

## **Doctoral thesis**

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### **The Application of Massively Parallel Next Generation Sequencing Technologies for Diagnosis of Hereditary Spastic Paraplegia:**

A Contribution to the Development of DNA Diagnosis of a Highly Heterogeneous Disease.

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## **Abstract**

The aim of this work is to map the genetical spectrum of hereditary spastic paraplegia in Czech patients. Hereditary spastic paraplegia (HSP) is an inherited neurological disorder, clinically characterized by progressive weakness and spasticity of the lower limbs leading to problems with gait and can cause the complete loss of the ability to walk. HSP is clinically and genetically very heterogenous; more than 90 HSP types and pathogenic variants in more than 70 genes have been described to date. HSP manifests as an uncomplicated (pure) form in the majority of HSP patients. Less often it manifests as a complicated phenotype with the associated clinical signs (mental impairment, epilepsy, ataxia, atrophy of the optic nerve).

We performed massively parallel sequencing (MPS) of uncomplicated HSP gene panel in a group of 96 Czech patients with suspected uncomplicated type of HSP. In all patients the most common HSP type SPG4 was previously excluded. HaloPlex Target Enrichment kit and SureSelect Target Enrichment kit (both Agilent Technologies, US) with custom probes designed was used for MPS. All coding and neighbouring regions of included genes was sequenced by MPS. We performed whole exome sequencing (WES) in four patients using SureSelect All Exon Kit v6 (Agilent Technologies, US). The presence of all found variants was confirmed by Sanger sequencing. Their pathogenicity was assessed in silico and by segregation analysis where possible.

The causal pathogenic variant was found in 22 patients from 96 examined using MPS sequencing of the gene panel. Our results represent 23 % of examined patients evaluated by this method (22 from 96). The percentage of evaluated patients was 29,4 % in the group of familial patients (15 from 55) and it was higher than in the group of sporadic patients with

15,5 % evaluated patients (7 from 45). Two patients were evaluated by WES from four patients examined. By using next generation sequencing methods (MPS of gene panel and WES) we found all together 10 types of HSP among the non-SPG4 Czech patients (SPG4, SPG5, SPG6, SPG7, SPG10, SPG11, SPG31, SPG35, SPG77 a IAHSPP, no patient with SPG3 was diagnosed); the most frequent is type SPG31 with pathogenic variants in the *REEP1* gene. SPG31 is the most frequent type among the autosomal dominant HSPs. The most frequent autosomal recessive type of HSP are SPG11 (slightly more frequent) and SPG7. We observe a low frequency of SPG3 (variants in *ATL1* gene) among the Czech HSP patients although this type was repeatedly described as the second most frequent type of HSP. We found rare types of HSP, such as SPG35, IAHSPP and especially SPG77. The Czech patient with SPG77 is the fourth patient worldwide. Twelve new variants in six genes together have been found. The number of new variants is highest in the *REEP1* gene.

In the more frequent types of HSP diagnosed in this work we discuss the phenotypic features characteristic for types of HSP. These data can be useful for further clinical diagnostics.

We summarized the results from this work and the group of previously evaluated patients with SPG4 and SPG3, which allows us to describe the genetical characteristics of the group of Czech HSP patients. Altogether 11 types of HSP have been found among Czech patients with hereditary spastic paraplegia to date (with SPG3 diagnosed previously). The most frequent type is SPG4 (70 % of all evaluated HSP patients). The other more frequent types are SPG31, SPG11, SPG7 and SPG10 in 8.3 %, 6.0 %, 4.8 % and 3.6 % from all evaluated patients. Sanger sequencing is effective only in *SPAST* gene, sequencing of *REEP1* gene can be more effective than sequencing of *ATL1* gene.

All these results represent the very first data concerning the genetic characteristics of hereditary spastic paraplegia in the Czech Republic and are thus unique with regard to epidemiological data about diagnosis HSP.