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ORIGINAL INVESTIGATION

Erik A. Sistermans · Ilse J. de Wijs · René F. M. de Coo
Leo M. E. Smit · Fred H. Menko · Bernard A. van Oost

A (G-to-A) mutation in the initiation codon of the proteolipid protein gene causing a relatively mild form of Pelizaeus-Merzbacher disease in a Dutch family

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Abstract Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive disorder that is characterized by dysmyelination of the central nervous system resulting from mutations in the proteolipid protein (PLP) gene. Mutations causing either overexpression or expression of a truncated form of PLP result in oligodendrocyte cell death because of accumulation of PLP in the endoplasmic reticulum. It has therefore been hypothesized that absence of the protein should result in a less severe phenotype. However, until now, only one patient has been described with a complete deletion of the PLP gene. We report a Dutch family with a relatively mild form of PMD, in which the disease cosegregates with a (G-to-A) mutation in the initiation codon of the PLP gene. This mutation should cause the total absence of PLP and is therefore in agreement with the hypothesis that absence of PLP leads to a mild form of PMD.

Introduction

Pelizaeus-Merzbacher disease (PMD) is an X-linked dysmyelinating disorder resulting from mutations in the proteolipid protein (PLP) gene. Different mutations have been found in the PLP gene (Gow et al. 1994), mostly leading to the expression of altered or truncated forms of PLP, or to overexpression of the protein. One patient has been described with a deletion of at least 29 kb that includes the complete PLP gene (Raskind et al. 1991). The clinical expression in this patient, who was 35 years old, was relatively mild as the life expectancies of the connatal and classical forms of PMD do not normally extend beyond the first and second decade, respectively. Here, we describe another patient (II-1) who suffers from a mild form of this disorder and who is now 37 years old.

E. A. Sistermans · I. J. de Wijs · R. F. M. de Coo
B. A. van Oost (✉)
Department of Human Genetics, University Hospital Nijmegen,
P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

L. M. E. Smit
Department of Pediatrics, Free University Hospital,
P. O. Box 7057, 1007 MB Amsterdam, The Netherlands

F. H. Menko
Department of Clinical Genetics, Free University Hospital,
P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

Case history

Motor dysfunction in patient II-1 was first noticed at the age of 4 years. A gradual retardation of motor and mental development resulted in mental deficiency and spastic paraplegia. He was admitted to an institution for the mentally disabled at age 14. At the age of 33 years, he came to medical attention again because of the slow deterioration of his mental condition and progression of his spastic tetraplegia. Neurologic evaluation revealed a spastic atactic tetraplegia. Cerebral fluid examination, metabolic screening, and enzyme activities of lysosomal enzymes showed no abnormalities. Electroencephalography (EEG) was normal, but cortical evoked potentials indicated slow central conduction velocities. Conduction velocities of the peripheral nerves were reduced, and magnetic resonance imaging (MRI) revealed extensive symmetric abnormalities of the white matter, with signs of leukodystrophy. Sural nerve biopsy showed reduced density of myelinated fibers and groups of small myelinating fibers indicative of regeneration. No signs of storage disease were found.

His sister (II-2) has an adequate mental function with intact coordination and balance functions. However, she has a pyramidal syndrome and complains about pains in her muscles and joints. An MRI scan of her cervical myelum revealed an area of enhanced signal intensity which might indicate a decrease in the amount of myelin.

This patient's son (III-1) was born after an uneventful 42-week pregnancy. Delivery and the subsequent neonatal period were free of complications, but the baby was irritable and had sleeping problems in the first few weeks of life. A convergent strabismus was apparent, his motor development was retarded, and he was hypotonic with a persistent headlag. An extension hypertonia gradually developed. At the age of 1 year, the child was alert and had good visual perception, but slightly atactic motor behavior was observed with an evident spastic tetraplegia and poor balance control. No abnormalities were revealed by laboratory investigations, including karyotyping, hematological screening, liver and kidney function tests, metabolic screening, and determination of peroxisomal and lysosomal enzyme activities. EEG and visual and auditive evoked potentials were normal, somatosensory evoked potentials were retarded. The motor conduction velocities of peripheral

nerves were within normal limits. MRI revealed hypo- and dysmyelination in T2 weighted and inverse recovery (IR) images. Subsequently, the clinical picture of moderate spastic tetraparesis with pseudobulbar symptomatology remained stationary; his mental development was moderately retarded. There was slow language development with an expressive articulation disorder. At the age of 6 years, the boy is dependent on help for activities of daily living (ADL) and wheelchair-bound, although he is able to crawl.

Results

Although the clinical course described here is not indicative of a progressive neurological disorder and does not meet all the clinical criteria of PMD, linkage analysis shows that the disease locus segregates with polymorphic Xq22 markers surrounding the PLP gene (Fig. 1A). However, the gene is not deleted and no mutations have been detected in exons 2–7. Gene duplication has not occurred either.

To test the possibility that a mutation lay in the small (4-nt) coding region of exon 1, a polymerase chain reaction (PCR)-directed site-specific mutagenesis (PDSM) test was developed. By using the mutated forward primer 749 (5´CGGCTACAATTGGAGTCAGAGTCCCAAAG-*CC* 3´; mutated nucleotide in italics) in combination with a reverse primer (5´ CCCGAACCCCAAAGTTGGAA 3´) in intron 1 (Strautnieks et al. 1992), a *Nco*I site overlapping the 4-nt coding region of exon 1 was introduced into the resulting 114-bp PCR fragment (5´ ACATGG 3´ → 5´ C↓CATGG 3´; PCR: 92°C for 1 min, 55°C for 2 min, 72°C for 3.5 min for 30 cycles). *Nco*I digestion of the PCR product from a wild-type allele should result in 85-bp and 29-bp fragments, whereas any mutation in the 4-nt coding region will prevent this digestion. When this PDSM test was performed for the individuals of the family, the two affected males (II-1 and III-1) showed a mutation. Their mothers (I-2 and II-2, respectively) were heterozygous, whereas the non-affected males (I-1, II-3, and II-4) did not carry the mutation (Fig. 1A). Finally, exon 1 of patient III-1 was sequenced bidirectionally and a G→A transition was found, changing the ATG initiation codon into ATA (Fig. 1B).

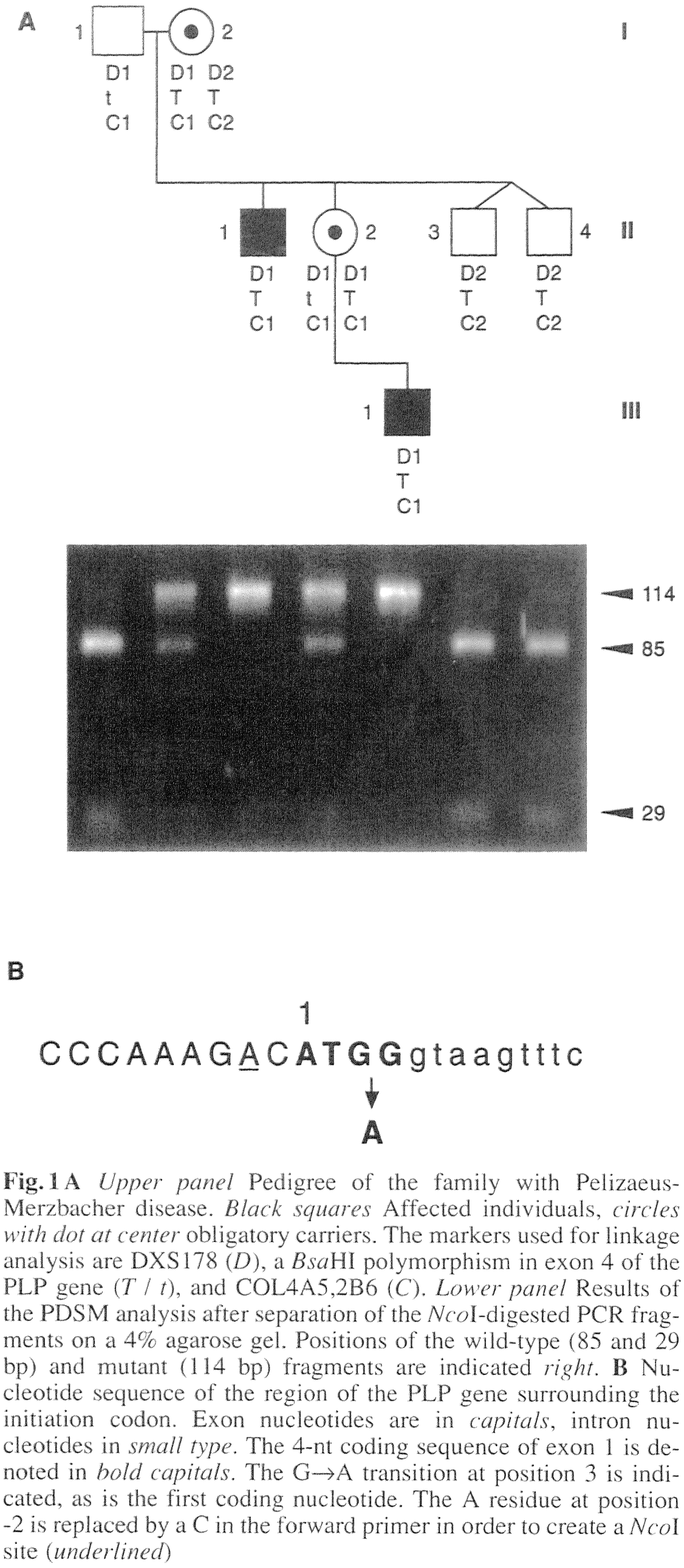

Fig. 1A *Upper panel* Pedigree of the family with Pelizaeus-Merzbacher disease. *Black squares* Affected individuals, *circles with dot at center* obligatory carriers. The markers used for linkage analysis are DXS178 (*D*), a *Bsa*HI polymorphism in exon 4 of the PLP gene (*T* / *t*), and COL4A5,2B6 (*C*). *Lower panel* Results of the PDSM analysis after separation of the *Nco*I-digested PCR fragments on a 4% agarose gel. Positions of the wild-type (85 and 29 bp) and mutant (114 bp) fragments are indicated *right*. **B** Nucleotide sequence of the region of the PLP gene surrounding the initiation codon. Exon nucleotides are in *capitals*, intron nucleotides in *small type*. The 4-nt coding sequence of exon 1 is denoted in *bold capitals*. The G→A transition at position 3 is indicated, as is the first coding nucleotide. The A residue at position -2 is replaced by a C in the forward primer in order to create a *Nco*I site (*underlined*)

Discussion

Many examples are known that demonstrate the pathogenic effects of mutations affecting the initiation codon. The G-to-A mutation that we describe here has been found, among other sites, in the phenylalanine hydroxylase gene causing phenylketonuria (Eiken et al. 1992) and in the beta-globin gene causing beta-thallassaemia (Saba et al. 1992). Furthermore, it is known to affect translation initiation in other organisms too, such as *Escherichia coli* (Usui et al. 1994) and *Saccharomyces cerevisiae* (Mukhopadhyay et al. 1994). We therefore conclude that it causes Pelizaeus-Merzbacher disease in this family. Because the next in-frame ATG codon is located at the 3´ end of exon 4, it is unlikely that translation initiation from this codon will occur, or will lead to the production of a functional protein. The observed phenotype in this family is in accordance with the hypothesis that an absence of PLP will cause a relatively mild form of PMD, compared with overexpression or expression of a mutated form of PLP. A possible explanation for this discrepancy is that the latter mutations result in oligodendrocyte cell death because of an accumulation of PLP in the endoplasmic

reticulum/Golgi apparatus system, whereas in the absence of PLP, oligodendrocytes survive and make a PLP-lacking compact form of myelin (Gow et al. 1994; Kagawa et al. 1994). As the patients described in this report do not meet all the clinical criteria for the diagnosis of PMD, we conclude from these results that the clinical spectrum of X-linked PMD is wider than originally thought, with the practical implication that patients who do not meet all the clinical criteria should nevertheless be candidates for mutation analysis of the PLP gene.

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