

## The mitochondrial m-AAA protease complex in the pathogenesis of hereditary spinocerebellar degenerations

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Autosomal dominant spinocerebellar ataxias (SCA) are a heterogeneous group of neurological disorders characterized by cerebellar dysfunction mostly due to Purkinje cell degeneration. We previously mapped SCA28 to chromosome 18p11 in a 4-generation Italian family. We have now discovered that *AFG3L2* (ATPase family gene 3-like 2) mutations cause SCA28. Along with paraplegin, which causes recessive spastic paraparesis SPG7, *AFG3L2* is a component of the mitochondrial *m*-AAA complex, an evolutionarily conserved metalloprotease involved in protein quality control. We have identified 9 heterozygous *AFG3L2* missense mutations in the original kindred and in other 4 unrelated SCA families. Interestingly, one index patient in this latter group was found to carry also a heterozygous SPG7 recessive mutation in the paraplegin gene. Segregation analysis of the *AFG3L2* and *SPG7* mutations in this family demonstrated a modulatory role of the *SPG7* mutation, with a full-blown phenotype in the *AFG3L2/SPG7* compound heterozygote and cerebellar atrophy in the *AFG3L2* heterozygotes. All the *AFG3L2* mutations are located in functional domains of the protein at highly conserved amino acids. Structural modeling in the eubacterial FtsH protease indicates that they may affect substrate interaction. *AFG3L2* protein and transcript were found to be highly and selectively expressed in cerebellar Purkinje cells. Expression of normal and mutant *AFG3L2* homocomplex in *m*-AAA-deficient yeast cells demonstrate that the mutations cause respiratory deficiency and defective processing of *m*-AAA substrates. Using antibodies raised in our laboratory, we have also investigated paraplegin expression in lymphocytes from a group of HSP patients (both sporadic and compatible with AR inheritance). *SPG7* gene was sequenced in the patients exhibiting absence or severe reduction of paraplegin protein. Overall, pathogenic mutations on both alleles were found in sporadic patients and in familial cases with an overall frequency of ~11%. The mutations were not found in a large control population. The majority of patients presented an adult-onset slowly progressive “complex” phenotype characterized by spastic gait and clinical and MRI signs of cerebellar involvement. Western blot analysis demonstrated the absence or a severe reduction of paraplegin not only in patients carrying two null alleles, but also in patients compound heterozygous for missense mutations and in a patient homozygous for the Ala510Val, previously described as a polymorphism, but very frequent in our series. Functional analysis in yeast of human *m*-AAA with mutant paraplegin allowed to discriminate between pathogenic mutations and rare benign variants and clearly showed that the Ala510Val substitution is a disease-causing mutation which produces a respiratory-deficient phenotype.

In conclusion, our work identifies *AFG3L2* as a novel cause of dominant neurodegenerative disease, indicating an essential role of the mitochondrial *AFG3L2* homocomplex in protecting the cerebellum against neurodegeneration and that deficiency of mitochondrial protein quality control is a frequent cause of hereditary motor neuron dysfunction.