Examination of the genes *DNM2*, *GARS, MORC2, TRPV4* and *SOD1* among Czech patients with hereditary neuropathy axonal type

For my PhD thesis I chose to work with patients with axonal form of CMT, because at that time axonal forms were less likely to be clarified by classical methods of molecular genetics. For further examination in patients with unclear cause of the axonal CMT, the genes *DNM2*, *GARS* and *TRPV4* were selected. The aim was to determine the significance of pathogenic mutations in these genes as the cause of CMT2 in Czech patients.

In the course, we identified causal variants in the genes *MORC2* and *SOD1* with WES. Therefore, we have tested additional CMT2 patients for the presence of these variants.

Using Sanger sequencing, I examined a representative set of patients for the *DNM2* (37), *GARS* (10) and *TRPV4* (24) genes without finding a causal mutation, then we investigated genes *SOD1* (43 patients) and *MORC2* (161 patients). The cohort (50 patients) was also subjected to MLPA analysis using a P406-A1 CMT2 duplication and deletion detection kit for genes *RAB7A*, *GARS, HSPB1*, *HSBP8* and *SPTLC1* (kit P406-A1 CMT2). At that time, massively parallel sequencing (MPS) was becoming important. We compared the cost of classical sequencing versus MPS, and accordingly, we decided that the genes *DNM2*, *GARS*, *MORC2*, *TRPV4* and *SOD1* would now be included in the panel of 97 genes associated with hereditary neuropathies. We examined 217 patients with MPS. This led to several interesting findings. In the CMT2 cohort the most important were:

1. Finding the NM_002047.2(GARS):c.2074A>G (p.Met692Val) mutation in the *GARS* gene, which is probably non-pathogenic, respectively is not the cause of CMT2 in the family, not segregate with the disease in the family.
2. In a patient with early onset neuropathy in childhood, scoliosis and dyspnoea, MPS helped to identify the cause of the disease as a result of the causal mutation NM_021625.4(TRPV4):**c.557G>A (p.Arg186Gln)** in exon 3 of the ***TRPV4* gene**.

Mutation has already been described as pathogenic (Landoure et al. 2010). We have published our case report in Neurologie pro praxi (Mazanec et al. 2016).

3. In the ***DNM2*** **gene**, MPS sequencing revealed variant NM_001005360.2(DNM2):**c.1102G>A (p.Glu368Lys)** in heterozygous state and NM_001005360.2(DNM2):**c.1393C>T (p.Arg465Trp)** which is associated with centronuclear myopathy (Bitoun et al. 2005) and the phenotype of our patients is explanable by the mutation.

4. MPS has allowed us to detect other variants to extend the spectrum of mutations associated with axonal CMT and HMN. For example variant in the ***MORC2*** **gene** NM_014941.2(MORC2):**c.568C>T (p.Arg190Trp)** in exon 10 was found in a heterozygous state in a patient with difficulty with walking, muscle weakness and disease progression, the variant being described as a mutation hotspot. Restriction analysis, designed to digest the mutant allele, in a group of 161 patients has found the variant in one other patient.

5. In a large Czech family with a clinical phenotype of slowly progressive familial ALS (FALS) and occurring in a total of 5 generations, WES analysis found that members of the family carry in exon 4 of the ***SOD1*** **gene** a mutation of NM_000454.4(SOD1):**c.140A>G (p.His47Arg)** in heterozygous state. It has already been described as pathogenic in Ostern et al. 2012.