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A novel *PLP1* mutation further expands the clinical heterogeneity at the locus

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Abstract

Objectives. To characterize at clinical and molecular levels a family presenting with X-linked recessive Hereditary Spastic Paraplegia (HSP). *Background:* HSPs are a large group of genetically heterogeneous neurodegenerative disorders characterized by progressive upper motor neuron signs. Mutations in the proteolipid protein (*PLP1*) gene have been identified in families linked to the SPG2 locus on chromosome Xq22. However, Pelizaeus-Merzbacher disease (PMD) is also an X-linked recessive neurological disorder caused by *PLP1* mutations. *Methods:* The SPG2 locus was investigated by linkage analysis in the family. The *PLP1* gene was screened by sequencing. We present findings in a large French-Canadian family with an X-linked recessive HSP. The proband presented early with developmental delay and developed progressive spastic paraplegia. He has been wheelchair-bound since the age of 3 years. At the latest follow-up, he was 20 years old and had severe spasticity predominantly affecting the lower extremities, moderate cerebellar dysfunction, and optic atrophy. *Results.* Linkage to SPG2 was established and a G to A mutation (M1R) in the initiation codon of the *PLP1* gene was identified, likely resulting in the complete absence of proteolipid protein. *Conclusions.* We report a new *PLP1* gene mutation in a patient with a clinical phenotype consistent with a PLP1 null syndrome.

Introduction

Hereditary spastic paraplegias (HSP) are a large group of clinically and genetically heterogeneous neurodegenerative disorders characterized by progressive upper motor neuron dysfunction involving preferentially the lower extremities leading to progressive gait spasticity. HSP are classified clinically as complicated or uncomplicated. The term "uncomplicated" or "pure" HSP is used when spastic paraparesis, with its associated upper motor neuron signs or symptoms (e.g. spastic bladder), are the only features. The term "complicated" or "complex" is used when additional clinical features are present, such as peripheral neuropathy, amyotrophy, sensorineural hearing loss, optic atrophy, seizures, mental retardation, dementia, and extrapyramidal signs. HSP are also classified according to their mode of inheritance: autosomal dominant, autosomal recessive and X-linked. To date, over 40 genetic loci and 17 genes have been identified. Four X-linked HSP loci have been reported and two causative genes have been identified. SPG1 (MIM 303350) is caused by mutation of the *L1CAM* gene on chromosome Xq28 (1). SPG2 (MIM 312920) on chromosome Xq22 results from mutation of the myelin proteolipid protein (*PLP1*) gene (2). No genetic defect has yet been identified in the remaining X chromosome loci SPG16 (MIM 300266) (3, 4) and SPG34 (MIM 300750) (5).

Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive hypomyelinating leukodystrophy caused by mutations of the proteolipid protein gene (*PLP1*) and is thus allelic to SPG2 (6). PMD typically presents in infancy or early childhood with developmental regression, nystagmus and hypotonia progressing to spastic quadraparesis(7). The most common mutation type is a gene duplication which results in a relatively mild form of the disease (8) (9). Interestingly, null mutations were reported in families with a mild form of the disease (10-12), while patients with point mutations present the most severe phenotypes (7).

Three subtypes of PMD have been described and are differentiated by their age of onset and severity (13-15). Connatal PMD has the most severe phenotype with onset in the neonatal period; these patients never achieve ambulation. Classic PMD has intermediate severity with onset in infancy or early childhood. These children eventually achieve ambulation with assistance but motor regression during childhood or adolescence results in the loss of ambulation skills. The least severe form of PMD is the PLP1 null syndrome which presents before five years of age and progresses more slowly. Children with this form do not develop nystagmus but do develop a peripheral neuropathy. Despite a mild spastic quadraparesis, they achieve independent ambulation, but with some degree of ataxia.

PLP

The gene that encodes the myelin proteolipid protein (*PLP1*) is alternatively spliced yielding two transmembrane proteins PLP and DM20(16). These proteins are among the most abundant in the central nervous system (CNS) constituting approximately 50% of the total myelin proteins. Both are transmembrane proteins and it is thought that PLP is probably involved in myelin compaction. Mutations of the *PLP1* gene lead to dysfunction and death of the oligodendrocytes.

PLP1 gene mutations have been described in both PMD and X-HSP families. To date, 118 mutations have been reported (Human Gene Mutation Database) and include gene duplication, deletion, missense and splicing mutations. Mutations are usually unique to a single family (7).

Subjects and Methods

Patients

This French-Canadian family has affected males in the last two generations. Inheritance of the disease is X-linked recessive as evidenced by the transmission of the disease through the unaffected maternal line. Family members were recruited and assessed by experienced neurologists. All patients provided informed consent according to the criteria of the Institutional Review Board of McGill University, Montreal, Quebec.

The proband is a male who was first seen in neurology at the age of 10 months for gross motor difficulties. His family history is significant for several family members, all males, affected by HSP (Figure 1). The patient's perinatal history was unremarkable. He presented in the first year of life with gross motor delay and was found to have spasticity of the lower extremities, hyperreflexia and bilateral Babinskis, along with axial hypotonia. He was diagnosed with probable X-linked recessive HSP. In the second year of life, he developed intermittent vertical nystagmus of the right eye; which disappeared on subsequent neuro-ophthalmological evaluations. He never achieved independent walking. Over the years, the spasticity in his lower extremities worsened and he developed upper motor neuron signs in the upper extremities, a hyperreflexic bladder, dysarthria, optic atrophy and scoliosis, for which he was operated on at the age of 16 years. At the latest neurological follow-up, at 20 years of age, the patient had mild cognitive dysfunction, significant dysarthria, restriction of extraocular movements, optic atrophy, slow rapid alternating movements, with spasticity and hyperreflexia involving the lower extremities much more than upper extremities. Extensive investigations were performed throughout the years. Of interest, a magnetic resonance imaging (MRI) of the brain was performed at the age of 12 years and showed hyperintense signal of the periventricular and deep white matter, better seen on T2-weighted imaging and fluid-attenuated inversion recovery (FLAIR) (Figure 2). His spinal MRI was normal. Electromyogram and nerve conduction studies showed a mild sensorimotor demyelinating polyneuropathy with decreased nerve conduction velocities.

Molecular Analysis

Blood was taken from informed, consenting individuals and DNA extracted following standard protocols. Linkage analysis was performed using microsatellite markers DXS990, DSX8020, DSX8089 and DXS8112 from the SPG2 locus. The model used for analysis was an X-linked recessive trait, with 75% penetrance, 0% phenocopy, and 0.01% allele frequency. PCR-amplified fragments of exon one of the *PLP1* gene were sequenced on both strands (forward primer: AGCCGGCTACAATTGGAGTCAGA , reverse primer: TAACTCCTTCTCCGCTGTACTAA) using a thermosequenase ^{33}P cycle sequencing kit (United States Biochemical, Cleveland, OH). Sequences were resolved on 6% polyacrylamide gels and detected by autoradiography.

Results

Linkage was established to the SPG2 locus with a maximum lod score of 4.24 at theta = 0.0 for the microsatellite DXS8020. Sequencing analysis of individuals Rou5886 and Rou5611 from this family (Figure 1) revealed a novel *PLP1* mutation (ATG->AGG shift) (Figure 3). Analysis of the DNA from all available family members confirmed the presence of this M1R mutation in all affected individuals. The mother of each affected boy also carried the mutation which was not detected in any unaffected individuals (Figure 1).

Discussion

Mutations in the *PLP1* gene are reported to cause the allelic disorders of PMD and X-HSP (SPG2). Duplication of the gene is the most common cause of PMD (12) and results in the intermediate phenotype known as classical PMD. Missense mutations in *PLP1* cause the most

severe subtype of PMD (connatal). Most of the SPG2 mutations reported are missense mutations. The majority of *PLP1* point mutations cause a more severe phenotype than null mutations suggesting that the aberrant protein has a deleterious effect.

To date, two mutations have been reported in the *PLP1* initiator codon, Met1Ile and Met1Thr (9, 10), both causing a mild PMD phenotype similar to the phenotype caused by *PLP1* gene deletion (11). In our family, we report a similar mutation (Met1Arg), expected to result in the total absence of PLP and DM20 proteins.

Our patient's clinical features and imaging findings are consistent with a clinical phenotype of PLP1 null syndrome but presents some clinical findings suggestive of a severe complicated HSP, such as the severe spasticity in the lower extremities, the hyperreflexic bladder and the absence of significant cognitive involvement. The PLP1 null syndrome and the complicated form of HSP-2 share several characteristics, such as age of onset, spasticity, and cerebellar involvement. However, the presence of nystagmus early in the course, the severity of spasticity as well as the MRI result are more consistent with a severe form of complicated HSP (17). The other clinical findings are more typical of the PLP1 null syndrome; the patient's motor milestones were delayed as early as the first year of life (14) and the presence of axial hypotonia early on(2). The patient nerve conduction studies and MRI results are also consistent with this diagnosis (18, 19). It is interesting to note that the only PLP1-related disease where ambulation is never achieved is the connatal form, which this patient certainly did not have. The age of death of the patient's uncle (17 years) is somewhat atypical for both diseases; death at that early age is typically reported only with the most severe form of PMD (connatal).

We believe that our patient raises the important question of whether PMD and SPG2 are two different allelic diseases or one disease with variable severity. The latter is probably more likely and our patient, presenting typical features of both PMD and SPG2 would be in the middle of the spectrum, both in terms of his clinical manifestations and in terms of his imaging findings. The identification of the causative mutation in this family provides further evidence of the significant overlap between the two disorders and further evidence for consideration as a single entity with a spectrum of phenotypes.

Figure 1: Pedigree of X-HSP family indicating affected individuals (solid symbols).
The proband is indicated by an arrow. The *PLP1* exon 1 mutation status is indicated for the individuals analyzed. To preserve confidentiality the symbols and/or mutation status of a number of individuals have been omitted from this pedigree. In the second generation there are four additional females who do not have affected children and so their mutation status has not been disclosed.

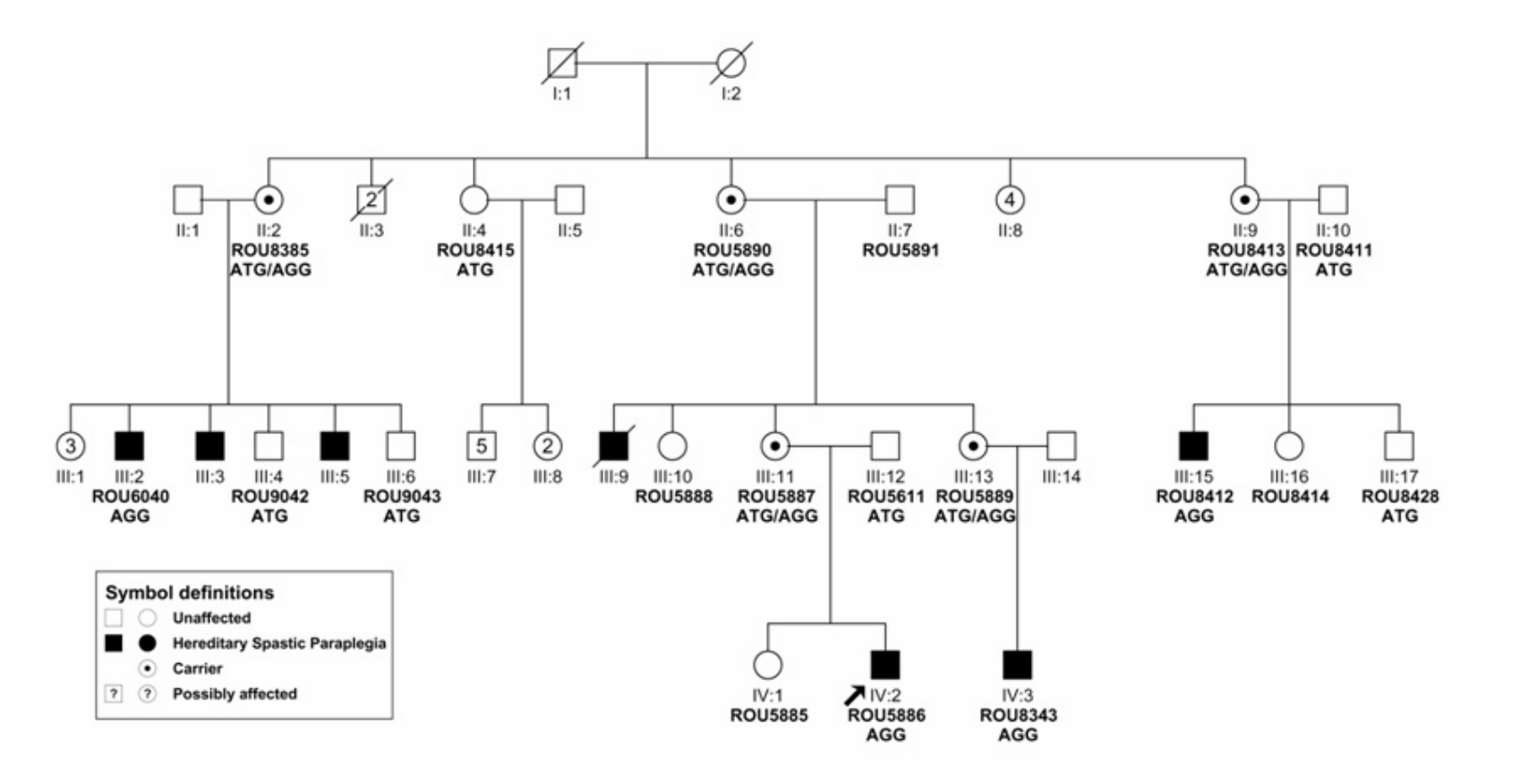


Figure 1: Pedigree of X-HSP family indicating affected individuals (solid symbols).

Figure 2: MRI performed at the age of 12 years.

A. Axial MRI, T2-weighted: normal white matter signal in the brainstem and cerebellum.

B. Axial MRI, T2-weighted: mild hyperintensities in the periventricular regions.

C. Axial MRI, T2-weighted: normal white matter signal in the cerebral hemispheres besides the periventricular regions.

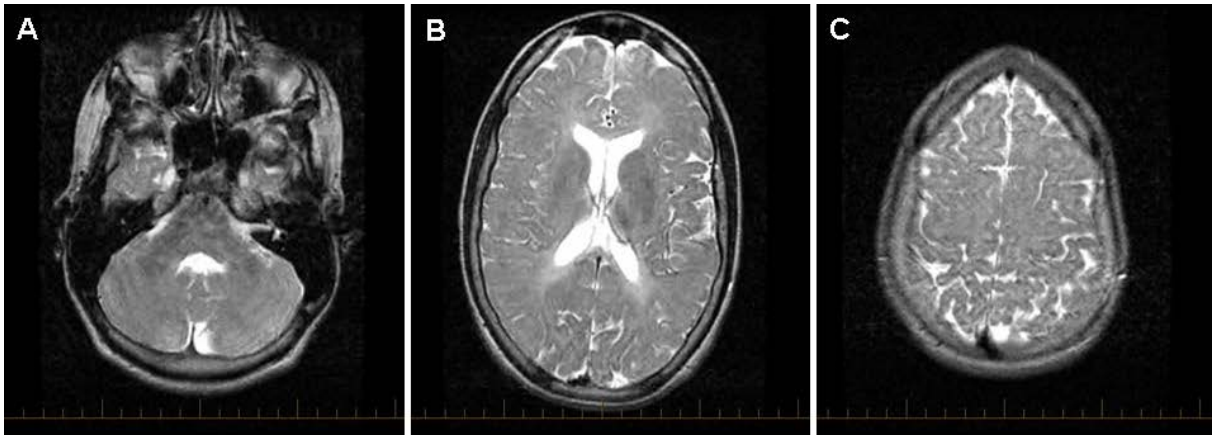
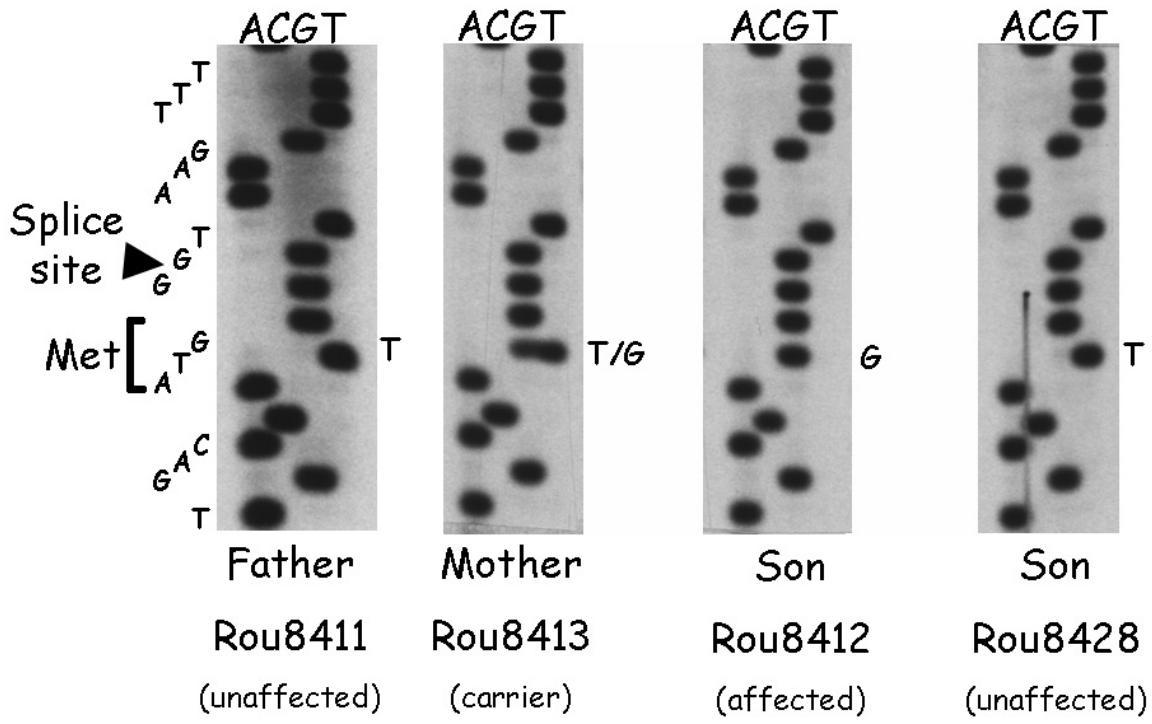


Figure 3: Sequence of *PLP1* exon 1 in the region of interest.

The father displays the normal sequence; the mother who is a carrier of the mutated gene is a M1R heterozygote and the site of the T->G shift is indicated. Their affected son carries the mutated initiator codon (AGG) while their unaffected son has a normal (ATG) copy of the codon. Met indicates the methionine start codon.



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