

## Set up of a clinical-molecular database for the definition of a diagnostic flow chart in hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a group of clinically and genetically heterogeneous neurodegenerative disorders, characterized by progressive lower extremity weakness and spasticity. HSP can be either pure or complicated by additional neurologic findings such as seizures, dementia, peripheral neuropathy. HSP displays both phenotypic and locus heterogeneity: autosomal and X-linked forms have been recognized and more than 33 loci and 17 genes identified to date. This indeterminacy produces in clinical practice an expensive and time-consuming molecular screening for patients, mostly following, as unique criteria, the incidence of the various forms of HSP.

In order to facilitate patient classification and address molecular diagnosis our group has started the development of a dedicated electronic database, gathering relevant clinical, instrumental, familial and molecular data of HSP patients. This will help to define a diagnostic flow-chart and establish a HSP network to provide efficient molecular diagnosis to most HSP patients. Moreover, the availability of well-characterized cohorts will allow stratification of patients, who might constitute potential targets for future treatments.

To date, we screened a large cohort of patients for point mutations in *SPAST* and *SPG7*, respectively encoding spastin, the most frequently altered gene in autosomal dominant HSP, and paraplegin, associated with autosomal recessive HSP. We identified mutations in the *SPAST* gene in 20 / 69 patients: 5 missense, 4 nonsense, 5 splicing, 3 small and two gross deletions and a small indel. Eight mutations were unreported to date: three missense in exon 9 (A408G) and exon 11 (E454G and G471V), one nonsense in exon 2 (Q167X), one altering intron 10 donor splicing site, two small deletions in exons 2 and 3 and a small indel in exon 6, leading to frameshift and formation of a premature stop codon. 50% of the mutations are located in the ATPase associated with various cellular activities (AAA) domain at C-terminus, as previously reported, but we also had an interesting percentage of mutations in the microtubule interacting and endosomal trafficking (MIT) domain at N-terminus. All the mutations were heterozygous, but one patient displayed two variants on the same allele associated with atypical neuromuscular involvement.

The analysis of the *SPG7* gene in 66 patients led to the identification of six novel mutations: three missense variants (G349S, G386S, I743T), one small and one gross deletion and a small insertion. Two patients were homozygous for the variation, in one case there was a compound heterozygosity and in three patients we were able to identify only a heterozygous variant. These data may indicate the presence of variations in other regions of the *SPG7* gene or in other genes possibly interacting with paraplegin. Interestingly, the same homozygous deletion in two unrelated patients may indicate a possible recombination hotspot.

These data underline that gene rearrangements in both genes are relatively frequent. Our data also point out that monoallelic variations in recessive disorders may not be an uncommon finding, prompting us to improve the sensitivity of molecular tests as well as to search for pathogenetic variants in other candidate genes. Negative patients will proceed molecular characterization following our flow chart in collaboration with other centers.