperuricemia, uric acid dose-dependent endothelial dysfunction, and all the features of MetS, all of which were also prevented by allopurinol *(Nakagawa et al., 2006)*.

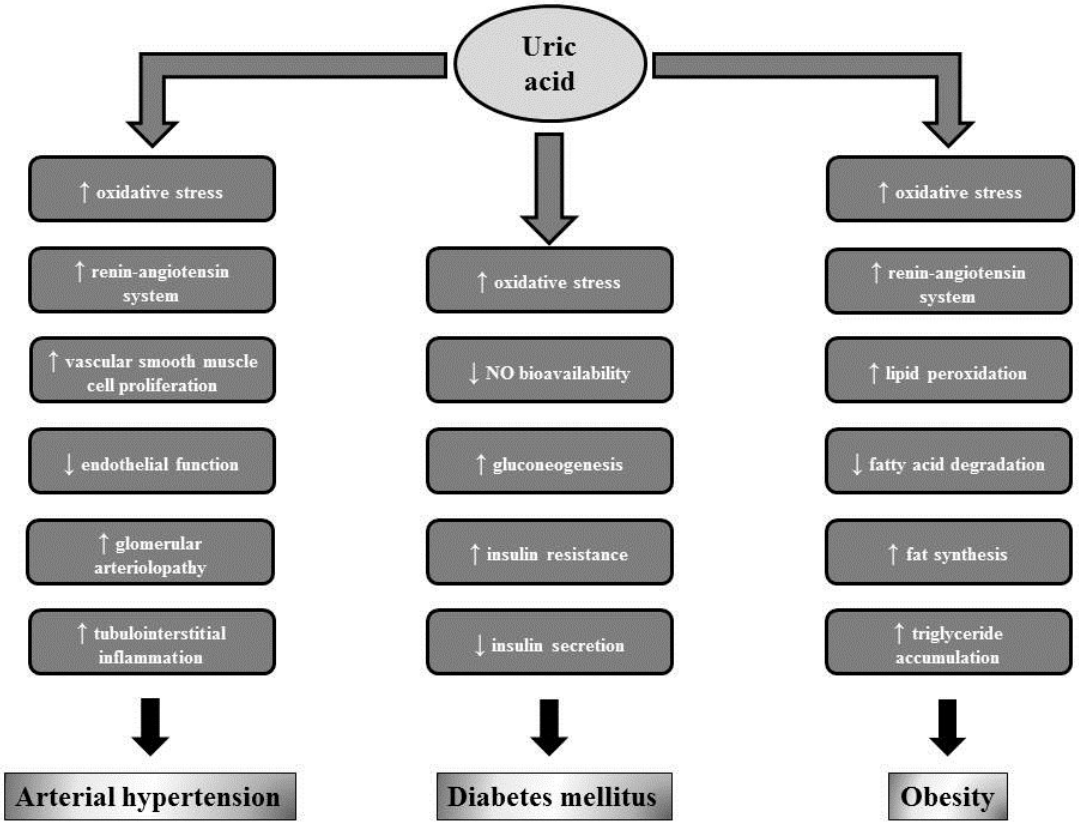


Figure 2. Schematic diagram of uric acid actions in the pathogenesis of arterial hypertension, diabetes mellitus, and obesity. Adapted from *(Kanbay et al., 2016)*.

1.10.3 Evidence from randomized trials

The BP-lowering and endothelial function improving the effect of allopurinol and probenecid (inhibitor of urate reabsorption) has been observed in two small randomized, double-blind, placebo-controlled trials in adolescents with pre-hypertension and hypertension *(Feig et al., 2008; Soletsky et al., 2012)*. Pilot studies in adults provide additional evidence that lowering of SUA levels may decrease BP *(Beattie et al., 2014)*, improve endothelial and kidney function *(Kanbay et al., 2011)*, slow down intima-media thickness progression *(Higgins et al., 2014)*, and even lead to weight loss independent of calory restriction *(Madero et al., 2015)*.

1.10.4 Evidence from genetic studies

As indicated in section 1.2.2, the vast majority of the BP heritability remains unexplained. On the contrary, evidence from epidemiological and functional studies suggests that uric acid intracellular localization, and thus the mechanisms leading to urate under-secretion or abnormal membrane transport may be in a causal pathway toward arterial hypertension. Still, large scale genome-wide association studies have failed to document an association between validated multiple genetic loci for uricemia and BP *(Caulfield et al., 2008; Vitart et al., 2008; Yang et al., 2010)*. Conversely, in a family-based study of a homogeneous population with common dietary habits in Southern Italy, a risk allele of the rs73455 polymorphism in the SLC2A9 gene was dose-dependently associated with increased SUA levels, clinic and white-coat SBP as well as with carotid atherosclerosis and arterial stiffness *(Mallamaci et al., 2014, 2015)*.

Mendelian randomization studies are capable of deciphering the causal relationship using a genetic marker or score as an un-confounded proxy of exposure to the phenotype of interest while rulling out reverse causality *(Lawlor et al., 2008)*. Using the Mendelian randomization principle, Parsa et al. provided additional support for SUA levels as a causal risk factor for hypertension *(Parsa et al., 2012)*. The strength of these findings over other analyses is that the study subjects, who were from an isolated founder population of the Amish community, underwent 24-hour ambulatory BP monitoring while staying on standardized high- and low-sodium diets. It was shown that each copy of the rs16890979 minor allele in the SLC2A9 gene conferred a significant decrease in

SUA level as well as a significant decrease in the 24-hour SBP of 2.2 and 1.5 mm Hg on the high- and low-sodium diet, respectively. No uric acid genotype-based association with clinic BP was observed when the subjects were on liberal diet *(Parsa et al., 2012)*. Other studies examining the uric acid genotype-based effect on BP under uncontrolled dietary settings *(Palmer et al., 2013)* as well as on MetS *(McKeigue et al., 2010)*, type 2 diabetes *(Pfister et al., 2011)* and CVD *(Stark et al., 2009)* provided limited support for the causal nature of these associations. Likewise, a recent meta-analysis using a multilocus genetic risk score demonstrated that sole gain/loss of function of urate transporters, and thus the increase in SUA levels, is likely to have only a modest causal effect in CVD *(White et al., 2016)*.

However, it should be noted that the failure to prove causation of uric acid in metabolic disorders through the Mendelian randomization approach could be due to three main reasons. First, the leading polymorphisms associated with extracellular SUA levels lie within the SLC2A9 gene, which codes for the transporter that transits uric acid out of the cell whereas the adverse effects on the cardiovascular system are exerted by intracellular uric acid. Second, the pleiotropic effect of the urate genetic instrument makes it difficult to prove causality between SUA levels *per se* and co-morbidities. For instance, SLC2A9 can exchange uric acid for glucose and fructose, whereas SLC22A12 exchanges uric acid for organic and anorganic anions. In addition, in the multilocus genetic risk score, each SNP may modulate the SUA levels by a different mechanism. Third, evaluated polymorphisms explain together only 3−7% of the variance of SUA levels *(Kanbay et al., 2013)*.

1.11 Pathophysiological link between uric acid and albuminuria

1.11.1 Epidemiological evidence

Observational trials documented that high SUA levels predict cardiovascular outcomes in the general population *(Bos et al., 2006)* as well as in hypertensive patients *(Hoieggen et al., 2004)*. However, not all studies published to date have reported that the association of SUA levels with cardiovascular outcomes is independent of the other risk factors *(Culleton et al., 1999; Wheeler et al., 2005)*. In line with this evidence are the findings by Scheven et al. on behalf of the PREVEND general population cohort study.

In terms of cardiovascular outcomes, the predictive power of baseline SUA levels remained significant, but decreased after adjusting for baseline albuminuria, and vice versa, which indicates that hyperuricemia and increased UAE might be related to CVD through an overlapping mechanism *(Scheven et al., 2014)*. In addition, the cross-sectional association between hyperuricemia and increased UAE has been documented in patients with hypertension *(Viazzi et al., 2005)*, type 2 diabetes *(Resl et al., 2012)*, and MetS *(Rodilla et al., 2009)*.

The association between SUA levels and albuminuria was explained by assuming that hyperuricemia induces oxidative stress and endothelial dysfunction, which results in albuminuria. Accordingly, Testa et al. demonstrated a synergistic interaction between a risk allele of the rs734553 polymorphism in the SLC2A9 gene and a major endogenous inhibitor of NO synthase (asymmetric dimethylarginine) in CKD progression, and concluded that reduced NO bioavailability is critical for uric acid to cause renal damage *(Testa et al., 2015)*. The other way round, a novel possible link between increased UAE and hyperuricemia was proposed in the above mentioned PREVEND study. In this observational study, UAE was associated with increased tubular uric acid reabsorption/decreased FEUA. This association was independent of potential confounders, and was reflected by an increase in SUA levels. The authors suggested tubular uric acid-albumin exchange and speculated that either the urate transporters could be up/down-regulated in the presence of albuminuria, or the association of albuminuria with uric acid reabsorption is mediated by sodium transport *(Scheven et al., 2014)*. However, as in this study only baseline levels of uric acid and albumin excretion were reported, it is very problematic to infer the causal direction of the reported association.

1.11.2 The role of renal sodium handling and urinary uromodulin excretion

Multiple lines of evidence suggest that sodium transport in epithelial tubular cells could serve as a potential link between decreased uric acid renal excretion and albuminuria. It has been hypothesized that hyperuricemia might have been beneficial to early hominoids because of its ability to sustain the BP level under low-salt diets via enhanced RAS stimulation and induce salt sensitivity *(Watanabe et al., 2002)*. Interestingly, in rats with experimetally induced hyperuricemia, hypertension was more

prominent in those on low-salt diet *(Mazzali et al., 2001)*. In turn, salt sensitivity in hypertensive patients increases the risk of albuminuria independent of their BP level *(Bigazzi et al., 1994)*. Furthermore, in the study using the Mendelian randomization principle by Parsa et al. high sodium intake accentuated the effect of genotype-based changes in SUA levels on BP *(Parsa et al., 2012)*.

It has been well established that fractional excretion of sodium (FENa) and FEUA positively correlate with each other under basal conditions as well as in hyperinsulinemia and sodium depletion *(Cunningham et al., 2007; Quinones Galvan et al., 1995; Ter Maaten et al., 1997)*. Thus, given an inverse correlation of uACR with FEUA, and a positive correlation of FEUA with FENa, one would assume that uACR and FENa are inversely correlated. However, studies evaluating the relationship between sodium intake/excrection and albuminuria reported either a positive, or no association *(Fox et al., 2006; A. Chang et al., 2013)*.

To add another piece of an already complex mosaic, it was later demonstrated that an increase in FEUA and/or FENa is associated with an increase in urinary uromodulin levels *(Padmanabhan et al., 2010; Troyanov et al., 2016)*. Uromodulin, known also as Tamm-Horsfall glycoprotein, is the most abundant tubular urine protein secreted exclusively by the epithelial cells of the TAL, and represents a marker of tubular function. As indicated in section 1.2.2, recent genome-wide association studies identified common variants near the uromodulin gene associated with hypertension *(Graham et al., 2014)* and CKD *(Kottgen et al., 2010; Pattaro et al., 2016)*. An increase in the urinary uromodulin/creatinine ratio (uUCR) has been associated with an increase in eGFR, FENa *(Padmanabhan et al., 2010; Troyanov et al., 2016)*, and dietary salt intake *(Torffvit et al., 2004)*. On the other hand, the evidence on the relationship of uromodulin excretion with albuminuria is limited. Still, reduced urinary uromodulin excretion was observed in diabetic nephropathy *(Bernard et al., 1987)*. Furthermore, minor G allele of the rs13333226 polymorphism in the uromodulin gene, which has been associated with a lower risk of hypertension and increased eGFR in another study *(Padmanabhan et al., 2010)*, has been shown to be protective against diabetic nephropathy *(Ahluwalia et al., 2011)*.

Uromodulin increases Na-K2-Cl-mediated NaCl reabsorption *(Mutig et al., 2011)*, and thus appears to have a role in BP regulation through modulation of sodium

handling in the TAL and, via tubulo-glomerular feedback, at the proximal tubular level *(Padmanabhan et al., 2014)*. Tubulo-glomerular feedback is one of the paracrine autoregulatory mechanisms maintaining the GFR at steady state despite the fluctuations in arterial BP. Elevated GFR with a subsequent increase in sodium chloride concentration in the tubular fluid is sensed by the macula densa, which is a collection of epithelial cells at the junction of the TAL and distal convoluted tubule located in the proximity to the glomerulus. This leads to release of vasoactive substances and afferent arteriole constriction, resulting in a drop in GFR. Decreased GFR leads to an opposite action. Thus, through its effect on sodium reabsorption in the TAL and macula densa sensitivity to luminal sodium chloride, uromodulin may modulate tubulo-glomerular feedback which in turn may lead to changes in GFR, rate of solute reabsortion in the proximal tubule, and BP.

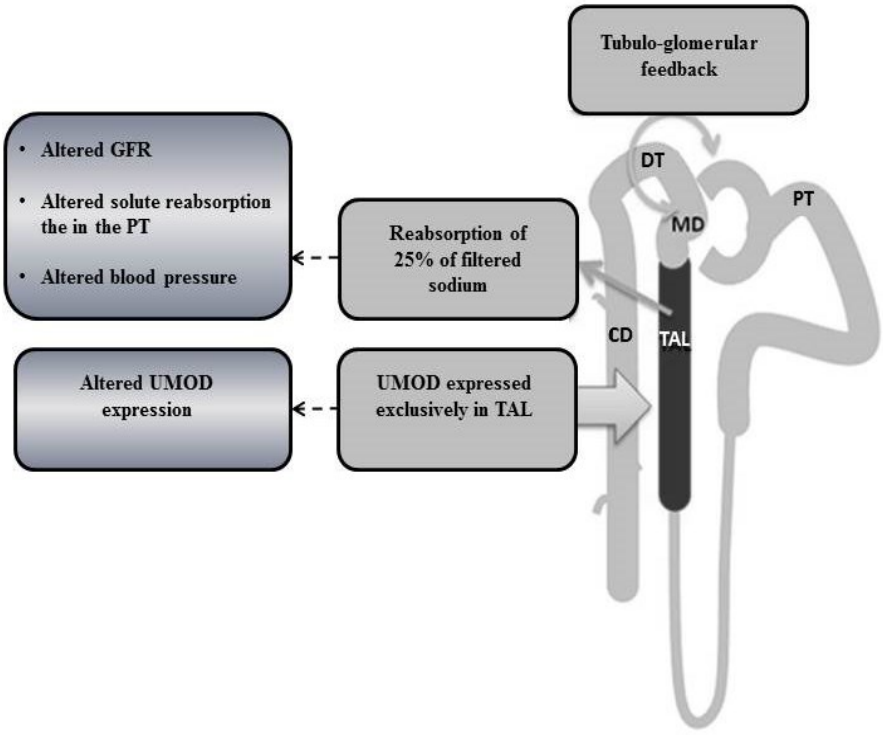


Figure 3. Putative mechanisms of uromodulin-related phenotypes. CD, collecting duct; DT, distal tubule; MD, macula densa; PT, proximal tubule; TAL, thick ascdending limb of the loop of Henle; UMOD, uromodulin. Adapted from *(Padmanabhan et al., 2014)*.

2 Aims and hypotheses

2.1 Hypotheses

It is conceivable that metabolic syndrome is not a uniform entity; however, its clinical expression is based on a set of variably linked pathophysiological mechanisms. Given that hyperuricemia may have a role in the pathogenesis of metabolic syndrome and there is a mechanism by which increased serum uric acid levels lead to albuminuria, then the serum uric acid levels may be related to urinary albumin excretion also in individuals without manifest metabolic syndrome by its arbitrary definition; however, with some metabolic derangements. Furthermore, it is credible that the association between urinary uric acid and albumin excretion reflects other pathophysiological processes, in which renal handling of solutes and urinary proteins are involved. In particular, we hypothesize that the effect of uric acid excretion on albumin excretion may be mediated by urinary uromodulin through modulation of sodium reabsorption in the thick ascending limb of the loop of Henle. Better clarification of the underlying pathophysiology may help to further elucidate the role of uric acid in hypertension, metabolic syndrome, and cardiovascular disease, and provide the basis for the development of targeted therapeutic approaches.

2.2 Aims of the thesis

The overall aim of this thesis is to evaluate the structure of clustering of metabolic phenotypes and assess the pathophysiological mechanisms explaining the relationship between uric acid excretion and albuminuria.

The specific aims are:

1. To evaluate the association of serum uric acid levels with urinary albumin excretion, and the modification effect of metabolic phenotypes on this association in individuals without manifest metabolic syndrome.

2. To evaluate the association between SLC2A9 candidate SNPs and urinary albumin excretion.

3. To specify the pathophysiological mechanism explaining the relationship between urinary uric acid and albumin excretion.

4. To propose and build a construct of metabolic syndrome.

3 Methods

3.1 The Czech post-MONICA study

3.1.1 Study population

The Czech post-MONICA study is a cross-sectional survey investigating the prevalence and treatment of cardiovascular risk factors in the general population of the Czech Republic. A one-percent random sample stratified by age and gender was selected from 9 Czech districts (Litomerice, Benesov, Cheb, Pardubice, Prague-East, Chrudim, Jindrichuv Hradec, Pilsen, and Kromeriz) using the General Health Insurance company registry, which contains data of all insurees in the country. Health insurance is mandatory for all Czech citizens and is paid by the employer/employee, or by the government (for children, retired, and unemployed persons). Therefore, the General Health Insurance company registry and the National Population Registry are basically identical. A total of 3612 individuals aged 25–64 years were examined in 2007–2009 (as previously reported) *(Cifkova et al., 2010 [a,b])*. All study participants were Caucasians and provided their written informed consent. Recruitment and sample collection were approved by the Joint Ethics Committee of the Institute for Clinical and Experimental Medicine and Thomayer Hospital (Prague, Czech Republic).

3.1.2 Examination and laboratory analyses

Waist circumference was measured in standing participants at midway between the inferior margin of the last rib and the top of the iliac crest in the horizontal plane. Blood pressure was measured in triplicate at one-minute intervals by standard mercury sphygmomanometers on the right arm. The mean of the second and third readings was retained. A sample of 12-h fasting blood and an early morning spot urine sample were collected for biochemical analyses. Determination of SUA, serum creatinine and lipids, plasma glucose, urinary albumin, and creatinine was performed in a central laboratory. The Centers for Disease Control and Prevention (Atlanta, GA, USA) continuously monitored the accuracy of analyses. Urinary albumin was determined using immunoturbidimetry, whereas urinary creatinine was determined using an enzymatic method, and the albumin/creatinine ratio was calculated. Albuminuria was defined as

uACR ≥ 3 mg/mmol *(CKD Work Group)*. To calculate eGFR, the CKD-EPI equation was used *(Matsushita et al., 2012)*. Individuals were diagnosed with hyperuricemia if the level of SUA was ≥ 420 μmol/L in men, and ≥ 360 μmol/L in women. Information on demographics, lifestyle habits, history of CVD, and current use of medication was obtained by a physician-completed questionnaire. Cardiovascular disease was defined as a self-reported history of myocardial infarction, coronary artery bypass grafting, percutaneous coronary intervention, stroke, or manifest peripheral arterial disease. Metabolic syndrome was defined according to the Joint Interim Statement by several professional societies as the presence of ≥ 3 of the following components: 1) waist circumference ≥ 102 cm (men) or ≥ 88 (women); 2) triglycerides ≥ 1.7 mmol/l, or drug treatment for elevated triglycerides; 3) HDL-cholesterol < 1.0 mmol/l (men) or < 1.3 mmol/l (women), or drug treatment for reduced HDL-cholesterol; 4) BP ≥ 130/85 mmHg, or drug treatment for elevated BP; 5) fasting plasma glucose ≥ 5.6 mmol/l, or drug treatment for elevated blood glucose *(Alberti et al., 2009)*. Individuals without MetS were categorized into 2 groups according to the presence of MetS components; 0 MetS component and 1–2 MetS component(s). In order to avoid the pleiotropic effect between metabolic phenotypes, persons with MetS are not included in this analysis.

3.1.3 Statistical analyses and data reporting

Individuals with incomplete data (n=170), MetS (n=1093), diabetes treated with glucose-lowering medication (n=19), eGFR < 60 ml/min/1.73m^2 (n=29), current use of diuretics (n=62), or allopurinol (n=13), and UAE below the detection limit of 1 mg (n=394) were excluded. This resulted in 1832 individuals for our analyses.

For further analyses, lipids and BP were adjusted for treatment effects using validated methods. Increments of 0.65 mmol/l, 0.21 mmol/l, or 0.75 mmol/l were added to measured triglycerides, whereas measured HDL-cholesterol levels were reduced by 0.15 mmol/l, 0.06 mmol/l, or 0.18 mmol/l in individuals treated by fibrates, statins, or combination therapy, respectively *(Wu et al., 2007)*. Visceral fat tissue function was assessed using an adapted equation of visceral adiposity index (VAI) developed by Amato et al. *(Amato et al., 2010)*. Usually, a VAI of 1 is deemed normal. In the interest

of straightforward interpretation, HDL-cholesterol was not included in the formula, and VAI was defined as:

Men:

$$VAI = \left(\frac{\text{Waist circumference}}{39.68 + (1.88 \times \text{BMI})} \right) \times \left(\frac{\text{Triglycerides}}{1.03} \right)$$

Women:

$$VAI = \left(\frac{\text{Waist circumference}}{36.58 + (1.89 \times \text{BMI})} \right) \times \left(\frac{\text{Triglycerides}}{0.81} \right)$$

Increments of 15/10 mmHg were added to the measured SBP/DBP of individuals treated by BP-lowering medication, respectively *(Tobin et al., 2005)*. Mean arterial pressure (MAP) was calculated by summing one third of the SBP and two thirds of the DBP.

All continuous variables were tested for normality and logarithmically transformed where applicable. Descriptive data are displayed as mean and standard deviation (SD), median and interquartile range for quantitative variables, or as frequency and percent for categorical variables. The two-tailed t-test for independent samples was used to compare the difference in age between the groups. The distribution of gender was compared by Pearson's chi-square test. The mean ± SD, median with interquartile range and prevalence of clinical characteristics were age- and gender-adjusted using weighted average of age- and gender-specific rates. The differences in clinical characteristics were compared by age- and gender-adjusted logistic regression. The independent association of uACR with SUA levels in different groups was assessed by multiple multivariate linear regression analyses.

Multiple generalized linear models were used to examine whether exposure to metabolic phenotypes modifies the association of uACR with SUA levels. Serum uric acid levels were used as a dependent variable, and pre-specified interaction terms of Ln-uACR * Ln-VAI, Ln-uACR * MAP and Ln-uACR * Ln-fasting glucose were included as independent predictors. All models were adjusted for Ln-uACR *per se*, Ln-VAI *per se*, MAP *per se*, and Ln-fasting glucose *per se*, respectively, as well as for the other potential

confounders of Ln-uACR and uric acid relationship, i.e., age, gender, smoking, education, HDL-cholesterol, eGFR, and CVD. In order to adjust for multiple comparisons, a Holm-Bonferroni-corrected significant difference was defined as two-sided p values <0.0167; <0.025; and <0.05. To further assess the direction of the interaction, persons with 1–2 MetS component(s) were subdivided by the medians of uACR, VAI, MAP, and fasting glucose. The differences in the adjusted means ± SD of SUA levels according to the median of uACR by the medians of VAI, MAP and glucose were assessed by generalized regression mixed-effects models. In these models, the intercept was treated as a random effect, while the median of uACR, median of the respective metabolic phenotype, the interaction term of the median of uACR and metabolic phenotype, and other covariates were treated as fixed effects. Reported are multivariate adjusted means ± SD and p values.

Calculations were performed using SPSS 16 software (SPSS Inc., Chicago, Illinois, USA). Unless otherwise stated, a significant difference was defined as a two-sided p value < 0.05.

3.2 The CARTaGENE study

3.2.1 Study population

The CARTaGENE study represents a population-based cohort consiting of men and women aged between 40 and 69 years and residing in the metropolitan areas of the Canadian province Quebec (Montreal, Quebec, Sherbrooke, and Saguenay) *(Awadalla et al., 2013; El-Bikai et al., 2015)*. Participants were randomly selected from the provincial health insurance registries [FIPA files, fichier administratif des inscriptions des personnes assurées de la Régie de l'assurance maladie du Québec (RAMQ)]. From August 2009 to October 2010, a total of 20 007 men and women consented to participate in the study. This analysis involves 737 individuals of French Canadian descent with available genotyping data and urinary samples *(Troyanov et al., 2016)*. The French Canadians of Quebec represent a population with the founder effect.

3.2.2 Examination and laboratory analyses

A validated interviewer-based questionnaire was used to capture socio-demographic factors, lifestyle habits, current medication use, and a detailed history of arterial hypertension, type 2 diabetes, and renal disease. During the clinic visit, anthropometric measurements were taken. Peripheral BP was measured by an Omron IntelliSense BP Monitor (HEM-907XL; Omron Healthcare, Inc., Lake Forest, Illinois, USA). Three BP measurements were performed on the nondominant arm using an appropriate cuff size with the participant in the sitting position. The readings were taken after an initial 10-minute rest period, and the mean of all available BP measurements was reported.

Determination of SUA, serum creatinine, lipids, electrolytes, and glucose was performed in the central laboratory in Chicoutimi, Quebec, Canada. Quality assurance tests demonstrated that the test-retest reliability measurements well exceeded 90%. Urinary analysis were perfomed in a spot urine sample and included glucose, electrolytes, uric acid, albumin, creatinine, and uromodulin. The urine samples were stored at −80ºC, and uromodulin was measured at the biochemical platform at the University of Zürich (Switzerland) using an ELISA assay as described previously

(Troyanov et al., 2016). Urinary creatinine was measured with the use of Beckman Coulter Synchron System Creatinine Assay. Albuminuria was defined as uACR ≥ 3 mg/mmol *(CKD Work Group)*. To determine eGFR, the CKD-EPI equation was used *(Matsushita et al., 2012)*.

3.2.3 Genotyping

Within the CARTaGENE cohort, 737 French Canadians were selected for genotyping from the extremes of the Framingham Risk Score and vascular rigidity index for both men and women. Genome-wide SNP genotyping was performed using the Illumina Omni2.5-8 BeadChip following the manusfacturer´s protocol. Genotype calls were determined using Illumina's BeadStudio software. Quality control of the data was carried out using the Plink 1.9 toolset *(C. C. Chang et al., 2015)*. PLINK´s identity by descent analysis was used to detect any hidden relatedness. An ethnicity check was performed using fastStructure to preserve only Caucasians *(Raj et al., 2014)*. None of the individuals were excluded due to hidden relatedness, non-Caucasian origin or the SNP-missingness < 0.03. Two individuals were excluded due to the called and self-reported gender mis-match. After filtering of the genotype data (call rate <96% across all individuals, call rate <99% and MAF <1%), we obtained a dataset of 2 179 801 autosomal SNPs in 735 samples. From this dataset, imputation of allele dosage of ungenotyped SNPs was performed with 1000 Genome Haplotypes phase 3 as the reference panel using ShapeIT and Impute2 *(Delaneau & Marchini, 2014; Howie et al., 2009)*. Quality control filtering of the imputed data (MAF <0.05, and imputation score <0.8) yielded a final genotype dataset of 6 662 672 SNPs in 735 samples. The population stratification was assessed using a principal component analysis (PCA)-based method as implemented in Eigenstrat software *(Price et al., 2006)*. Single-nucleotide polymorphisms with departure from Hardy-Weinberg equilibrium at p<0.0001 were excluded.

We selected two SNPs within the SLC2A9 gene for further analyses based on their associations with SUA levels and replicability in different populations. A common missense rs16890979 variant has been associated with SUA levels in different ethnicities *(Dehghan et al., 2008)* as well as in an isolated founder population of the Old Order

Amish. On the other hand, a common non-coding rs13129697 variant has been associated with urate levels in the meta-analysis of 5 population-based cohorts pooling a total of 28 thousand Caucasians *(Yang et al., 2010)*. The rs16890979 variant was genotyped, whereas the rs13129697 variant was imputed with the imputation score of 0.98.

3.2.4 Statistical analyses and data reporting

In total, 688 individuals had genotyping and urinary data available. Further, we excluded individuals with an eGFR < 60 ml/min/1.73m^2 (n=20), glycosuria (n=24), current use of diuretics (n=42), allopurinol (n=3), and uricosuric drugs (fenofibrate, losartan; n=6), as these conditions and medications may alter uric acid and uromodulin excretion. This resulted in 593 individuals available for our analyses.

In analogy to the Czech post-MONICA study, BP levels, lipids, and glucose were adjusted for treatment effects using validated methods. Increments of 15/10 mmHg were added to the measured systolic/diastolic BP if individuals were treated by BP-lowering medication, respectively *(Tobin et al., 2005)*. Mean arterial pressure was calculated by summing one third of the SBP and two thirds of the DBP. Increments of 0.65 mmol/l, 0.21 mmol/l, or 0.75 mmol/l were added to measured triglycerides, whereas measured HDL-cholesterol levels were reduced by 0.15 mmol/l, 0.06 mmol/l, or 0.18 mmol/l if individuals were treated by fibrates, statins, or combination therapy, respectively *(Wu et al., 2007)*. To adjust glucose for antidiabetic medication, the non-parametric method described by Tobin et al. was used *(Tobin et al., 2005)*.

Urinary albumin and uromodulin were expressed as a ratio to creatinine, whereas urinary uric acid and sodium were expressed as fractional excretion based on a previous publication *(Troyanov et al., 2016)*. Fractional excretion of solutes was calculated as [($Solute_{urine}$/$Solute_{serum}$ divided by $creatinine_{urine}$/$creatinine_{serum}$)]*100.

Continuous variables were tested for normality and variables with the skewed distribution were logarithmically transformed. For descriptive statistics, the two-tailed t-test for independent samples, Mann-Whitney's U test, Kruskal-Wallis test, and Pearson's

chi-squared test were used where applicable. Descriptive data are displayed as mean ± SD, median and interquartile range, or as frequency and percent.

To evaluate the association of the SLC2A9 candidate SNPs with SUA levels, FEUA, and Ln-uACR, linear regression with the additive genetic model was used. Imputed genotypes were coded as dosage. Covariates for each regression model were added in a stepwise fashion. Analyses were performed using the Plink 1.9 toolset *(C. C. Chang et al., 2015)*.

To test whether urinary uric acid excretion modifies the relationship of uACR with urate SNP, we included the interaction term of the genotype*FeUA tertiles as an independent factor in the generalized linear mixed model for Ln-uACR. The model was adjusted for the genotype *per se* and FEUA tertiles *per se* as well as for the other significant confounders. Here, a simple 0, 1, 2 allele dosage coding was used. The intercept was treated as a random effect while the genotype, FEUA tertiles, the interaction term of the genotype with FEUA tertiles, and other covariates were treated as fixed effects. To further assess the direction of the interaction, the differences in the multivariate adjusted means ± SD of Ln-uACR according to the genotype and FEUA tertiles are reported.

We used the backward stepwise linear regression model to test the independent association of FEUA with Ln-uACR. Further, we examined the role of Ln-uUCR and FENa as potential mediators of the relationship between urinary uric acid and albumin excretion. In cross-sectional studies, mediation analysis can be used to suggest causal links between the variables. Shortly, if an independent variable and mediator are associated (path a), and a mediator and dependent variable are associated (path b), the total effect of the independent variable on the dependent variable is the result of path a × path b. At the same time, the product of path a × path b can be expressed as total effect−direct effect (Figure 4) *(Preacher & Kelley, 2011)*. In other words, after adding the mediator to the linear regression model, the relationship between the independent variable and dependent variable becomes attenuated or eliminated, as the mediating variable is located causally between the independent and dependent variables. The FEUA, Ln-uUCR and FENa were standardized to a mean of zero and SD of one to enable a comparison of regression coefficients. We applied the multiple mediation procedure using an SPSS macro developed by Preacher and Hayes *(Preacher & Hayes,*

2008) and calculated the percentage decrease for regression coefficients when controlling for mediator(s). Finally, we confirmed the association of Ln-uUCR with albuminuria defined as a categorical trait using logistic regression. Calculations were performed using SPSS 16 software (SPSS Inc., Chicago, Illinois, USA). A significant difference was defined as a two-sided p value < 0.05.

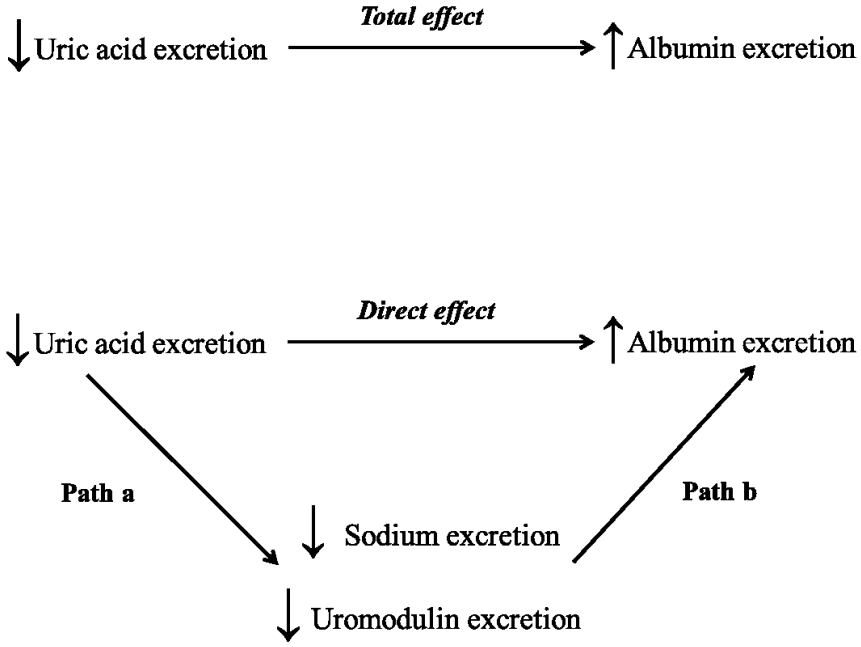


Figure 4. Schematic diagram of the relationship between urinary uric acid and albumin excretion mediated by urinary sodium and uromodulin excretion. Total effect; association of uric acid excretion with albumin excretion without controlling for mediator(s). Direct effect; association of uric acid excretion with albumin excretion after controlling for mediator(s). Path a; direct effect of uric acid excretion on mediator(s). Path b; direct effect of mediator(s) on albumin excretion after controlling for uric acid excretion.

3.3 Principal component analysis

MetS defined by distinct cut-off points may lead to misclassification of the risk as well as a huge loss of power by dichotomizing data. To overcome this limitation, PCA was used to develop a continuous MetS score. The PCA reduces a number of variables to a minor set of components that capture the maximum of the variance in data. The PCA-derived score can be used as a progressive function of the exposure of the constituting variables. The PCA-derived MetS score showed impressive validity in both genders and highly correlated with MetS as defined by the International Diabetes Federation (IDF) *(Wijndaele et al., 2006)*.

In total, 678 CARTaGENE individuals had all the metabolic phenotypes and urinary data available. Further, we excluded individuals with an eGFR < 60 ml/min/1.73m^2 (n=18), glycosuria (n=21), current use of diuretics (n=41), allopurinol (n=2), and uricosuric drugs (fenofibrate, losartan; n=6). This resulted in 590 CARTaGENE individuals available for PCA.

Of the total of 3612 participants of Czech post-MONICA study, we excluded those with incomplete data (n=164), diabetes treated with glucose-lowering medication (n=200), eGFR < 60 ml/min/1.73m^2 (n=63), current use of diuretics (n=255), and allopurinol (n=51). This resulted in 2930 individuals available for PCA in the Czech post-MONICA study.

Adjustment for other treatment effects was performed using validated methods as already described in sections 3.1.3 and 3.2.4. All variables were regressed on age and gender before applying PCA. Two parallel PCAs were performed separately in both studies *(Wijndaele et al., 2006)*. First, we applied PCA to the defining variables of MetS. Second, we applied PCA to the defining variables of MetS after the addition of (a) uACR and FEUA (CARTaGENE study), and uACR and SUA levels (Czech post-MONICA study). The proportion of variance explained by each principal component was assessed by eigenvalues, which denote the sum of the squared component loadings. Principal components with eigenvalues >1 were extracted. Principal component scores were derived using linear regression. We used direct oblimin rotation, as this method permits correlation of the principal components. Finally, we evaluated the role of Ln-uUCR and FENa as mediators of the relationship between the principal component scores using

mediation analysis approach. Calculations were performed using SPSS 16 software (SPSS Inc., Chicago, Illinois, USA) and SPSS macro developed by Preacher and Hayes *(Preacher & Hayes, 2008)*. A significant difference was defined as a two-sided p value < 0.05.

4 Results

4.1 The Czech post-MONICA study

4.1.1 Characteristics

Of the 1832 individuals without manifest MetS, 64.1% (n=1174) presented with either one or two MetS component(s), whereas 35.9% (n=658) were metabolically healthy. Among individuals with 1−2 MetS component(s), 667 (56.8%) presented with a single one, while 507 (43.2%) presented with a combination of 2 components. The frequency of MetS components, presenting either solely or in combination, is shown in Table 3. The most frequent MetS component combination was increased BP together with increased waist circumference (29.4%), followed by increased BP together with increased fasting glucose (23.5%), and increased BP together with increased triglycerides (13.8%). The frequency of the remaining combinations was under 10%.

Table 3. The frequency of MetS components in the 1−2 MetS component(s) group

MetS component	**Frequency, n (%)**
Waist circumference ≥ 102 cm (men) or ≥ 88 (women)	351 (29.9%)
Triglycerides ≥ 1.7 mmol/l, or drug treatment for elevated triglycerides	235 (20.0%)
HDL cholesterol < 1.0 mmol/l (men) or < 1.3 mmol/l (women), or drug treatment for reduced HDL	137 (11.7%)
BP ≥ 130/85 mmHg, or drug treatment for elevated BP	673 (57.3%)
Fasting plasma glucose ≥ 5.6 mmol/l, or drug treatment for elevated blood glucose	285 (24.3%)
Total	1174 (100%)

Adapted from *(Krajcoviechova et al., 2016)*.

The demographic, clinical, and biochemical characteristics of the groups are summarized in Table 4. Although persons with 1–2 MetS component(s) were almost equally represented by both genders (51.7% of men), they were older and more often men compared to those with 0 MetS component. Therefore, the subsequent comparisons were age- and gender-adjusted. Individuals without any MetS component, but on BP-

lowering or lipid-lowering mediaction were taking this medication for reasons other than arterial hypertension or metabolic dyslipidemia (beta-blockers and statins).

Table 4. Characteristics of the Czech post-MONICA study participants without MetS

	0 MetS component n=658	**1−2 MetS component(s)** n=1174	**P value**
Age, years	39.4±10.0	46.3±11.2	<0.001
Gender (male), n (%)	225 (34.2)	607 (51.7)	<0.001
Current smoker, n (%)	199 (30.0)	379 (32.4)	0.46*
Education			
Basic, n (%)	206 (30.0)	534 (44.5)	0.012*
Secondary, n (%)	320 (50.0)	466 (40.4)	
University, n (%)	132 (20.0)	172 (15.1)	
Waist circumference, cm	78.0±8.9	90.0±11.7	<0.001*
Serum triglycerides, mmol/L	0.86 [0.65-1.14]	1.18 [0.88-1.63]	<0.001*
HDL-cholesterol, mmol/L	1.69±0.34	1.52±0.37	<0.001*
Visceral adiposity index	0.95 [0.72-1.26]	1.35 [0.73-1.83]	<0.001*
SBP, mmHg	113 [107-119]	127 [117.3-139]	<0.001*
DBP, mmHg	74.2±6.0	83.6±10.3	<0.001*
MAP, mmHg	87.0±6.3	98.9±12.0	<0.001*
Fasting glucose, mmol/L	4.9 [4.5-5.2]	5.1 [4.8-5.5]	<0.001*
uACR, mg/mmol	0.3 [0.1-0.8]	0.3 [0.1-0.74]	0.261*
SUA, μmol/L	241.1±72.3	279.2±79.5	<0.001*
eGFR, ml/min/1.73m^2	94.6±15.8	92.4±15.8	0.063*
BP-lowering medication, n (%)	5 (1.1%)	194 (15.2%)	<0.001*
Lipid-lowering medication, n (%)	14 (2.2%)	75 (5.6%)	0.181*
CVD, n (%)	5 (1.1%)	41 (3.5%)	0.187*

Mean±SD and number (percentage) are presented for age and gender, respectively. Number and age- and gender-adjusted percentages are presented for categorical variables. Age- and gender-adjusted mean±SD, median and interquartile range are presented for continuous variables.

* P value obtained using age- and gender-adjusted logistic regression.

Adapted from *(Krajcoviechova et al., 2016)*.

4.1.2 Determinants of SUA levels

In a fully adjusted model for uricemia, Ln-uACR was an independent factor for an increase in SUA levels in individuals with 1–2 MetS component(s) (Sβ 0.048; p=0.024); however, not in those without any MetS component. Male gender, younger age, decrease in eGFR, and increases in waist circumference, serum triglycerides, and SBP were independent factors for an increase in SUA levels in both groups, whereas lower education was associated with an increase in SUA levels only in the 1−2 MetS component(s) group (Table 5).

Table 5. Multiple linear regression models for SUA levels

	0 MetS component n=658		**1−2 MetS component(s)** n=1174	
	Sβ	**P value**	**Sβ**	**P value**
Age, years	−0.206	<0.001	−0.258	<0.001
Gender (male)	0.587	<0.001	0.482	<0.001
Current smoker	0.010	0.715	0.026	0.219
Education†	−0.018	0.517	0.044	0.039
Waist circumference, cm	0.160	<0.001	0.256	<0.001
Ln-triglycerides, mmol/L	0.105	<0.001	0.072	0.001
HDL-cholesterol, mmol/L	0.029	0.341	−0.020	0.436
Ln-SBP, mmHg	0.086	0.015	0.068	0.044
DBP, mmHg	0.001	0.987	−0.044	0.168
Ln-fasting glucose, mmol/L	−0.017	0.545	0.024	0.279
Ln-uACR, mg/mmol	0.030	0.264	0.048	0.024
eGFR, ml/min/1.73m^2	−0.339	<0.001	−0.427	<0.001
CVD	0.023	0.389	0.039	0.071

† University as reference category

Adapted from *(Krajcoviechova et al., 2016)*.

4.1.3 Impact of metabolic phenotypes on the association of SUA levels with uACR

Among individuals with 1–2 MetS component(s), SUA levels increased by the interaction of Ln-uACR with Ln-VAI (Sβ 0.06; p=0.012), and of Ln-uACR with MAP (Sβ 0.05; p=0.009). Surprisingly, we observed no interaction between Ln-uACR and Ln-fasting glucose in relation to uricemia (Sβ 0.008; p=0.705). Differences in multivariate adjusted means ± SD of SUA according to the median of uACR in interaction with the medians of VAI, MAP, and fasting glucose are shown in Table 6 and Figure 5.

Table 6. Adjusted means ± SD of SUA levels by the medians of uACR and metabolic phenotypes in the 1–2 MetS component(s) group

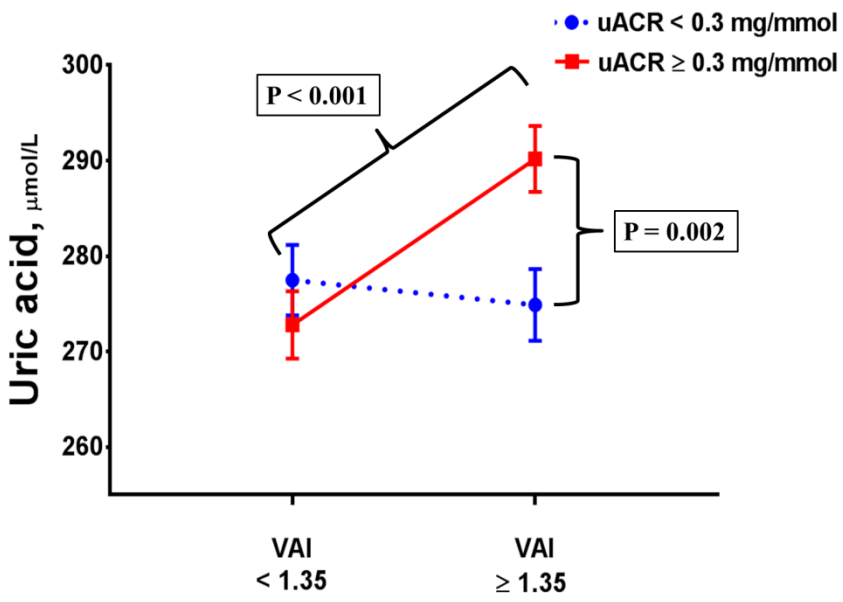
Multivariate adjusted means ± SD of uric acid, µmol/L						
uACR < 0.3 mg/mmol	*VAI < 1.35*	277.47±3.71	*MAP < 98 mmHg*	277.92±3.55	*Glucose < 5.2 mmol/L*	275.50±3.9
	VAI ≥ 1.35	274.89±3.76	*MAP ≥ 98 mmHg*	274.57±3.91	*Glucose ≥ 5.2 mmol/L*	277.22±3.6
uACR ≥ 0.3 mg/mmol	*VAI < 1.35*	272.80±3.54	*MAP < 98 mmHg*	276.79±3.59	*Glucose < 5.2 mmol/L*	278.76±3.43
	VAI ≥ 1.35	290.17±3.46	*MAP ≥ 98 mmHg*	285.53±3.43	*Glucose ≥ 5.2 mmol/L*	284.42±3.59

Adjusted for the median of uACR *per se*, the respective median of VAI *per se*, MAP *per se*, and glucose *per se,* as well as age, gender, smoking status, education, HDL-cholesterol, eGFR, and CVD.

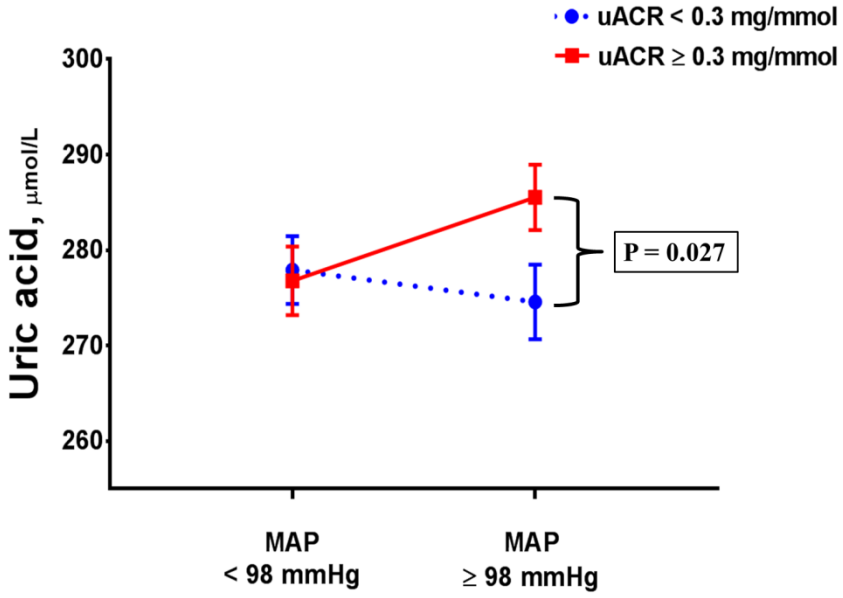
Adapted from *(Krajcoviechova et al., 2016)*.

Figure 5. Differences in multivariate adjusted means ± SD of SUA levels in individuals with 1–2 MetS component(s) by the median of uACR in interaction with the median of (a) VAI, (b) MAP, (c) and fasting glucose.

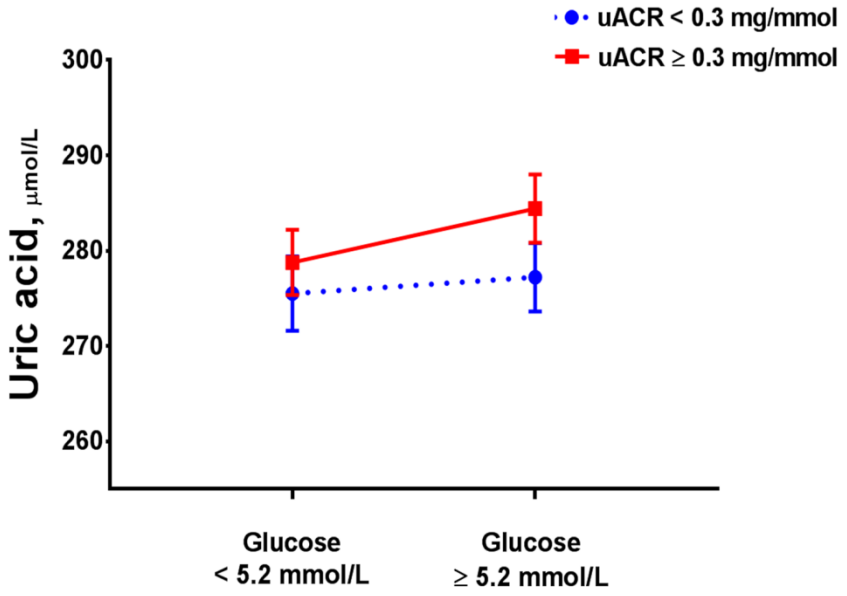
(a)



(b)



(c)



Adjusted for the median of uACR *per se*, the respective median of VAI *per se*, MAP *per se*, and glucose *per se,* as well as age, gender, smoking status, education, HDL-cholesterol, eGFR, and CVD.

Adapted from *(Krajcoviechova et al., 2016)*.

We further confirmed the effect modification of VAI and MAP on the association of SUA levels and uACR in separate linear regression analyses. Effect modification occurs when the magnitude of an association differs depending on the level of a third or fourth variable. One common way of dealing with effect modification is to examine the association separately for each level of the modifying variable. We therefore subdivided the individuals with 1−2 MetS component(s) into 4 groups defined by the medians of VAI and MAP. Then, we performed 4 separate linear regression models for SUA levels. Our independent variable of interest, Ln-uACR, was entered by default while the other potential confounders were included in a stepwise fashion. Interestingly, the positive association of Ln-uACR with SUA levels was exclusively confined to individuals whose VAI together with MAP were greater than the group median (Sβ 0.190; $p<0.001$) (Table 7). Of note, other factors increasing SUA levels, such as younger age, male gender, and decrease in eGFR contributed similarly to the variance of SUA levels in all subgroups. In

other words, the association of uACR and SUA levels was only observed when VAI and MAP were concomitantly increased.

Table 7. Multiple linear regression analyses for SUA levels by the medians of VAI and MAP in the 1−2 MetS component(s) group

	VAI < 1.35 & MAP < 98 mmHg n=287		**VAI ≥ 1.35 & MAP < 98 mmHg n=304**		**VAI < 1.35 & MAP ≥ 98 mmHg n=300**		**VAI ≥ 1.35 & MAP ≥ 98 mmHg n=283**	
	Sβ	**P value**	**Sβ**	**P value**	**Sβ**	**P value**	**Sβ**	**P value**
Ln-uACR, mg/mmol	0.064	0.108	0.048	0.268	0.024	0.571	0.19	<0.001
Gender (male)	0.468	<0.001	0.487	<0.001	0.501	<0.001	0.485	<0.001
eGFR, ml/min/1.73m^2	-0.406	<0.001	-0.321	<0.001	-0.443	<0.001	-0.489	<0.001
Waist circumference, cm	0.296	<0.001	0.226	<0.001	0.321	<0.001	0.248	<0.001
Age, years	-0.182	<0.001	-0.165	0.001	-0.275	<0.001	-0.286	<0.001
Ln-triglycerides, mmol/L	0.107	0.010	0.126	0.009	-	-	0.104	0.027

Ln-uACR was entered by default, while other potential covariates were included in a stepwise fashion.

Adapted from *(Krajcoviechova et al., 2016)*.

4.2 The CARTaGENE study

4.2.1 Characteristics

Five hundred and ninety three (n=593) French Canadians participating in the CARTaGENE study had available genotyping and urinary measurements, were free of eGFR < 60 ml/min/1.73m2, glycosuria, and use of diuretics, allopurinol, and uricosuric drugs. The demographic, clinical, and urinary characteristics of included individuals are shown in Tables 8 and 9.

Table 8. Demographic and clinical characteristics of the CARTaGENE study participants

Age, years	54.3±8.6
Gender (men), n (%)	270 (45.5)
Current smoker, n (%)	172 (29.1)
Education	
Basic, n (%)	13 (2.2)
Secondary, n (%)	357 (60.3)
University, n (%)	222 (37.5)
Waist circumference, cm	90.9±13.5
Serum triglycerides, mmol/L	1.4 [0.9−2.2]
Ln-triglycerides, mmol/L	0.40±0.61
HDL-cholesterol, mmol/L	1.27±0.42
SBP, mmHg	123.5±18.3
DBP, mmHg	72.1±11.2
MAP, mmHg	89.2±12.8
Glucose, mmol/L	5.2 [4.7−5.9]
Ln-glucose, mmol/L	1.68±0.21
SUA, µmol/L	282.2±72.2
eGFR, ml/min/1.73m^2	90.8±12.3

Mean±SD, median and interquartile range, and number (percent) are presented for continuous variables with normal distribution, for continuous variables with positive skewed distribution, and for categorical variables, respectively.

Among the total of individuals taking lipid-lowering medication (n=90), 86 were taking statins, 3 fibrates, and 6 ezetimibe, either solely or in combination (Table 9). Among total of individuals taking BP-lowering medication (n=59), 32 were taking beta-blockers, 7 calcium-channel blockers, 30 RAS inhibitors, and 7 alpha-blockers. Seventeen individuals were on oral antidiabetics, whereas one was on insulin treatment.

Table 9. Urinary characteristics and medication use of the CARTaGENE study participants

History of CVD, n (%)	32 (5.4%)
BP-lowering medication, n (%)	59 (9.9%)
Lipid-lowering medication, n (%)	90 (15.2%)
Glucose-lowering medication, n (%)	18 (3%)
uACR, mg/mmol	0.30 [0.15−0.52]
Ln-uACR, mg/mmol	−1.20±1.11
Albuminuria, n (%)	23 (3.9%)
uUCR, mg/g creatinine	27.1 [11.4−43.2]
Ln-uUCR, mg/g creatinine	3.1±0.96
FEUA, %	10.0±4.21
FENa, %	0.81±0.43

Mean±SD, median and interquartile range, and number (percent) are presented for continuous variables with normal distribution, for continuous variables with positive skewed distribution, and for categorical variables, respectively.

4.2.2 Association of SLC2A9 SNPs with FEUA

We confirmed the associations of SLC2A9 candidate SNPs with SUA levels and FEUA (Tables 10 and 11). The association of both SNPs with SUA levels and FEUA was strong, and outreached the genome-wide significance threshold of $p<5*10^{-8}$, although this was not required for our candidate SNP analyses.

Table 10. Association of SLC2A9 candidate SNPs with SUA levels (μmol/L)

	Chr	Position	A1	A2	MAF	β	SE	P value
rs13129697	4	9926967	T	G	0.69	31.509	3.578	1.55×10^{-17}
rs16890979	4	9922167	C	T	0.75	28.676	3.796	1.70×10^{-13}

Final model adjusted for age, gender, waist circumference, serum triglycerides, MAP, eGFR. Chr, chromosome; A1, major allele; A2, minor allele; MAF, major allele frequency.

Table 11. Association of SLC2A9 candidate SNPs with FEUA (%)

	Chr	Position	A1	A2	MAF	β	SE	P value
rs13129697	4	9926967	T	G	0.69	−1.706	0.251	2.67×10^{-11}
rs16890979	4	9922167	C	T	0.75	−1.649	0.263	7.61×10^{-10}

Final model adjusted for age, gender, waist circumference, HDL-cholesterol and eGFR. Chr, chromosome; A1, major allele; A2, minor allele; MAF, major allele frequency.

4.2.3 Clinical and urinary univariate correlates for uACR

Women showed a slight trend toward higher Ln-uACR compared with men; however, the difference between the genders was not significant (Table 12). While Ln-uACR did not differ between smoking and education categories (Table 12), it did increase with age, serum triglycerides, BP, and glucose (Table 13). Systolic, mean, and diastolic BP levels showed the strongest association with Ln-uACR (r=0.209; r=0.191; r=0.157, respectively). As expected, Ln-uACR increased with the decrease in urinary uric acid excretion (Table 13). Interestingly, a decrease in urinary uromodulin as well as sodium excretion was associated with an increase in Ln-uACR. In fact, the association between urinary uromodulin and Ln-uACR was among the strongest ones reported here (r -0.130). However, we found no association of Ln-uACR with SUA levels and eGFR.

Table 12. Demographic correlates for Ln-uACR (mg/mmol)

		Median [interquartile range]	**P value**
Gender	Men	0.25 [0.13−0.50]	0.164*
	Women	0.28 [0.16−0.54]	
Current smoker	Yes	0.28 [0.15−0.53]	0.622*
	No	0.26 [0.15−0.51]	
Education	Basic	0.48 [0.20−0.90]	0.364[&]
	Secondary	0.26 [0.15−0.53]	
	University	0.26 [0.15−0.50]	
History of CVD	Yes	0.40 [0.22−0.85]	0.028*
	No	0.26 [0.15−0.51]	

*P value obtained using the Mann-Whitney U test
[&]P value obtained using the Kruskal-Wallis test

Table 13. Clinical and urinary univariate correlates for Ln-uACR (mg/mmol)

	r	P value
Age, years	0.136	0.001
Waist circumference, cm*	0.045	0.284
Ln-triglycerides, mmol/L	0.110	0.007
HDL-cholesterol, mmol/L	−0.057	0.169
SBP, mmHg	0.209	<0.001
DBP, mmHg	0.157	<0.001
MAP, mmHg	0.191	<0.001
Ln-glucose, mmol/L	0.114	0.005
SUA, µmol/L	0.028	0.499
eGFR, ml/min/1.73m^2	0.025	0.550
Ln-uUCR, mg/g creatinine	−0.130	0.002
FEUA, %	−0.096	0.020
FENa, %	−0.106	0.010

*Please note that only 575 individuals had waist circumference available.

4.2.4 Association of SLC2A9 SNPs with uACR

Table 14. Association of SLC2A9 candidate SNPs with Ln-uACR (mg/mmol)

	Crude			Multivariate adjusted[#]		
	β	**SE**	**P value**	**β**	**SE**	**P value**
rs13129697, T	−0.144	0.074	0.051	−0.181	0.075	0.016
rs16890979, C	−0.094	0.077	0.220	−0.121	0.077	0.119

[#]Final model adjusted for gender, MAP, glucose, and FeUA; further adjustment for age, uUCR, and FENa did not change the results.

In the linear regression model for Ln-uACR, the non-synonymous rs16890979 SNP was not associated with Ln-uACR either crude or multivariate adjusted (Table 14). On the other hand, the absolute beta (β) coefficient of rs13129697 for Ln-uACR showed a strong tendency toward statistical significance (β=−0.144±0.074; p=0.051), and further increased after multivariate adjustment (β=−0.181±0.075; p=0.016). Specifically, it was the inclusion of FEUA in the multivariate model, which increased the strength of association of each copy of the rs13129697 T allele with a decrease in Ln-uACR (Table 14). This suggests that the relationship between rs13129697 genotype and uACR is modified by the level of FEUA. Furthermore, the rs13129697 T allele, which was associated with an increase in SUA levels and a decrease in FEUA (Tables 10 and 11), was associated with a decrease in Ln-uACR (Table 14). This was unexpected, as a decrease in 24-hour UAE was associated with a decrease in SUA levels and an increase in FEUA according to Scheven et al. *(Scheven et al., 2014)*. Therefore, we included the interaction term of rs13129697 genotype (TT, TG, GG)*FeUA tertiles as an independent factor in the generalized linear mixed model for Ln-uACR. The model was adjusted for rs13129697 genotype *per se* and FEUA tertiles *per se*, as well as for the other significant confounders, i.e., gender, MAP and plasma glucose. Interestingly, the Ln-uACR increased by the interaction of rs13129697 GG genotype with FEUA tertiles (p=0.002). Among individuals within the 1st and 2nd FEUA tertiles, rs13129697 G homozygotes had higher estimated mean of Ln-uACR compared to heterozygotes (p=0.006; p=0.005, respectively) as well as compared to T homozygotes (p=0.004; p=0.001, respectively).

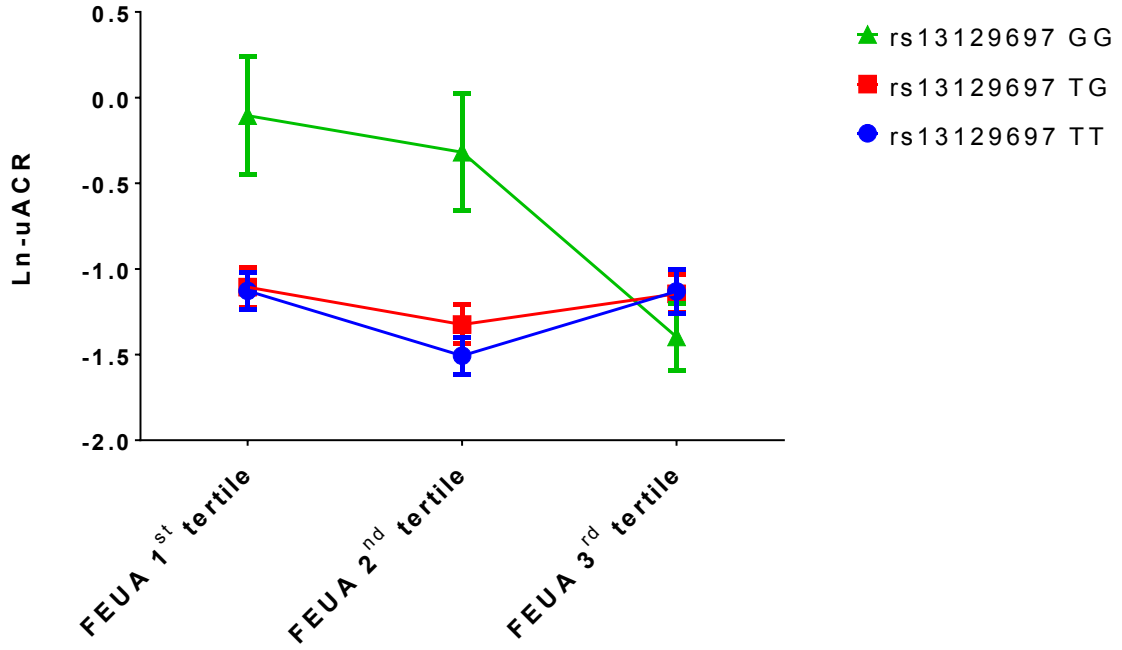
Further, among the rs13129697 G homozygotes, those within the 1st and 2nd FEUA tertiles had higher estimated mean of Ln-uACR compared to those within the 3rd FEUA tertile (p=0.001; p=0.006, respectively) (Table 15, Figure 6). At the same time, the estimated mean of Ln-uACR increased with a decrease in FEUA tertiles *per se* (p=0.020), and with the presence of each copy of the rs13129697 minor G allele *per se* (p=0.002) (Table 16, Figure 7). In pairwise comparison, rs13129697 G homozygotes had higher estimated mean of Ln-uACR compared to heterozygotes (p=0.002) as well as compared to T homozygotes (p<0.001). Separately, individuals within the 1st FEUA tertile had higher estimated mean of Ln-uACR compared to those in the 3rd FEUA tertile (p=0.005) (Table 16). When further adjusting the model for Ln-uUCR and FENa, the interaction between rs13129697 GG genotype and FEUA tertiles on Ln-uACR remained strong and significant (p=0.004); however the association between FEUA tertiles and Ln-uACR became insignificant (p=0.278). The frequencies of rs13129697 genotypes were in Hardy-Weinberg equilibrium (p=0.129).

Table 15. Multivariate adjusted means ± SD of Ln-uACR (mg/mmol) by the interaction of rs13129697 TT, TG, and GG genotypes with FEUA tertiles

FEUA tertile	rs13129697 TT n=270	rs13129697 TG n=272	rs13129697 GG n=51
1st	−1.128±0.108	−1.106±0.117	−0.105±0.343
2nd	−1.507±0.108	−1.324±0.114	−0.319±0.341
3rd	−1.131±0.131	−1.145±0.111	−1.397±0.195

Adjusted for rs13129697 genotype *per se*, FEUA tertiles *per se*, gender, MAP, and glucose.

Figure 6. Multivariate adjusted means ± SD of Ln-uACR (mg/mmol) by the interaction of rs13129697 TT, TG, and GG genotypes with FeUA tertiles.



Adjusted for rs13129697 genotype *per s,* FEUA tertiles *per se*, gender, MAP, and glucose.

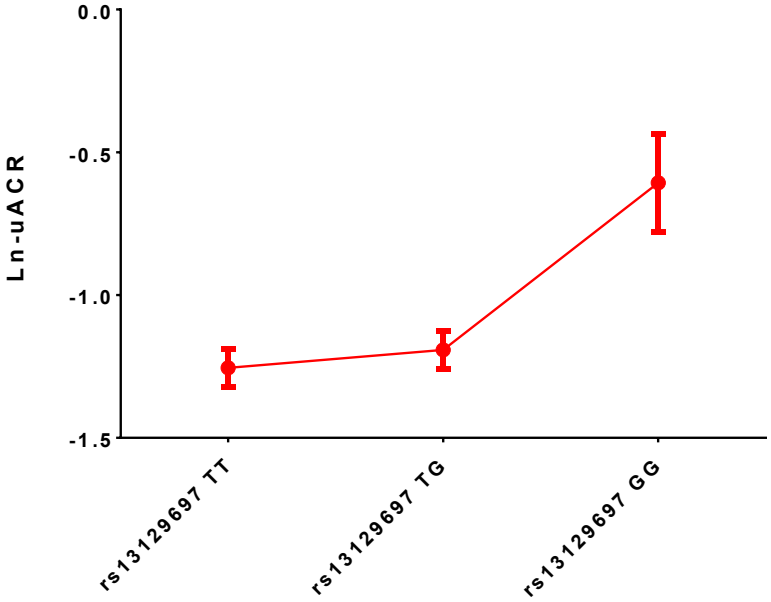
Table 16. Multivariate adjusted means ± SD of Ln-uACR (mg/mmol) by (a) rs13129697 TT, TG, and GG genotypes *per se* and (b) FEUA tertiles *per se*

Ln-uACR	**a)**		
	rs13129697 TT	**rs13129697 TG**	**rs13129697 GG**
	n=270	**n=272**	**n=51**
	−1.255±0.066	−1.192±0.065	−0.607±0.172
	b)		
	FEUA	**FEUA**	**FEUA**
	1st tertile	**2nd tertile**	**3rd tertile**
	−0.780±0.128	−1.050±0.125	−1.224±0.089

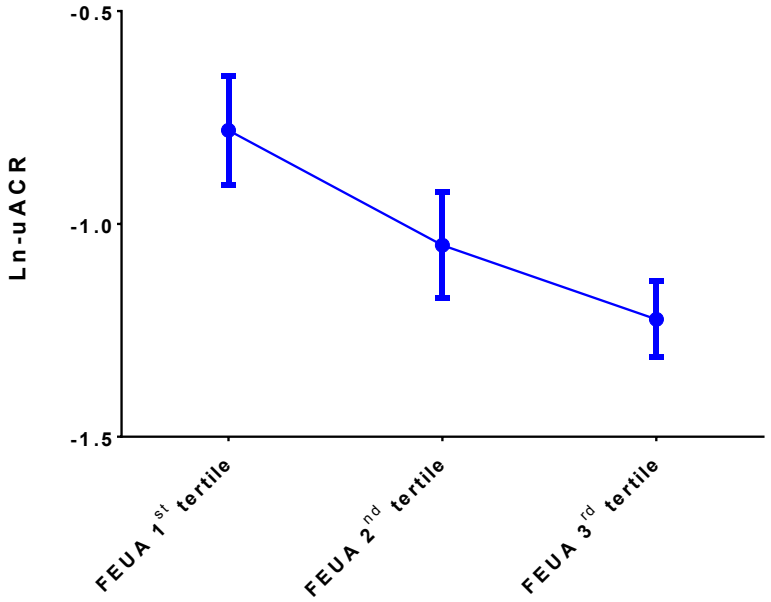
Adjusted for rs13129697 genotype *per se*, FEUA tertiles *per se*, gender, MAP, and glucose.

Figure 7. Multivariate adjusted means ± SD of Ln-uACR (mg/mmol) by (a) the rs13129697 TT, TG, and GG genotypes *per se* and (b) FeUA tertiles *per se*.

(a)



−(b)



Adjusted for rs13129697 genotype *per se*, FEUA tertiles *per se*, gender, MAP, and glucose.

4.2.5 Pathophysiology of the link between urinary uric acid and albumin excretion

First, we confirmed a linear association between urinary uric acid and albumin excretion. In a multivariate linear regression model for Ln-uACR, all univariate or previously reported correlates for Ln-uACR were considered as covariates, except for Ln-uUCR and FENa (Table 17). Covariates and our independent variable of interest (FEUA) were included in a stepwise backward fashion. An increase in MAP and plasma glucose, a decrease in FEUA, and female gender were independently associated with an increase in Ln-uACR, whereas carriage of each copy of the rs13129697 T allele was associated with a decrease in Ln-uACR.

We further evaluated the role of urinary uromodulin and sodium excretion as potential mediators of the relationship between FEUA and Ln-uACR. Mediation analysis enables drawing causal inferences between independent variable of interest and dependent variable in cross-sectional studies. As a prerequisite for mediation analysis, we confirmed that each mediator of interest (Ln-uUCR, FENa) is associated with independent (FEUA) and dependent (Ln-uACR) variable after adjusting for age and gender. Both potential mediators highly correlated with FEUA and Ln-uACR (Table 18).

Table 17. Multivariate linear regression model for Ln-uACR (mg/mmol) uncontrolled for mediator(s)

	β	**SE**	**Sβ**	**P value**
MAP, mmHg	0.014	0.004	0.172	<0.001
rs13129697, T	−0.181	0.075	−0.099	0.016
FEUA, %	−0.113	0.050	−0.101	0.023
Ln-glucose, mmol/L	0.458	0.215	0.086	0.034
Gender (male)	−0.188	0.096	−0.084	0.050

Variables were included in a stepwise backward fashion.

FEUA was standardized to a mean of 0 and SD 1 in order to enable the comparison of regression coefficients with Ln-uUCR and FENa.

Table 18. Age- and gender-adjusted Pearson correlations between potential mediators with FEUA and Ln-uACR

	FEUA, %		Ln-uACR, mg/mmol	
	Pearson correlation	**P value**	**Pearson correlation**	**P value**
Ln-uUCR	0.281	<0.001	−0.136	0.001
FENa	0.374	<0.001	−0.107	0.009

FEUA and potential mediators were standardized in order to enable the comparison of their regression coefficients.

Next, we used the linear regression model for Ln-uACR (see Table 17) as a reference model. When each of the potential mediator, and mediators together were added to the reference model, the regression coefficients of FEUA for Ln-uACR decreased and became non-significant. Specifically, Ln-uUCR explained 32% (model 2), FENa 44% (model 3), and Ln-uUCR together with FENa explained 70% (model 4) of the relationship between Ln-uACR with FEUA (reference model 1) (Table 19). This suggests that the total effect of FEUA on uACR may be almost fully mediated by urinary uromodulin and sodium excretion. Furthermore, the role of both mediators was justified in bootstrapping analysis. In addition, when we included an interaction term of rs13129697*FEUA in the model, β coefficients of Ln-uUCR and FENa for Ln-uACR remained unchanged (−0.112±0.046; −0.110±0.048, respectively). Finally, we confirmed a strong association of Ln-uUCR with albuminuria defined as a categorical trait. The odds of albuminuria decreased with an increase in each unit of Ln-uUCR in crude (OR 0.52; 95% CI 0.35−0.78; p=0.002) and multivariate adjusted analysis (OR 0.54; 95% CI 0.35−0.82; p=0.004). However, we observed no association of FENa with categorical albuminuria.

Table 19. Multiple multivariate models for Ln-uACR (mg/mmol) showing mediators of the relationship between urinary uric acid and albumin excretion

		β	SE	P value	Bootstraping	
					Point estimate	**95% CI**
Reference model 1 (from Table 17)						
	TOTAL EFFECT*					
	FEUA	−0.113	0.050	0.023	-	-
Model 2						
	DIRECT EFFECT[#]					
	FEUA	−0.077	0.051	0.133	-	-
	Path b[##]					
	Ln-uUCR	−0.120	0.046	0.010	−0.036	−0.070/-−0.011
Model 3						
	DIRECT EFFECT[#]					
	FEUA	−0.063	0.053	0.241	-	-
	Path b[##]					
	FENa	−0.117	0.049	0.016	−0.050	−0.099/−0.008
Model 4						
	DIRECT EFFECT[#]					
	FEUA	−0.034	0.055	0.540	-	-
	Path b[##]					
	Ln-uUCR	−0.112	0.046	0.016	−0.033	−0.070/−0.008
	FENa	−0.108	0.048	0.027	−0.046	−0.095/−0.003

All models were adjusted for gender, MAP, plasma glucose, and rs13129697 TT, TG, GG genotype. FEUA and potential mediators were standardized in order to enable a comparison of their regression coefficients.

*Effect of FEUA on uACR without controlling for mediator(s)

[#]Direct effect of FEUA on uACR after controlling for mediator(s)

[##]Direct effect of mediator(s) on uACR

4.3 The construct of the metabolic syndrome

A comparison of the CARTaGENE and Czech post-MONICA study individuals included in PCA is shown in Table 20. The CARTaGENE study individuals were older, had higher serum triglycerides, lower HDL-cholesterol, higher glucose levels, and were more often treated for dyslipidemia; however, they had lower MAP and were less often treated for arterial hypertension compared to the Czech post-MONICA study individuals. There was no difference in gender, smoking habits, waist circumference, SUA levels, uACR, and eGFR between the cohorts (Table 20).

First, we applied PCA to the defining variables of MetS separately in both studies. Second, we applied PCA to the defining variables of MetS after the addition of (a) Ln-uACR and FEUA (CARTaGENE study), and (b) Ln-uACR and SUA levels (Czech post-MONICA study). The correlation coefficients between variables and principal components are shown in Table 21. Each PCA exhibited a satisfactory value of sampling adequacy (Kaiser-Meyer-Olkin of 0.685 to 0.727) and met the criteria for Bartlett´s Test of Sphericity, ($p<0.001$). This implies that there are correlations in the dataset that are appropriate for PCA.

When PCA was applied to the established variables of MetS (5 variables), the single principal component was identified in both studies. The derived score explained 42.6% and 41.2% of the variance of included variables in CARTaGENE, and in the Czech post-MONICA study, respectively. The absolute loadings of waist circumference, triglycerides, and HDL-cholesterol to the derived score were high, comparable and ranged between 0.69 and 0.77 in both studies, followed by a MAP of around 0.5. The loadings of glucose to a single component were 0.38 in the CARTaGENE and 0.45 in the Czech post-MONICA studies (Table 21). Overall, the absolute loadings of established MetS variables indicate a high correlation with the single principal component.

Table 20. Distribution of metabolic and renal phenotypes between studies

	CARTaGENE n=590	Czech post-MONICA n=2930	P value
Age, years	52.9±8.5	45.7±11.2	<0.001
Gender (men), n (%)	270 (45.8)	1419 (48.4)	0.237
Current smoker, n (%)	167 (28.4)	936 (31.9)	0.086
Waist circumference, cm	91.0±13.6	90.8±14.0	0.742
Serum triglycerides, mmol/L	1.4 [0.9-2.2]	1.2 [0.9-1.8]	<0.001
Ln-triglycerides, mmol/L	0.38±0.61	0.25±0.56	<0.001
HDL-cholesterol, mmol/L	1.27±0.43	1.47±0.39	<0.001
MAP, mmHg	90.1±13.0	97.9±13.1	<0.001
Glucose, mmol/L	5.2 [4.7-5.9]	5.2 [4.8-5.6]	0.021
Ln-glucose, mmol/L	1.68±0.21	1.65±0.13	<0.001
SUA, µmol/L	281.7±77.1	288.5±87.9	0.082
FEUA, %	10.0±4.2	-	
eGFR, ml/min/1.73m^2	91.1±12.3	91.6±15.1	0.412
uACR, mg/mmol	0.27 [0.15-0.51]	0.2 [0.1-0.6]	0.359
Ln-uACR, mg/mmol	-1.20±1.10	−1.20±1.15	0.879
uUCR, mg/g creatinine	27.1 [11.2-43.2]	-	
Ln-uUCR, mg/g creatinine	3.1±1.0	-	
FENa, %	0.8±0.4	-	
BP-lowering medication, n (%)	60 (10.2)	459 (15.7)	<0.001
Lipid-lowering medication, n (%)	86 (14.6)	209 (7.2)	<0.001

Mean±SD, median and interquartile range and number (percent) are presented for continuous variables with normal distribution, for continuous variables with not normal distribution, and for categorical variables, respectively.

Table 21. PCA using traditional defining variables of the MetS alone and after the addition of (a) uACR and FEUA (CARTaGENE study), and (b) uACR and SUA levels (Czech post-MONICA study).

	The CARTaGENE study			The Czech post-MONICA study		
	5 variables	7 variables		5 variables	7 variables	
	Single PC	1st PC	2nd PC	Single PC	1st PC	2nd PC
% of variance explained	42.6	32.3	15.1	41.2	33.2	14.6
Eigenvalue	2.13	2.26	1.06	2.06	2.33	1.02
Waist circumference, cm	0.76	0.76	0.26	0.75	0.75	0.03
Ln-triglycerides, mmol/L	0.77	0.77	0.18	0.75	0.74	0.08
HDL cholesterol, mmol/L	−0.70	−0.77	−0.07	−0.69	−0.66	0.06
MAP, mmHg	0.51	0.40	0.62	0.52	0.49	0.36
Ln-glucose, mmol/L	0.38	0.38	0.12	0.45	0.40	0.007
Ln-uACR, mg/mmol	-	0.03	0.84	-	−0.004	0.95
FEUA, %	-	−0.36	−0.36	-	-	-
SUA, μmol/L	-	-	-	-	0.62	0.10

PC, principal component. Displayed are correlation coefficients between included variables and principal components.

When applying PCA to the established variables of MetS together with Ln-uACR and FEUA in the CARTaGENE, and with Ln-uACR and SUA levels in the Czech post-MONICA study (7 variables), two principal components were identified. The second principal component accounted for around 15% of the explained variance in both studies, while the total explained variance of the included variables increased up to 47.4% in the CARTaGENE and 47.8% in the Czech post-MONICA studies. The loading factors of the established MetS variables to the first principal component were comparable to the loading factors to the single component of an un-enriched score in both studies, whereas

the loading of Ln-uACR to the first principal component was marginal. On the contrary, Ln-uACR exhibited a very high and comparable loadings to the second principal component in both studies (r=0.84; r=0.95, respectively). Besides Ln-uACR, the second principal component was reasonably loaded by MAP (r>0.3) in both studies. In the CARTaGENE study, FEUA loaded negatively and identically to the first and second principal components (r=−0.36) whereas, in the Czech post-MONICA study, SUA levels loaded only to the first principal component (r=0.62). Overall, despite slight disparities, the principal components exhibited comparable loading patterns between the studies. As direct Oblimin rotation was used, the first and second principal component scores correlated in the CARTaGENE (r=0.265), as well as in the Czech post-MONICA studies (r=0.095). Of note, all scores were normally distributed.

Then, we evaluated the relationship of the principal component scores with Ln-uUCR and FENa in the CARTaGENE study. Each unit decrease in Ln-uUCR was associated with an increase of 0.096 in the single principal component score (β −0.096±0.041; p=0.020), an increase of 0.117 in the first principal component score (β −0.117±0.041; p=0.005), and an increase of 0.203 in the second principal component score (β −0.203±0.040; p<0.001). Each unit decrease in FENa was associated with an increase of 0.121 in the second principal component score (β −0.121±0.041; p=0.003), whereas no association with the single principal component score (β 0.043±0.041; p=0.297) and the first principal component score was observed (β −0.013±0.041; p=0.754).

Finally, we evaluated whether urinary uromodulin could serve as a potential mediator of the relationship between the first and second principal component scores in the CARTaGENE study. When adding uUCR to the reference model, the regression coefficients of the first principal component for the second principal component decreased while remaining still highly significant. Unequivocally, Ln-uUCR explained 9.1% (model 2) of the relationship between the first and second principal component scores (Table 22). Furthermore, the mediating role of uUCR was confirmed in bootstrapping analysis.

Table 22. Multivariate models for the second principal component score

	β	SE	P value	Bootstrapping	
				Point estimate	**95% CI**
Reference model					
TOTAL EFFECT*					
1st PC score	0.263	0.041	<0.001	-	-
Model 2					
DIRECT EFFECT#					
1st PC score	0.239	0.040	<0.001	-	-
Path b##					
Ln-uUCR	−0.184	0.040	<0.001	0.025	0.010/0.046

All models were adjusted for eGFR, current smoking, and education.

PC, principal component.

*Effect of 1st PC on 2nd PC without controlling for mediator

#Direct effect of 1st PC on 2nd PC after controlling for mediator

##Direct effect of mediator on 2nd PC

5 Discussion

This thesis evaluated the structure of clustering of metabolic phenotypes and assessed the pathophysiological mechanisms underlying the relationship between urinary uric acid and albumin excretion. Data from two population-based studies were analyzed. Due to the high prevalence of arterial hypertension (men 50.2%, women 37.2%) *(Cifkova et al., 2010[a])*, dyslipidemia (men 73.8%, women 66%), and body mass index ≥ 30 kg/m^2 (men 33.6%, women 30.1%) *(Cifkova et al., 2010[b])*, the Czech post-MONICA study is well-suited for evaluation of the modification effects of metabolic phenotypes on the relationship of SUA levels with uACR. On the other hand, the major strength of the CARTaGENE study is the biobank of urine samples enabling determination of a number of urinary solutes and protein concentrations at a population level, thus enabling further insight into renal pathophysiology *(Awadalla et al., 2013)*.

Our major findings can be summarized in four parts:

1. In the cross-sectional analysis of data obtained in a large representative population survey (Czech post-MONICA study), we showed that uACR was an independent factor for the increase in SUA levels in adults without manifest MetS, but with 1–2 MetS component(s) *(Krajcoviechova et al., 2016)*. There was solid evidence for synergistic interaction of uACR with visceral adiposity and BP on the increase in SUA levels.
2. In the population-based cohort of French Canadians (CARTaGENE study), we showed that the serum urate-increasing rs13129697 T allele was associated with a paradoxical decrease in uACR. The reason for this discrepant finding is the interaction between rs13129697 genotype and the current rate of FEUA.
3. We confirmed that the decrease in urinary uric acid excretion is associated with an increase in uACR in the French Canadian cohort. Using the mediation analysis approach, we further suggest that decreased urinary uromodulin and sodium excretion may be in a causal pathway between decreased urinary uric acid excretion and albuminuria.
4. Finally, by additing uACR together with SUA levels (Czech post-MONICA study), and uACR together with FEUA (CARTaGENE study) to the established variables of MetS, we identified two correlated, but still unique principal

components. With slight disparities, the first and second principal component scores exhibited comparable loading patterns between the studies. In the CARTaGENE study, urinary uromodulin explained 9% of the correlation between the principal component scores.

5.1 Modification effect of visceral adiposity and BP on the relationship between uACR and SUA levels

In the Czech post-MONICA cohort, SUA levels increased by a synergistic interaction of uACR with visceral adiposity and BP, which supports the hypothesis of obesity-related hypertension with altered renal hemodynamics as the primary mechanism *(Vaneckova et al., 2014)*. Although several studies have shown the association between SUA levels and albuminuria in patients with hypertension, pre-hypertension, and diabetes, their relationship in subjects with increased adiposity was unknown *(Lee et al., 2006; Resl et al., 2012; Viazzi et al., 2005)*. On the other hand, in a small cross-sectional study of 429 hypertensive patients, coexistence of MetS and hyperuricemia increased 4 times the odds of being microalbuminuric *(Rodilla et al., 2009)*. Even though one may reason that our results only confirm the relationship between uric acid and BP, an increase in MAP only, or an increase in VAI only, did not modify the association of SUA levels with uACR.

Oxidative stress induced by intracellular uric acid with subsequently decreased NO bioavailability and enhanced RAS activation could be one of the molecular mechanisms responsible for the observed link between increased SUA levels and albuminuria in obesity and hypertension *(Krajcoviechova et al., 2017; Marseglia et al., 2014)*. In line with the detrimental actions of intracellular uric acid, animal models describe the mechanisms of hyperuricemia-induced MetS and arterial hypertension *(Mazzali et al., 2001; Nakagawa et al., 2006)*. Besides, ectopic fact accumulation, particularly in the visceral compartment, serves as an active endocrine organ releasing adipocytokines or adipokines, which further induce local and systemic ROS production and low-grade inflammation. In turn, adipose tissue inflammation indicates transition from simple obesity to arterial hypertension, insulin resistance, and microvascular damage *(Nishimura et al., 2009)*.

In the past years, hyperuricemia in MetS was attributed to decreased uric acid excretion due to hyperinsulinemia and decreased GFR, or due to enhanced reabsorption. However, the status of GFR does not provide complete justification for the relationship between SUA levels and MetS *(See et al., 2009)*. It has been documented that high-normal SUA levels increase the risk of early GFR decline in type 1 diabetic patients *(Ficociello et al., 2010)*. In our analyses, the inverse association of eGFR with SUA levels was consistent and of comparable magnitude between the 0 MetS component and 1−2 MetS component(s) groups, which implies that metabolic phenotypes might have no modification effect on this association. Furthermore, we observed no interaction of eGFR with VAI (Sβ -0.024; p=0.303), eGFR with MAP (Sβ 0.002; p=0.924), and eGFR with glucose (Sβ -0.023; p=0.330) in relation to SUA levels. These results remained unchanged even after including individuals with eGFR < 60 ml/min/1.73m^2 and diabetes without MetS (total subjects analyzed n=1211). On the other hand, our study suggests a link between SUA levels and uACR in a condition that may hypothetically evolve into MetS. Although a clustering of metabolic risk factors is undeniable, the concept of a syndrome with insulin resistance as a solely underlying mechanism has been challenged *(Kahn et al., 2005)*. However, if hyperuricemia may induce MetS, and there is a link between uricemia and albuminuria, one may hypothesize that both SUA levels and uACR are involved in the pathogenesis of MetS. Our findings indirectly support this hypothesis by showing the interaction between metabolic phenotypes with UAE in adults without manifest MetS by its arbitrary definition.

5.2 The association between SLC2A9 SNP and uACR

In a cross-sectional analysis of the available genotyping and urine data of the French Canadian cohort (CARTaGENE study), we confirmed an association between both our SLC2A9 candidate SNPs and SUA levels, with the direction and magnitude of the effect consistent with previous reports *(Dehghan et al., 2008; Huffman et al., 2015; Karns et al., 2012; McArdle et al., 2008; Yang et al., 2010)*. The role of SLC2A9 as a high-capacity urate transporter has been well documented *(Caulfield et al., 2008; Preitner et al., 2009)*. The major alleles of several noncoding variants of the SLC2A9 gene have also been associated with a decrease in urate excretion (*Caulfield et al., 2008;*

Kottgen et al., 2013; Vitart et al., 2008). But, none of these analyses included our selected candidate SNPs. In this thesis, we showed an association of the rs13129697 and rs16890979 major alleles with decreased FEUA, with the strength of associations well over-reaching the genome-wide significance. The French Canadians represent a population with a founder effect. This means that the founder population may have less genetic variation, as well as that the allele frequencies may differ compared to the original population. However, the allele frequencies of our candidate SNPs among French Canadians were comparable to those previously reported in the 1000 Genomes Project for populations of European descent *(Auton et al., 2015)*.

Due to the strong and independent association of uric acid with current and future MetS, it has been of interest for many researchers whether variants of the SLC2A9 gene, the major determinant of SUA levels, also associate with metabolic phenotypes. Overall, these studies yielded controversial results. On the contrary, evidence on the association of SLC2A9 variants with albuminuria is scarce. Voruganti et al. showed that the rs13129697 minor allele was associated with uACR in Mexican Americans; however, the direction of the effect was not reported *(Voruganti et al., 2013)*. Similarly, several non-coding SLC2A9 SNPs (rs6832439, rs13131257, rs737267) were associated with uACR at a p value with a strong tendency towards statistical significance (p 0.050−0.056) in American Indians *(Voruganti et al., 2014)*.

In the general population of the city of Groningen, the Netherlands, Scheven et al. have reported that the 24-hour UAE is positively associated with tubular uric acid reabsorption and SUA levels. The authors suggested tubular uric acid-albumin exchange and speculated that the association of albuminuria with uric acid reabsorption may be mediated by sodium transport *(Scheven et al., 2014)*. In line with Scheven et al., we have confirmed that a decrease in FEUA is associated with an increase in uACR in a representative sample of French Canadians, free of eGFR < 60ml/min/1.73m^2, glycosuria, and confounding medication. It has been shown that the rs13129697 G allele decreases SUA levels (Yang et al., 2010). However, it was the rs13129697 T allele, which was associated with a decrease in uACR in our analyses. The reason for this discrepant finding is an interaction between rs13129697 GG genotype and the current rate of FEUA. In particular, it appears that rs13129697 GG genotype may be "dysfunctional" under specific circumstances and FEUA is decreased, which may lead to

increased UAE. This finding further supports the hypothesis of tubular uric acid/albumin exchange. However, the conditions leading to the paradoxical decrease in FEUA in homozygotes for the rs13129697 G allele are unclear. It has been shown that SLC2A9 exerts a gender-specific effect, with the rs13129697 T allele increasing SUA levels to a considerably greater extent in women than in men *(Karns et al., 2012)*. Accordingly, it may also be that rs13129697 is associated with uACR with a gender-specific effect; however, a greater sample size than ours would be required to test these associations separately in both genders. On the other hand, albuminuria may modify the expression of genes encoding for tubular uric acid transporters the same as albumin modifies the function of several non-urate transporters in the proximal tubular cells *(Scheven et al., 2014)*.

It has been estimated that 90% of clinical hyperuricemia cases result from impaired urinary uric acid excretion *(Le et al., 2008)*. Despite an inverse association between FEUA and uACR, we found no association between SUA levels and uACR in French Canadians. There are two explanations for this discrepancy. First, the SLC2A9 gene, a major determinant of FEUA, has two transcript variants. The long isoform is expressed mostly in basolateral membranes, whereas the short one is expressed only in apical membranes of the proximal tubular cells *(Augustin et al., 2004)*. A hypothetical condition or defect of the long isoform with decreased uric acid reabsorption from the epithelial cells to the blood, whereas a normal function of the short isoform would result in relative accumulation of uric acid within the proximal tubular cells, reduced FEUA, and normal SUA levels. Second, SLC2A9 is not only a urate transporter, but also a hexose transporter. However, the rates of urate transport by both SLC2A9 isoforms are 45- to 60-fold faster than those of glucose or fructose *(Caulfield et al., 2008)*. Caulfield et al. proposed that SLC2A9 can exchange extracellular (urinal) glucose and fructose for intracellular urate when secreting urate from the blood into the urine, and assumed that glycosuria with reduced SUA levels seen in type 2 diabetes may facilitate urate efflux into the urine due to trans-stimulation of urate transport at the apical membrane of the proximal tubular cells *(Caulfield et al., 2008)*. By contrast, in the absence of glycosuria, uric acid excretion would be relatively reduced and uric acid may accumulate within the epithelial cells of the proximal tubules, where it may induce oxidative stress with all the previously discussed consequences. This mechanism goes in line with the inverse

correlation between FEUA and uACR in the absence of glycosuria in the French Canadian cohort.

5.3 Role of urinary uromodulin and sodium excretion

A large body of evidence from animal studies corroborates uric acid as a causal factor in the development of MetS and hypertension. As has been already discussed above, Scheven et al. suggested that the association of albuminuria with uric acid reabsorption may be mediated by sodium transport *(Scheven et al., 2014)*. Our data provide further support for this hypothesis. Using the mediation analysis approach, we suggest that the effect of decreased urinary uric acid excretion on increase in uACR may be mediated by decreased uromodulin and sodium excretion. There are several aspects of the proposed mechanism which should be discussed in the context of current knowledge on uromodulin excretion and tubular physiology.

Uromodulin is a high-molecular-weight polymer secreted exclusively by the epithelial cells of the TAL and represents the most abundant protein in the urine. When secreted, it is first anchored to the apical membrane of the epithelial cells, from where it is cleaved into the tubular fluid. Uromodulin facilitates the translocation of the Na-K2-Cl co-transporter to the apical surface of the TAL and increases Na-K2-Cl-mediated NaCl reabsorption *(Mutig et al., 2011)*. Accordingly, uromodulin has been suggested to have a role in BP regulation through modulation of salt and water handling in the TAL (*Padmanabhan et al., 2014)*.

In epidemiological studies, urinary uromodulin excretion positively correlated with eGFR, dietary salt intake, FENa, FEUA, urinary volume, and ultrasound-assessed renal length and volume, and negatively correlated with age and diabetes *(Padmanabhan et al., 2010; Pruijm et al., 2016; Troyanov et al., 2016; Torffvit et al., 2004)*. Furthermore, when added to the established cardiovascular scores, serum uromodulin improved the risk prediction in patients undergoing coronary angiography *(Delgado et al., 2017)*. Collectively, uromodulin appears to be a marker of tubular mass and function, as well as cardiovascular health.

As expected, FEUA positively correlated with FENa and uromodulin excretion in our analyses. The explanation for the link between uromodulin excretion, FENa, and FEUA was that with low uromodulin production there is less sodium reabsorption in the TAL, followed by a compensatory increase in sodium reabsorption in the proximal tubule and a decrease in FENa *(Padmanabhan et al., 2010; Bleyer et al., 2016)*. Indeed, only 25% of the total sodium reabsorbtion takes place in the TAL, whereas around 69% of the total sodium is reabsorbed in the proximal tubule. Proximal sodium and urate reabsorption are directly related through a tertiary active transport process *(Bobulescu & Moe, 2012)*. On the other hand, Troyanov et al. showed that there is also an association between uricosuric medication use and urinary uromodulin, suggesting that tubular uric acid may affect uromodulin excretion and TAL activity *(Troyanov et al., 2016)*. This could be explained by the finding that increased dietary salt intake, and thus the amount of sodium in the tubular fluid, increases uromodulin expression and excretion in rats as well as in humans *(Torffvit et al., 2004; Ying & Sanders, 1998)*. Thus, lower FEUA with coupled lower FENa may decrease uromodulin excretion and TAL activity. Accordinly, with less uromodulin excretion, there is less sodium reabsorption in the TAL and more sodium reabsorption in the proximal tubule.

Unlike some other authors, we showed an independent and inverse association of uUCR with uACR, and an inverse association of FENa with uACR. In addition, we confirmed the inverse independent association between uUCR and albuminuria defined as a categorical trait. These findings merit greater attention. In the study of two population-based cohorts including a total of 6500 individuals, both 24-hour uromodulin excretion and spot uromodulin concentration were positively associated with eGFR and sodium excretion; however, no association with albuminuria was observed *(Pruijm et al., 2016)*. On the contrary, using a case-cohort design, Garimella et al. have shown that individuals with higher uromodulin excretion had higher eGFR, which is consistent with other reports, but they also had lower uACR, lower SBP, and lower prevalence of diabetes *(Garimella et al., 2017)*, which goes in line with our findings. Furthermore, reduced urinary uromodulin excretion was observed in diabetic nephropathy *(Bernard et al., 1987)*. Also, minor G allele of the rs13333226 polymorphism in the uromodulin gene, which has been associated with a lower risk of hypertension and increased eGFR in

another study *(Padmanabhan et al., 2010)*, has been shown to be protective against diabetic nephropathy *(Ahluwalia et al., 2011)*.

The explanation of the lack of an association between uromodulin excretion and albuminuria put forth in other studies was that uromodulin represents a marker of tubular mass and function, whereas albuminuria results from glomerular damage. On the other hand, increasing evidence emphasizes the role of the proximal tubule in albumin reabsorption *(Dickson et al., 2014)*. Thus, if the amount of uromodulin excretion represents a proxy of tubular function, it may well be that it also somehow reflects the activity of the proximal tubule, where albumin is reabsorbed.

The inverse association of FENa with uACR appears to be counter-intuitive, as a high dietary sodium intake reflected in higher FENa is a risk factor for hypertension and albuminuria. Accordingly, studies evaluating the relationship between sodium intake/excrection and albuminuria reported either a positive *(Forman et al., 2012; Fox et al., 2006)*, or no association *(Chang et al., 2013)*. On the contrary, with less uromodulin excretion, there is less sodium reabsorption in the TAL and more sodium reabsorption in the proximal tubule. Overall, less sodium is excreted, which may result in an increase in BP and uACR. This proposed mechanism also explains the inverse association of uUCR with uACR. Accordingly, in rats with experimentally induced hyperuricemia, the effect of increased SUA levels on the increase in BP was more pronounced with salt restriction, which was explained by enhanced RAS activity (Mazzali et al., 2001). In turn, enhanced RAS activation is associated with increased UAE.

5.4 Construct of the metabolic syndrome

In order to update the construct of the MetS, identification of additional risk phenotypes and continuous score modeling have been recommended *(Kahn et al., 2005)*. First, we derived age- and gender-adjusted principal component scores of MetS by applying PCA to the established MetS variables as described by Wijndaele et al. *(Wijndaele et al., 2006)*. Here, a single principal component was identified, explaining 42.6% of the variance in the data. Similarly, we applied PCA to the established variables of the MetS enriched with uACR together with SUA levels (Czech post-MONICA study) or with FEUA (CARTaGENE study). Here, two principal components were identified,

increasing the overall explained variance up to 47−48%, a figure comparable to the previously reported ones by Wijndaele et al. or Hillier et al. (50%) *(Wijndaele et al., 2006; Hillier et al., 2006)*.

In recent decades, factor analysis and PCA have been widely used to describe the underlying structure of the MetS. Several studies showed that there are at least two factors explaining the overall correlation between the syndrome variables *(Graziano et al., 2015; Wijndaele et al., 2006)*. On the other hand, some other studies reported only one factor of the syndrome, mainly loaded by adiposity measures, similar to the single principal component of our un-enriched score *(Gurka et al., 2014; Hillier et al., 2006)*. However, none of these studies included SUA levels, FEUA or uACR.

There are several findings from PCA that should be highlighted. First, the single principal component derived from the established variables of MetS, as well as the first principal component of an enriched score, were mainly loaded with the variables representing abdominal obesity in both studies. Second, uACR was the main loading factor to the second principal component, followed by BP in both studies. Third, the FEUA loaded evenly to the first and second principal components in the CARTaGENE study, whereas SUA levels loaded reasonably ($r>0.3$) only to the first principal component in the Czech post-MONICA study. It should be noted that, despite the high correlation ($r=0.589$), SUA levels and FEUA are two diferrent phenotypes, and may reflect different actions of uric acid. To support this assumption, we performed an additional PCA in the CARTaGENE study, in which we included SUA levels instead of FEUA, while keeping the remaining variables unchanged. Here, SUA levels loaded reasonably only to the first principal component with a magnitude comparable to that we reported in the Czech post-MONICA study ($r=0.70$ vs. 0.62). Fourth, the two principal component scores were correlated in both studies, we assume that there are at least two inter-related pathophysiological mechanisms underlying the clinical expression of the syndrome. While trying to reduce them into a single entity, substantial information may be neglected. The two unique principal component scores question the presence of a single unifying etiology of MetS. Interestingly, urinary uromodulin explained a part of the correlation between the principal component scores with a direction consistent with the effect of FEUA on uromodulin, and uromodulin on uACR. Fifth, more than half of the variance of the included variables remained unexplained, which suggests greater

complexity of the syndrome. Overall, despite slight disparities, our findings provide further support for the common variance in cardio-renal phenotypes in diverse populations.

5.5 Strength and limitations

Both studies represent a large cross-sectional survey of health determinants in a randomly selected population sample using a standardized protocol and detailed phenotyping. In addition, all biochemical analyses were performed in the central laboratories using standardized methods. The major strength of our analyses is the availability of stored urine samples in the French Canadian cohort, in which the concentrations of a number of solutes and proteins were measured. Finally, large-scale information is available on a number of covariates, including medication use, which allows adjustment for treatment effects.

Several limitations of our analyses should also be mentioned. Even though we used a mediation analysis approach, the cross-sectional nature of both studies limits causal inferences. In addition, uACR and uUCR measurements were based on a single morning spot urine sample. However, this method is accepted in epidemiological studies, and both uACR and uUCR were shown to be in good agreement with 24-hour uromodulin and albumin excretion, respectively *(Dyer et al., 2004; Pruijm et al., 2016)*. Furthermore, only uACR was available in the urine samples from the Czech post-MONICA study, while other solutes like uromodulin, uric acid, and sodium were not determined. Finally, inclusion of Caucasians only limits the applicability of the findings to different ethnicities.

6 Conclusions

In this thesis, data from two population-based studies were analyzed. In the Czech post-MONICA study, we showed that the urinary albumin/creatinine ratio was an independent factor for the increase in serum uric acid levels in adults without manifest metabolic syndrome, but with 1–2 syndrome component(s). Furthermore, serum uric acid levels increased by the synergistic interaction of urinary albumin/creatinine ratio with visceral adiposity and blood pressure, which may suggest obesity-related hypertension with altered renal hemodynamics as the primary mechanism. The mechanism underlying the relationship between urinary uric acid and albumin excretion was further evaluated in a representative population sample of French Canadians (CARTaGENE study) with more detailed urine biochemical analyses available. First, we confirmed that the decrease in fractional excretion of uric acid is associated with an increase in urinary albumin/creatinine ratio. Second, using the mediation analysis approach, we suggest that the effect of uric acid excretion on the albumin excretion may be mediated by urinary uromodulin through modulation of sodium reabsorption in the thick ascending limb of the loop of Henle. Indeed, the uromodulin and sodium excretion explained the major part of the relationship between urate and albumin excretion, which further supports the role of altered blood pressure regulation in this relationship. Uromodulin may not only be in a causal pathway between urate and albumin excretion but, also, to a much lesser extent, between the principal components of the metabolic syndrome. Due to the cross-sectional design, these findings require validation in prospective clinical and experimental studies.

In conclusion, the kidney plays a central role in blood pressure regulation. However, impaired urine solute and protein excretion represent not only a renal manifestation of arterial hypertension, but may also reflect processes which underlie the metabolic syndrome.

7 References

Ahluwalia TS, Lindholm E, Groop L, Melander O. Uromodulin gene variant is associated with type 2 diabetic nephropathy. J Hypertens 2011;29(9):1731-1734.

Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120(16):1640-1645.

Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15(7):539-553.

Amato MC, Giordano C, Galia M, Criscimanna A, Vitabile S, Midiri M, Galluzzo A. Visceral Adiposity Index: a reliable indicator of visceral fat function associated with cardiometabolic risk. Diabetes Care 2010;33(4):920-922.

Arnlov J, Evans JC, Meigs JB, Wang TJ, Fox CS, Levy D, et al. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study. Circulation 2005;112(7): 969-75.

Assmann G, Guerra R, Fox G, Cullen P, Schulte H, Willett D, Grundy SM. Harmonizing the definition of the metabolic syndrome: comparison of the criteria of the Adult Treatment Panel III and the International Diabetes Federation in United States American and European populations. Am J Cardiol 2007;99(4):541-548.

Augustin R, Carayannopoulos MO, Dowd LO, Phay JE, Moley JF, Moley KH. Identification and characterization of human glucose transporter-like protein-9 (GLUT9): alternative splicing alters trafficking. J Biol Chem 2004;279(16):16229-16236.

Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. A global reference for human genetic variation. Nature 2015;526(7571):68-74.

Awadalla P, Boileau C, Payette Y, Idaghdour Y, Goulet JP, Knoppers B, Hamet P, Laberge C. Cohort profile of the CARTaGENE study: Quebec's population-based biobank for public health and personalized genomics. Int J Epidemiol 2013;42(5):1285-1299.

Babio N, Martinez-Gonzalez MA, Estruch R, Warnberg J, Recondo J, Ortega-Calvo M, et al. Associations between serum uric acid concentrations and metabolic syndrome and its components in the PREDIMED study. Nutr Metab Cardiovasc Dis 2015;25(2):173-180.

Beattie CJ, Fulton RL, Higgins P, Padmanabhan S, McCallum L, Walters MR, et al. Allopurinol initiation and change in blood pressure in older adults with hypertension. Hypertension 2014;64(5):1102-1107.

Bernard AM, Ouled AA, Lauwerys RR, Lambert A, Vandeleene B. Pronounced decrease of Tamm-Horsfall proteinuria in diabetics. Clin Chem 1987;33(7):1264.

Bigazzi R, Bianchi S, Baldari D, Sgherri G, Baldari G, Campese VM. Microalbuminuria in salt-sensitive patients. A marker for renal and cardiovascular risk factors. Hypertension 1994; 23(2):195-199.

Bleyer AJ, Kmoch S. Tamm Horsfall glycoprotein and uromodulin: It is all about the tubules! Clin J Am Soc Neprol 2016;11(1):6-8.

Bobulescu IA, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. Adv Chronic Kidney Dis 2012;19(6):358-371.

Borghi C, Rosei EA, Bardin T, Dawson J, Dominiczak A, Kielstein JT, et al. Serum uric acid and the risk of cardiovascular and renal disease. J Hypertens 2015;33(9):1729-1741.

Bos MJ, Koudstaal PJ, Hofman A, Witteman JC, Breteler MM. Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. Stroke 2006;37(6):1503-1507.

Bruck K, Stel VS, Gambaro G, Hallan S, Volzke H, Arnlov J, et al. CKD Prevalence Varies across the European General Population. J Am Soc Nephrol 2016;27(7):2135-2147.

Caulfield MJ, Munroe PB, O'Neill D, Witkowska K, Charchar FJ, Doblado M, et al. SLC2A9 is a high-capacity urate transporter in humans. PLoS Med 2008;5(10):e197.

Cicerchi C, Li N, Kratzer J, Garcia G, Roncal-Jimenez CA, Tanabe K, et al. Uric acid-dependent inhibition of AMP kinase induces hepatic glucose production in diabetes and starvation: evolutionary implications of the uricase loss in hominids. FASEB J 2014;28(8):3339-3350.

Cifkova R, Fodor G, Wohlfahrt P. Changes in Hypertension Prevalence, Awareness, Treatment, and Control in High-, Middle-, and Low-Income Countries: An Update. Curr Hypertens Rep 2016;18(8):62.

Cifkova R[a], Skodova Z, Bruthans J, Holub J, Adamkova V, Jozifova M, Galovcova M, Wohlfahrt P, Krajcoviechova A, Petrzilkova Z, Lanska V. Longitudinal trends in cardiovascular mortality and blood pressure levels, prevalence, awareness, treatment, and control of hypertension in the Czech population from 1985 to 2007/2008. J Hypertens 2010;28(11):2196-2203.

Cifkova R[b], Skodova Z, Bruthans J, Adamkova V, Jozifova M, Galovcova M, Wohlfahrt P, Krajcoviechova A, Poledne R, Stavek P, Lanska V. Longitudinal trends in major cardiovascular risk factors in the Czech population between 1985 and 2007/8. Czech MONICA and Czech post-MONICA. Atherosclerosis 2010;211(2):676-681.

Comper WD, Hilliard LM, Nikolic-Paterson DJ, Russo LM. Disease-dependent mechanisms of albuminuria. Am J Physiol Renal Physiol 2008;295(6):F1589-1600.

Culleton BF, Larson MG, Kannel WB, Levy D. Serum uric acid and risk for cardiovascular disease and death: the Framingham Heart Study. Ann Intern Med 1999;131(1):7-13.

Cunningham R, Brazie M, Kanumuru S, E X, Biswas R, Wang F, et al. Sodium-hydrogen exchanger regulatory factor-1 interacts with mouse urate transporter 1 to regulate renal proximal tubule uric acid transport. J Am Soc Nephrol 2007;18(5):1419-1425.

Currie G, Delles C. Proteinuria and its relation to cardiovascular disease. Int J Nephrol Renovasc Dis 2013;7:13-24.

de Zeeuw D, Parving HH, Henning RH. Microalbuminuria as an early marker for cardiovascular disease. J Am Soc Nephrol 2006;17(8):2100-2105.

Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage. The Steno hypothesis. Diabetologia 1989;32(4):219-226.

Dehghan A, Kottgen A, Yang Q, Hwang SJ, Kao WL, Rivadeneira F, et al. Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. Lancet 2008;372(9654):1953-1961.

Delaneau O, Marchini J. Integrating sequence and array data to create an improved 1000 Genomes Project haplotype reference panel. Nat Commun 2014;5:3934.

Delgado GE, Kleber ME, Scharnagl H, Kramer BK, Marz W, Scherberich JE. (2017). Serum Uromodulin and Mortality Risk in Patients Undergoing Coronary Angiography. J Am Soc Nephrol 2017; Feb 27:Epub ahead of print.

Dickson LE, Wagner MC, Sandoval RM, Molitoris BA. The proximal tubule and albuminuria: really! J Am Soc Nephrol 2014;25(3):443-453.

Dyer AR, Greenland P, Elliott P, Daviglus ML, Claeys G, Kesteloot H, et al., J. Estimating laboratory precision of urinary albumin excretion and other urinary measures in the International Study on Macronutrients and Blood Pressure. Am J Epidemiol 2004;160(3):287-294.

Dzau VJ, Antman EM, Black HR, Hayes DL, Manson JE, Plutzky J, et al.n, W. The cardiovascular disease continuum validated: clinical evidence of improved patient outcomes: part I: Pathophysiology and clinical trial evidence (risk factors through stable coronary artery disease). Circulation 2006;114(25):2850-2870.

Ehret GB, Caulfield MJ. Genes for blood pressure: an opportunity to understand hypertension. Eur Heart J 2013;34(13):951-961.

El-Bikai R, Tahir MR, Tremblay J, Joffres M, Seda O, Sedova L, Awadalla P, Laberge C, Knoppers BM, Dumas P, Gaudet D, Ste-Marie LG, Hamet P. Association of age-dependent height and bone mineral density decline with increased arterial stiffness and rate of fractures in hypertensive individuals. J Hypertens 2015;33(4):727-735.

Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. JAMA 1991;266(21):3008-3011.

Fanelli C, Zatz R. Linking oxidative stress, the renin-angiotensin system, and hypertension. Hypertension 2011;57(3):373-374.

Feig DI, Soletsky B, Johnson RJ. Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. JAMA 2008;300(8):924-932.

Festa A, D'Agostino R Jr., Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000, 102(1):42-47.

Festa A, D'Agostino R Jr., Mykkanen L, Tracy RP, Zaccaro DJ, Hales CN, Haffner SM. Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose tolerance. The Insulin Resistance Atherosclerosis Study (IRAS). Arterioscler Thromb Vasc Biol 1999;19(3):562-568.

Ficociello LH, Rosolowsky ET, Niewczas MA, Maselli NJ, Weinberg JM, Aschengrau A, et el. High-normal serum uric acid increases risk of early progressive renal function loss in type 1 diabetes: results of a 6-year follow-up. Diabetes Care 2010;33(6):1337-1343.

Forman JP, Scheven L, de Jong PE, Bakker SJ, Curhan GC, Gansevoort RT. (2012). Association between sodium intake and change in uric acid, urine albumin excretion, and the risk of developing hypertension. Circulation 2012;125(25):3108-3116.

Fox CS, Larson MG, Hwang SJ, Leip EP, Rifai N, Levy D, et al. Cross-sectional relations of serum aldosterone and urine sodium excretion to urinary albumin excretion in a community-based sample. Kidney Int 2006; 69(11):2064-2069.

Garimella PS, Jaber BL, Tighiouart H, Liangos O, Bennett MR, Devarajan P, et al. Association of Preoperative Urinary Uromodulin with AKI after Cardiac Surgery. Clin J Am Soc Nephrol 2017;12(1):10-18.

Graessler J, Graessler A, Unger S, Kopprasch S, Tausche AK, Kuhlisch E, Schroeder HE. Association of the human urate transporter 1 with reduced renal uric acid excretion and hyperuricemia in a German Caucasian population. Arthritis Rheum 2006;54(1):292-300.

Graham LA, Padmanabhan S, Fraser NJ, Kumar S, Bates JM, Raffi HS, et al. W. Validation of uromodulin as a candidate gene for human essential hypertension. Hypertension 2014;63(3):551-558.

Graziano F, Grassi M, Sacco S, Concas MP, Vaccargiu S, Pirastu M, Biino G. Probing the factor structure of metabolic syndrome in Sardinian genetic isolates. Nutr Metab Cardiovasc Dis 2015;25(6):548-555.

Gurka MJ, Lilly CL, Oliver MN, DeBoer MD. An examination of sex and racial/ethnic differences in the metabolic syndrome among adults: a confirmatory factor analysis and a resulting continuous severity score. Metabolism 2014;63(2):218-225.

Guyton AC. Renal function curves and control of body fluids and arterial pressure. Acta Physiol Scand Suppl 1990;591:107-113.

Hamet P, Haloui M, Harvey F, Marois-Blanchet FC, Sylvestre MP, Tahir MR, et al. PROX1 gene CC genotype as a major determinant of early onset of type 2 diabetes in slavic study participants from Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation study. J Hypertens 2017;35 Suppl 1:S24-S32.

Higgins P, Walters MR, Murray HM, McArthur K, McConnachie A, Lees KR, Dawson J. Allopurinol reduces brachial and central blood pressure, and carotid intima-media thickness progression after ischaemic stroke and transient ischaemic attack: a randomised controlled trial. Heart 2014;100(14):1085-1092.

Hillege HL, Janssen WM, Bak AA, Diercks GF, Grobbee DE, Crijns HJ, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. J Intern Med 2001, 249(6):519-526.

Hillier TA, Rousseau A, Lange C, Lepinay P, Cailleau M, Novak M, et al.u, B. Practical way to assess metabolic syndrome using a continuous score obtained from principal components analysis. Diabetologia 2006;49(7):1528-1535.

Hoieggen A, Alderman MH, Kjeldsen SE, Julius S, Devereux RB, De Faire U, et al. The impact of serum uric acid on cardiovascular outcomes in the LIFE study. Kidney Int 2004; 65(3):1041-1049.

Horner D, Fliser D, Klimm HP, Ritz E. Albuminuria in normotensive and hypertensive individuals attending offices of general practitioners. J Hypertens 1996;14(5):655-660.

Hottenga JJ, Boomsma DI, Kupper N, Posthuma D, Snieder H, Willemsen G, Geus EJ. Heritability and stability of resting blood pressure. Twin Res Hum Genet 2005;8(5):499-508.

Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 2009;5(6):e1000529.

Huffman JE, Albrecht E, Teumer A, Mangino M, Kapur K, Johnson T, et al. V. Modulation of genetic associations with serum urate levels by body-mass-index in humans. PLoS One 2015;10(3):e0119752.

Chang A, Van Horn L, Jacobs DR Jr., Liu K, Muntner P, Newsome B, et al. Lifestyle-related factors, obesity, and incident microalbuminuria: the CARDIA (Coronary Artery Risk Development in Young Adults) study. Am J Kidney Dis 2013;62(2):267-275.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 2015;4:7.

Charles BA, Shriner D, Doumatey A, Chen G, Zhou J, Huang H, et al. N. A genome-wide association study of serum uric acid in African Americans. BMC Med Genomics 2011;4:17.

Cheung KJ, Tzameli I, Pissios P, Rovira I, Gavrilova O, Ohtsubo T, et al.J. M. Xanthine oxidoreductase is a regulator of adipogenesis and PPARgamma activity. Cell Metab 2007;5(2):115-128.

Jalal DI, Rivard CJ, Johnson RJ, Maahs DM, McFann K, Rewers M, Snell-Bergeon JK. Serum uric acid levels predict the development of albuminuria over 6 years in patients with type 1 diabetes: findings from the Coronary Artery Calcification in Type 1 Diabetes study. Nephrol Dial Transplant 2010;25(6):1865-1869.

Johnson RJ, Herrera-Acosta J, Schreiner GF, Rodriguez-Iturbe B. Subtle acquired renal injury as a mechanism of salt-sensitive hypertension. N Engl J Med 2002;346(12):913-923.

Johnson RJ, Rodriguez-Iturbe B, Kang DH, Feig DI, Herrera-Acosta J. A unifying pathway for essential hypertension. Am J Hypertens 2005;18(3):431-440.

Judd E, Calhoun DA. Management of hypertension in CKD: beyond the guidelines. Adv Chronic Kidney Dis 2015;22(2):116-122.

Kahn R, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2005;28(9):2289-2304.

Kanbay M, Huddam B, Azak A, Solak Y, Kadioglu GK, Kirbas I, et al. A randomized study of allopurinol on endothelial function and estimated glomular filtration rate in asymptomatic hyperuricemic subjects with normal renal function. Clin J Am Soc Nephrol 2011;6(8):1887-1894.

Kanbay M, Jensen T, Solak Y, Le M, Roncal-Jimenez C, Rivard C, et al. Uric acid in metabolic syndrome: From an innocent bystander to a central player. Eur J Intern Med 2016;29:3-8.

Kanbay M, Segal M, Afsar B, Kang DH, Rodriguez-Iturbe B, Johnson RJ. The role of uric acid in the pathogenesis of human cardiovascular disease. Heart 2013;99(11):759-766.

Kang DH, Han L, Ouyang X, Kahn AM, Kanellis J, Li P, et al. Uric acid causes vascular smooth muscle cell proliferation by entering cells via a functional urate transporter. Am J Nephrol 2005;25(5):425-433.

Karns R, Zhang G, Sun G, Rao Indugula S, Cheng H, Havas-Augustin D, et al. Genome-wide association of serum uric acid concentration: replication of sequence variants in an island population of the Adriatic coast of Croatia. Ann Hum Genet 2012;76(2):121-127.

Kazancioglu R. Risk factors for chronic kidney disease: an update. Kidney Int Suppl 2013;3(4):368-371.

Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013;3:1-150.

Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, et al., C. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet 2009;5(6):e1000504.

Kottgen A, Albrecht E, Teumer A, Vitart V, Krumsiek J, Hundertmark C, et al.Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nat Genet 2013;45(2):145-154.

Kottgen A, Hwang SJ, Larson MG, Van Eyk JE, Fu Q, Benjamin EJ, et al., C. S. Uromodulin levels associate with a common UMOD variant and risk for incident CKD. J Am Soc Nephrol 2010;21(2):337-344.

Krajcoviechova A, Tremblay J, Wohlfahrt P, Bruthans J, Tahir MR, Hamet P, Cifkova R. The Impact of Blood Pressure and Visceral Adiposity on the Association of Serum Uric Acid With Albuminuria in Adults Without Full Metabolic Syndrome. Am J Hypertens 2016;29(12):1335-1342.

Krajcoviechova A, Tremblay J, Wohlfahrt P, Bruthans J, Tahir MR, Hamet P, Cifkova R. Response to Letter to the Editor entitled Oxidative Stress Participates in the Associations Between Serum Uric Acid and Albuminuria in Obesity. Am J Hypertens 2017;30(3):e2-e3.

Lanaspa MA, Sanchez-Lozada LG, Choi YJ, Cicerchi C, Kanbay M, Roncal-Jimenez CA, et al.J. (2012). Uric acid induces hepatic steatosis by generation of mitochondrial oxidative stress: potential role in fructose-dependent and -independent fatty liver. J Biol Chem 2012;287(48):40732-40744.

Lash JP, Go AS, Appel LJ, He J, Ojo A, Rahman M, et al.H. I. Chronic Renal Insufficiency Cohort (CRIC) Study: baseline characteristics and associations with kidney function. Clin J Am Soc Nephrol 2009;4(8):1302-1311.

Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008;27(8):1133-1163.

Le MT, Shafiu M, Mu W, Johnson RJ. SLC2A9-a fructose transporter identified as a novel uric acid transporter. Nephrol Dial Transplant 2008;23(9):2746-2749.

Lee JE, Kim YG, Choi YH, Huh W, Kim DJ, Oh HY. Serum uric acid is associated with microalbuminuria in prehypertension. Hypertension 2006;47(5):962-967.

Li S, Sanna S, Maschio A, Busonero F, Usala G, Mulas A, et al. R. The GLUT9 gene is associated with serum uric acid levels in Sardinia and Chianti cohorts. PLoS Genet 2007;3(11):e194.

Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell 2001;104(4):545-556.

Lipkowitz MS. Regulation of uric acid excretion by the kidney. Curr Rheumatol Rep 2012; 14(2):179-188.

Lu W, Song K, Wang Y, Zhang Q, Li W, Jiao H, et al. Relationship between serum uric acid and metabolic syndrome: an analysis by structural equation modeling. J Clin Lipidol 2012; 6(2):159-167.

Lv Q, Meng XF, He FF, Chen S, Su H, Xiong J. High serum uric acid and increased risk of type 2 diabetes: a systemic review and meta-analysis of prospective cohort studies. PLoS One 2013;8(2):e56864.

Madero M, Rodriguez Castellanos FE, Jalal D, Villalobos-Martin M, Salazar J, Vazquez-Rangel A, et al., L. G. A pilot study on the impact of a low fructose diet and allopurinol on clinic blood pressure among overweight and prehypertensive subjects: a randomized placebo controlled trial. J Am Soc Hypertens 2015;9(11):837-844.

Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. Int J Cardiol 2016;213:8-14.

Mallamaci F, Testa A, Leonardis D, Tripepi R, Pisano A, Spoto B, et al. A polymorphism in the major gene regulating serum uric acid associates with clinic SBP and the white-coat effect in a family-based study. J Hypertens 2014;32(8):1621-1628.

Mallamaci F, Testa A, Leonardis D, Tripepi R, Pisano A, Spoto B, et al. A genetic marker of uric acid level, carotid atherosclerosis, and arterial stiffness: a family-based study. Am J Kidney Dis 2015;65(2):294-302.

Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, et al. F. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2013;31(7):1281-1357.

Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, et al. Oxidative stress in obesity: a critical component in human diseases. J Mol Sci 2014;16(1):378-400.

Matsushita K, Coresh J, Sang Y, Chalmers J, Fox C, Guallar E, et al. Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: a collaborative meta-analysis of individual participant data. Lancet Diabetes Endocrinol 2015;3(7):514-525.

Matsushita K, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, et al. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. JAMA 2012;307(18):1941-1951.

Mazzali M, Hughes J, Kim YG, Jefferson JA, Kang DH, Gordon KL, et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension 2001;38(5):1101-1106.

Mazzali M, Kanellis J, Han L, Feng L, Xia YY, Chen Q, et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. Am J Physiol Renal Physiol 2002;282(6):F991-997.

McArdle PF, Parsa A, Chang YP, Weir MR, O'Connell JR, Mitchell BD, Shuldiner AR. Association of a common nonsynonymous variant in GLUT9 with serum uric acid levels in old order amish. Arthritis Rheum 2008;58(9):2874-2881.

McKeigue PM, Campbell H, Wild S, Vitart V, Hayward C, Rudan I, et al. Bayesian methods for instrumental variable analysis with genetic instruments ('Mendelian randomization'): example with urate transporter SLC2A9 as an instrumental variable for effect of urate levels on metabolic syndrome. Int J Epidemiol 2010;39(3):907-918.

Mennuni S, Rubattu S, Pierelli G, Tocci G, Fofi C, Volpe M. Hypertension and kidneys: unraveling complex molecular mechanisms underlying hypertensive renal damage. J Hum Hypertens 2014;28(2):74-79.

Miall WE, Oldham PD. The hereditary factor in arterial blood-pressure. Br Med J 1963; 1(5323):75-80.

Mills KT, Bundy JD, Kelly TN, Reed JE, Kearney PM, Reynolds K, et al. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. Circulation 2016;134(6):441-450.

Mills KT, Xu Y, Zhang W, Bundy JD, Chen CS, Kelly TN, et al. A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010. Kidney Int 2015;88(5):950-957.

Munzel T, Gori T, Bruno RM, Taddei S. Is oxidative stress a therapeutic target in cardiovascular disease? Eur Heart J 2010;31(22):2741-2748.

Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380(9859):2197-2223.

Mutig K, Kahl T, Saritas T, Godes M, Persson P, Bates J, et al. Activation of the bumetanide-sensitive Na+,K+,2Cl- cotransporter (NKCC2) is facilitated by Tamm-Horsfall protein in a chloride-sensitive manner. J Biol Chem 2011;286(34):30200-30210.

Nakagawa T, Hu H, Zharikov S, Tuttle KR, Short RA, Glushakova O, et al. A causal role for uric acid in fructose-induced metabolic syndrome. Am J Physiol Renal Physiol 2006;290(3):F625-631.

Nishimura S, Manabe I, Nagai R. Adipose tissue inflammation in obesity and metabolic syndrome. Discov Med 2009;8(41):55-60.

Norden AG, Unwin RJ. Is the albumin retrieval hypothesis a paradigm shift for nephrology? J Am Soc Nephrol 2012;23(4):569-571.

Padmanabhan S, Graham L, Ferreri NR, Graham D, McBride M, Dominiczak AF. Uromodulin, an emerging novel pathway for blood pressure regulation and hypertension. Hypertension 2014;64(5):918-923.

Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, et al. Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet 2010;6(10):e1001177.

Padmanabhan S, Newton-Cheh C, Dominiczak AF. Genetic basis of blood pressure and hypertension. Trends Genet 2012;28(8):397-408.

Palmer TM, Nordestgaard BG, Benn M, Tybjaerg-Hansen A, Davey Smith G, Lawlor DA, Timpson NJ. Association of plasma uric acid with ischaemic heart disease and blood pressure: mendelian randomisation analysis of two large cohorts. BMJ 2013;347:f4262.

Parsa A, Brown E, Weir MR, Fink JC, Shuldiner AR, Mitchell BD, McArdle PF. Genotype-based changes in serum uric acid affect blood pressure. Kidney Int 2012;81(5):502-507.

Parving HH, Mogensen CE, Jensen HA, Evrin PE. Increased urinary albumin-excretion rate in benign essential hypertension. Lancet 1974;1(7868):1190-1192.

Pattaro C, Teumer A, Gorski M, Chu AY, Li M, Mijatovic V, et al. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. Nat Commun 2016;7:10023.

Peng TC, Wang CC, Kao TW, Chan JY, Yang YH, Chang YW, Chen WL. Relationship between hyperuricemia and lipid profiles in US adults. Biomed Res Int 2015;2015:127596.

Pfister R, Barnes D, Luben R, Forouhi NG, Bochud M, Khaw KT, et al. No evidence for a causal link between uric acid and type 2 diabetes: a Mendelian randomisation approach. Diabetologia 2011;54(10):2561-2569.

Ponnuchamy B, Khalil RA. Cellular mediators of renal vascular dysfunction in hypertension. Am J Physiol Regul Integr Comp Physiol 2009;296(4):R1001-1018.

Poulter NR, Prabhakaran D, Caulfield M. Hypertension. Lancet 2015;386(9995):801-812.

Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods 2008;40(3):879-891.

Preacher KJ, Kelley K. Effect size measures for mediation models: quantitative strategies for communicating indirect effects. Psychol Methods 2011;16(2):93-115.

Preitner F, Bonny O, Laverriere A, Rotman S, Firsov D, Da Costa A, et al. Glut9 is a major regulator of urate homeostasis and its genetic inactivation induces hyperuricosuria and urate nephropathy. Proc Natl Acad Sci U S A 2009;106(36):15501-15506.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38(8):904-909.

Pruijm M, Ponte B, Ackermann D, Paccaud F, Guessous I, Ehret G, et al.Associations of Urinary Uromodulin with Clinical Characteristics and Markers of Tubular Function in the General Population. Clin J Am Soc Nephrol 2016;11(1):70-80.

Quinones Galvan A, Natali A, Baldi S, Frascerra S, Sanna G, Ciociaro D, Ferrannini E. Effect of insulin on uric acid excretion in humans. Am J Physiol 1995;268(1 Pt 1):E1-5.

Raj A, Stephens M, Pritchard JK. fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics 2014;197(2):573-589.

Rao DC, Laskarzewski PM, Morrison JA, Khoury P, Kelly K, Glueck CJ. The Clinical Lipid Research Clinic Family Study: familial determinants of plasma uric acid. Hum Genet 1982;60(3):257-261.

Resl M, Clodi M, Neuhold S, Kromoser H, Riedl M, Vila G, et al. Serum uric acid is related to cardiovascular events and correlates with N-terminal pro-B-type natriuretic peptide and albuminuria in patients with diabetes mellitus. Diabet Med 2012;29(6):721-725.

Rodilla E, Perez-Lahiguera F, Costa JA, Gonzalez C, Miralles A, Moral D, Pascual JM. Association between serum uric acid, metabolic syndrome and microalbuminuria in previously untreated essential hypertensive patients. Med Clin 2009;132(1):1-6.

Romundstad S, Holmen J, Kvenild K, Hallan H, Ellekjaer, H. Microalbuminuria and all-cause mortality in 2,089 apparently healthy individuals: a 4.4-year follow-up study. The Nord-Trondelag Health Study (HUNT), Norway. Am J Kidney Dis 2003;42(3):466-473.

Russo LM, Sandoval RM, Campos SB, Molitoris BA, Comper WD, Brown D. Impaired tubular uptake explains albuminuria in early diabetic nephropathy. J Am Soc Nephrol 2009;20(3):489-494.

Sautin YY, Nakagawa T, Zharikov S, Johnson RJ. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. Am J Physiol Cell Physiol 2007;293(2):C584-596.

Scott FW, Trick KD, Stavric B, Braaten JT, Siddiqui, Y. Uric acid-induced decrease in rat insulin secretion. Proc Soc Exp Biol Med 1981;166(1):123-128.

See LC, Kuo CF, Chuang FH, Li HY, Chen YM, Chen HW, Yu KH. (2009). Serum uric acid is independently associated with metabolic syndrome in subjects with and without a low estimated glomerular filtration rate. J Rheumatol 2009;36(8):1691-1698.

Scheven L, Joosten MM, de Jong PE, Bakker SJ, Gansevoort RT. The association of albuminuria with tubular reabsorption of uric acid: results from a general population cohort. J Am Heart Assoc 2014, 3(2):e000613.

Schmidt MI, Watson RL, Duncan BB, Metcalf P, Brancati FL, Sharrett AR, et al. Clustering of dyslipidemia, hyperuricemia, diabetes, and hypertension and its association with fasting insulin and central and overall obesity in a general population. Atherosclerosis Risk in Communities Study Investigators. Metabolism 1996;45(6):699-706.

Simoes ESAC, Flynn JT. The renin-angiotensin-aldosterone system in 2011: role in hypertension and chronic kidney disease. Pediatr Nephrol 2012;27(10):1835-1845.

Soletsky B, Feig DI. Uric acid reduction rectifies prehypertension in obese adolescents. Hypertension 2012;60(5):1148-1156.

Stark K, Reinhard W, Grassl M, Erdmann J, Schunkert H, Illig T, Hengstenberg C. Common polymorphisms influencing serum uric acid levels contribute to susceptibility to gout, but not to coronary artery disease. PLoS One 2009;4(11):e7729.

Ter Maaten JC, Voorburg A, Heine RJ, Ter Wee PM, Donker AJ, Gans RO. Renal handling of urate and sodium during acute physiological hyperinsulinaemia in healthy subjects. Clin Sci 1997;92(1):51-58.

Testa A, Mallamaci F, Leonardis D, Spoto B, Pisano A, Sanguedolce MC, et al. Synergism between asymmetric dimethylarginine (ADMA) and a genetic marker of uric acid in CKD progression. Nutr Metab Cardiovasc Dis 2015;25(2):167-172.

Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. Stat Med 2005;24(19):2911-2935.

Torffvit O, Melander O, Hulten UL. Urinary excretion rate of Tamm-Horsfall protein is related to salt intake in humans. Nephron Physiol 2004;97(1):31-36.

Troyanov S, Delmas-Frenette C, Bollee G, Youhanna S, Bruat V, Awadalla P, et al. Clinical, Genetic, and Urinary Factors Associated with Uromodulin Excretion. Clin J Am Soc Nephrol 2016;11(1):62-69.

United States Renal Data System. 2016 USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2016.

van der Velde M, Bello AK, Brantsma AH, El Nahas M, Bakker SJ, de Jong PE, Gansevoort RT. Do albuminuria and hs-CRP add to the International Diabetes Federation definition of the metabolic syndrome in predicting outcome? Nephrol Dial Transplant 2012;27(6):2275-2283.

Vaneckova I, Maletinska L, Behuliak M, Nagelova V, Zicha J, Kunes J. Obesity-related hypertension: possible pathophysiological mechanisms. Endocrinol 2014;223(3):R63-78.

Viazzi F, Parodi D, Leoncini G, Parodi A, Falqui V, Ratto E, et al. Serum uric acid and target organ damage in primary hypertension. Hypertension 2005;45(5):991-996.

Vitart V, Rudan I, Hayward C, Gray NK, Floyd J, Palmer CN, et al. SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nat Genet 2008, 40(4):437-442.

Voruganti VS, Franceschini N, Haack K, Laston S, MacCluer JW, Umans JG, et al. Replication of the effect of SLC2A9 genetic variation on serum uric acid levels in American Indians. Eur J Hum Genet 2014;22(7):938-943.

Voruganti VS, Kent JW Jr., Debnath S, Cole SA, Haack K, Goring HH, et al. Genome-wide association analysis confirms and extends the association of SLC2A9 with serum uric acid levels to Mexican Americans. Front Genet 2013;4:279.

Wadei HM, Textor SC. The role of the kidney in regulating arterial blood pressure. Nat Rev Nephrol 2012;8(10):602-609.

Waken RJ, de Las Fuentes L, Rao DC. A Review of the Genetics of Hypertension with a Focus on Gene-Environment Interactions. Curr Hypertens Rep 2017;19(3):23.

Wallace C, Newhouse SJ, Braund P, Zhang F, Tobin M, Falchi M, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. Am J Hum Genet 2008;82(1):139-149.

Wang J, Qin T, Chen J, Li Y, Wang L, Huang H, Li J. Hyperuricemia and risk of incident hypertension: a systematic review and meta-analysis of observational studies. PLoS One 2014;9(12):e114259.

Watanabe S, Kang DH, Feng L, Nakagawa T, Kanellis J, Lan H, et al.son, R. J. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. Hypertension 2002;40(3):355-360.

Weir MR. Microalbuminuria and cardiovascular disease. Clin J Am Soc Nephrol 2007;2(3):581-590.

Wheeler JG, Juzwishin KD, Eiriksdottir G, Gudnason V, Danesh J. Serum uric acid and coronary heart disease in 9,458 incident cases and 155,084 controls: prospective study and meta-analysis. PLoS Med 2005;2(3):e76.

White J, Sofat R, Hemani G, Shah T, Engmann J, Dale C, et al. Plasma urate concentration and risk of coronary heart disease: a Mendelian randomisation analysis. Lancet Diabetes Endocrinol 2016;4(4):327-336.

Whitfield JB, Martin NG. Inheritance and alcohol as factors influencing plasma uric acid levels. Acta Genet Med Gemellol 1983;32(2):117-126.

Wijndaele K, Beunen G, Duvigneaud N, Matton L, Duquet W, Thomis M, et al. A continuous metabolic syndrome risk score: utility for epidemiological analyses. Diabetes Care 2006;29(10): 2329.

Wu J, Province MA, Coon H, Hunt SC, Eckfeldt JH, Arnett DK, et al. An investigation of the effects of lipid-lowering medications: genome-wide linkage analysis of lipids in the HyperGEN study. BMC Genet 2007;8:60.

Yang Q, Kottgen A, Dehghan A, Smith AV, Glazer NL, Chen MH, et al. Multiple genetic loci influence serum urate levels and their relationship with gout and cardiovascular disease risk factors. Circ Cardiovasc Genet 2010;3(6):523-530.

Yarnell JW, Patterson CC, Bainton D, Sweetnam PM. Is metabolic syndrome a discrete entity in the general population? Evidence from the Caerphilly and Speedwell population studies. Heart 1998;79(3):248-252.

Ying WZ, Sanders PW. Dietary salt regulates expression of Tamm-Horsfall glycoprotein in rats. Kidney Int 1998;54(4):1150-1156.

Yu MA, Sanchez-Lozada LG, Johnson RJ, Kang DH. Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction. J Hypertens 2010;28(6):1234-1242.

Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19(4):972-978.

Zdrojewski L, Zdrojewski T, Rutkowski M, Bandosz P, Krol E, Wyrzykowski B, Rutkowski B. Prevalence of chronic kidney disease in a representative sample of the Polish population: results of the NATPOL 2011 survey. Nephrol Dial Transplant 2016;31(3):433-439.

Zhang JX, Zhang YP, Wu QN, Chen B. Uric acid induces oxidative stress via an activation of the renin-angiotensin system in 3T3-L1 adipocytes. Endocrine 2015;48(1):135-142.

Zhi L, Yuzhang Z, Tianliang H, Hisatome I, Yamamoto T, Jidong C. High Uric Acid Induces Insulin Resistance in Cardiomyocytes In Vitro and In Vivo. PLoS One 2016;11(2):e0147737.

8 Appendix – own publications related to the topic

1 Krajcoviechova A, Tremblay J, Wohlfahrt P, Bruthans J, Tahir MR, Hamet P, Cifkova R. The Impact of Blood Pressure and Visceral Adiposity on the Association of Serum Uric Acid With Albuminuria in Adults Without Full Metabolic Syndrome. Am J Hypertens 2016;29(12):1335-1342.

2 Krajcoviechova A, Tremblay J, Wohlfahrt P, Bruthans J, Tahir MR, Hamet P, Cifkova R. Response to Letter to the Editor entitled Oxidative Stress Participates in the Associations Between Serum Uric Acid and Albuminuria in Obesity. Am J Hypertens 2017;30(3):e2-e3.

3 Cifkova R, Skodova Z, Bruthans J, Holub J, Adamkova V, Jozifova M, Galovcova M, Wohlfahrt P, Krajcoviechova A, Petrzilkova Z, Lanska V. Longitudinal trends in cardiovascular mortality and blood pressure levels, prevalence, awareness, treatment and control of hypertension in the Czech population from 1985 to 2007/08. J Hypertens 2010;28(11):2196-203.

4 Cifkova R, Skodova Z, Bruthans J, Adamkova V, Jozifova M, Galovcova M, Wohlfahrt P, Krajcoviechova A, Poledne R, Stavek P, Lanska V. Longitudinal trends in major cardiovascular risk factors in the Czech population between 1985 and 2007/8. Czech MONICA and Czech post-MONICA. Atherosclerosis 2010;211(2):676-681.