Hereditary spastic paraplegia and prominent sensorial involvement: think MAG mutations!

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Abstract
Homozygous mutations in MAG, encoding the myelin-associated glycoprotein, a transmembrane component of the myelin sheath, have been associated with SPG75 recessive spastic paraplegia. Here, we report the first patient with two compound heterozygous novel MAG mutations (p.A151V and p.S373R) and early developmental delay with a progressive complex phenotype characterized by spastic paraplegia, peripheral sensorimotor neuropathy, intellectual disability, and sensorial dysfunctions with severe optic atrophy and hearing involvement. Brain imaging showed progressive global cerebellar atrophy. We propose that complex hereditary spastic paraplegia, with axonal and demyelinating polyneuropathy, sensorial impairment and intellectual disability might suggest MAG mutations.

Introduction
Hereditary spastic paraplegias (HSP) are a heterogeneous group of neurodegenerative diseases clinically characterized by progressive lower extremity weakness and spasticity, which may be isolated (pure HSP) or combined to other neurological or non-neurological signs (complex HSP). More than 70 genes have been implicated, involved in interconnected biological processes including axonal transport, reticulum endoplasmic function, mitochondrial metabolism, vesicle formation, membrane trafficking and myelin formation and maintenance. The Myelin-Associated Glycoprotein MAG is a membrane-bound adhesion protein implicated in myelin function and glial-axon interactions, which interacts with SPG2-related membrane proteolipid. Recently, homozygous mutations in MAG have been reported in patients with complex HSP; here, we describe a patient with heterozygous composite MAG mutations and a complex HSP phenotype associated to severe sensorial symptoms.

Material and Methods
Standard protocol approvals, registrations, and patient consents
The study was carried out in accordance with the Declaration of Helsinki and was approved by the local ethical committee. Informed consent was obtained from the patient’s legal representative.

Genetic analyses
Analysis of single-nucleotide polymorphism with a mean resolution of 18 kilo-bases was performed on the
proband DNA. Whole exome sequencing (WES) was performed on the same DNA sample by Aros Company-LTD. Variants with a frequency higher than 1% were discarded. Special attention was focused on variants in the SPG, COG, and ALG genes and all other genes reported to be involved in inherited Spastic Paraplegia, Congenital Disorders of Glycosylation and Friedreich Ataxia.

**Brain imaging**

CT scan was performed on a 64-section CT scanner (Discovery750 HD; GE Healthcare, Milwaukee, Wisconsin). Magnetic resonance images were acquired on a 1,5-T system (AVENTO, Siemens medical solutions, Erlangen, Germany) as follows: axial slices T2-weighted, T2*-weighted, FLAIR, SWI sequences and sagittal slices T1-weighted sequences.

**Results**

**Case report**

The patient is a girl of an unrelated couple from North Africa. Her three brothers are healthy (Fig. 1A). Pregnancy and delivery were uneventful. Slow progression of the psychomotor achievements was noticed from the first months of life. The girl was able to sit unaided at 10 months and managed to walk at 21 months, with an unsteady and spastic gait. Clinical examination at the age of 4 showed a broad-based gait, upper limbs dysmetria, and coordination impairment. Lower limbs examination revealed spasticity with exaggerated osteotendinous reflexes. From the age of 7, the parents reported distal lower limbs vegetative disturbances, with frequent cold feet or pale blue feet. Mild atrophy of the calf muscles and diminished ankle reflexes were noticed. Distal weakness of the lower limbs progressed slowly resulting in gait worsening; from the age of 9, the girl needed support to walk and falls occurred occasionally. At the age of 10, clinical examination showed ataxic drop-foot gait, with knee flessum; lower limbs pyramidal tract signs were less obvious. Distal sensory assessment, as vibration and pin-prick sensations, could not be performed due to the patient understanding difficulties. Upper limbs dysmetria was improved, and the patient could eat and drink alone; nevertheless, her gestures were still very slow and uncoordinated. Some degree of facial akinesia was noticed. Height, weight, and cranial perimeter growth were normal. The patient had a severe intellectual disability; she could produce three words sentences and needed help for most of the daily living activities; she was admitted in an institution for disabled children.

Peripheral motor and sensory nerve conduction studies were normal at 4 years of age, and revealed a mild mixed axonal and demyelinating sensorimotor polyneuropathy at the age of 6, without worsening at the age of 10 (Table S1).

Funduscopic examination performed at 4 years disclosed bilateral severe optic atrophy that remained stable (Fig. 2A). Visual acuity was 4.5/10 on both eyes. Color vision, visual field, and pattern visual evoked potentials were not performed as the patient was uncooperative. Flash visual evoked potentials were normal. Brainstem auditory evoked potentials showed reduced amplitudes of waves I, III, and V on both sides, without prolonged latencies.

Brain MRI performed at 4 years of age showed increased visibility of the cerebellar sulci (data not shown). Brain MRI performed at 6 years showed severe global cerebellar atrophy, with mild medullar and severe optic tract atrophy; discrete hyper signal of the periventricular white matter were also noticed. Brain imaging at 10 years showed progression of the cerebellar atrophy, while the other findings remained unchanged (Fig. 2B–F). Spectroscopic MRI of the brain was normal.

Metabolic investigations including amino acid screening, urine organic acid screening, serum lipid profile, vitamin E dosage, acyl carnitine and long-chain fatty acid screening, cerebrospinal fluid analysis (cell count, protein level, glycorrachia/glycemia ratio, neurotransmitter screening), lysosomal enzyme activities and biochemical Congenital Disorder of Glycosylation testing in plasma were normal. Mitochondrial enzymatic activities on a muscle biopsy were normal.

**Genetics**

Analysis of single-nucleotide polymorphisms, performed with a mean resolution of 18 kilo-bases on the DNA from the index case did not disclose loss of heterozygosity, excluding consanguinity between the parents. Exome sequencing was then performed, using routine experimental protocols and pipeline analyses. Variants were screened for unknown damaging dominant mutations and for rare (frequency lower than 1/100) heterozygous bi-allelic mutations. No pertinent dominant mutation was found in genes related to inherited neurological diseases, while two mutations c.452C>T (p.A151V) and c.1117A>C (p.S373R) were found in MAG. They co-segregate in the index case, while both parents were carrying a single heterozygous variant, and none was found in the healthy brother (Fig. 1A and B). No alternative pair of relevant heterozygous mutations was found in another gene.
Both mutations affect one of the five extracellular immunoglobulin-related domains, but different from those altered by the pathogenic homozygous mutations identified so far in MAG (Fig. 1C). The c.452C > T mutation (rs144553163) is reported with a frequency of 3.8 x 10^{-9} and 4.4 x 10^{-9} in Gnomad exomes and genomes respectively, and leads to the p.A151V amino-acid change in the second immunoglobulin domain. The c.1117A > C mutation (rs142375870) is reported with a frequency of 2.4 x 10^{-9} and 9.7 x 10^{-9} in Gnomad exomes and genomes respectively, and leads to the p.S373R amino-acid change in the fourth immunoglobulin domain. Both mutations are predicted to be damaging by the Sift, Polyphen, Mutation-Taster and LRT prediction tools and were reported as variant with uncertain clinical significance in a heterozygous state in three patients with hereditary spastic paraplegia.

Discussion

We describe a young girl with the first compound heterozygous mutations in MAG, who presented a complex form of HSP. The clinical picture described fits with the previous seven reported cases with MAG homozygous mutations (1) development delay noticed in the first 2 years in all but one patients (2) spastic paraplegia with sensorial visual and hearing involvement and peripheral neuropathy; (3) progressive motor deterioration; (4) variable cognitive impairment with cognitive function ranging from normal to severe mental disability; and (5) cerebellar atrophy and white matter abnormal signal concordant with myelin disorder according to the Knapp leukodystrophy classification. Nevertheless, the severity and some singularities of the case reported here must be highlighted: only two patients, including this one, managed to walk independently; among the four of five patients with available follow-up information, all experienced motor deterioration after adolescence, while the one described here had earlier loss of motor autonomy at 7 years. In addition, our patient exhibited a severe cognitive impairment compared to the mild intellectual disability reported for all the others (Table S2).

This clinical presentation is due to compound heterozygous mutations affecting the second and fourth immunoglobulin domains of the MAG protein. MAG
Hereditary Spastic Paraplegia Related to MAG Mutations
functions are associated with the control of myelin production and maintenance, and to the adhesion and signaling between myelinating oligodendrocyte and Schwann cells and axons.4 These functions are related to the binding of sialic acid moieties on the neuronal surface by the Siglec first immunoglobulin domain, to the interaction of the first three immunoglobulin domains with the RTN4R protein, and to homo-dimerization through the fourth and fifth immunoglobulin domains.13 Thus, the two MAG mutations identified here should disorganize the adhesion and signaling processes between myelinating cells and the axons, and affect the dimer stability, but this remains to be challenged in silico by structural modeling of the amino-acid changes, and demonstrated in vivo. Importantly, although these mutations affect different domains than the ones affected by the former 3 MAG mutations yet identified, the reported clinical presentations share strong similarities, suggesting a common conserved pathophysiological mechanism. In this respect, the phenotype of the MAG KO mice disclosed progressive peripheral neuropathy, central nervous system myelin deterioration with aging, and abnormal optic nerve myelination,4,14 a combination of symptoms very consistent with the ones disclosed in our patient.

To conclude, hereditary spastic paraplegia comprises a wide group of inherited neurodegenerative and neurodevelopmental disorders, resulting from primary retrograde dysfunction of the long descending fibers of the corticospinal tract. Given the growing number of HSP genetic forms and their overlapping features, clinical characterization remains a challenge before genetic testing.3 Combined CNS and PNS involvement, including spastic-ataxic gait, mixed axonal, and demyelinating polyneuropathy, and evidence of visual and auditory tract impairments, should suggest MAG mutations.

**Acknowledgments**

We are indebted to INSERM, CNRS, the University of Montpellier, the University of Angers, the CHU of Angers, the region Pays de la Loire, Angers Loire Metropole, the Fondation pour les Maladies Rares and Fondation pour la Recherche Médicale, and the following patient associations: Retina France, UNADEV, Ouvrir Les Yeux, foundation VISIO. We thank Lagavulin for constructive discussions.

**Authors’ Contributions**

A. Roubertie and G. Lenaers conceptualized and designed the study, interpreted clinical and genetic data, drafted the initial manuscript, reviewed, and revised the manuscript.

M. Charif, A. Guichet and G. Manes carried out genetic analysis and critically reviewed the manuscript.

P. Meyer and N. Leboucq collected brain imaging data and revised the manuscript.

I. Meunier collected ophthalmological data and reviewed the manuscript.

G. Taieb, R. Junta Morales and F. Rivier collected clinical data and reviewed the manuscript.

C. Delettre and E. Sarzi interpreted the data and substantively revised the manuscript.

All authors read and approved the final manuscript.

**Conflict of Interest**

The authors have no conflict of interest to disclose.

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Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Nerve conduction studies concordant with mixed axonal and demyelinating sensorimotor polyneuropathy. (ms: millisecond; mV: millivolt, m/s: meter per second).
Table S2. Main clinical findings in published patients and in the present case harboring MAG mutations. NK: not known.