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Letter to the Editor

A novel *PLP1* deletion causing classic Pelizaeus-Merzbacher disease

Dear Editor,

Pelizaeus-Merzbacher disease and its allelic disorder, spastic paraplegia type 2 are X-linked recessive dysmyelinating disorders affecting the central nervous system. Pelizaeus-Merzbacher disease is caused in most cases by either duplication or point mutations in the *PLP1* gene. Less frequently it has been reported the presence of large deletions of the gene. This disease has a wide clinical spectrum and its causing mutations act through different molecular mechanisms. The *PLP1* gene is composed of seven exons encoding two proteins expressed mainly in oligodendrocytes, the proteolipidprotein (PLP) and its differently spliced isoform DM20. Both proteins, PLP and DM20, are the major protein components of myelin in the central nervous system playing a major role in myelin sheath formation by promoting sheath compaction. More than 300 different mutations have been reported in the *PLP1* gene [1] the duplication of the gene accounting for around 60% of the total [2]. Patients present clinical forms ranking from the more severe connatal to the mild spastic paraplegia 2 form (SPG2). Connatal forms are associated with missense mutations whereas a later onset is usually due to duplication of the *PLP1* gene suggesting that overdose can be a cause of PMD. A few cases of large deletions affecting the *PLP1* gene have been reported, leading to a complete deficiency of PLP and DM20 proteins. Complete deletion of *PLP1* gene causes a relatively mild syndrome [3–5]. In this work we have characterized a large deletion in PLP1 gene that we had reported before [6] in a PMD patient. In this work we have identify the breakpoints of the deletion and analyse the probable mechanisms for the deletion.

1. Case report

The patient, a 16-year-old boy, was born at full term to unrelated Spanish parents. He is the only child and there were no other cases of neurological disease in the family. He presented delay in motor development during the first year of life (head control at 6–7 months, sitting position at 13 months), hypotonia and horizontal nystagmus. He also showed slow conduction time on auditory evoked potentials. There was no history of seizures. When he was 9 years old he underwent evaluation of his neurological status and T2-weighted MRI of the brain revealed diffuse periventricular hypomyelination. He developed spastic tetraparesis, had slow understandable speech, and he could stand still but could not walk. This clinical form corresponds with the classic form of Pelizaeus-Merzbacher disease [7].

Informed consent was obtained from the parents for blood extraction and DNA analysis of the patient. The study was approved by the ethics committee of Hospital Sant Joan de Déu and Hospital Universitario La Paz and complied with the principles of the declaration of Helsinki. Genomic DNA was extracted from peripheral blood leucocytes by standardized procedures (commercial Kit Purogene from Qiagen, Maryland, USA). DNA sample was screened for *PLP1* gene dosage using the Multiplex Ligation-dependent Probe Amplification technique (MLPA) following the instructions given by the manufacturer (SALSA P022, MRC Holland, Amsterdam, The Netherlands). Oligonucleotide primers were designed to amplify *PLP1* exons, flanking intronic sequences and intergenic regions. Amplified DNA products were sequenced by the di-deoxy termination method (Applied Biosystems, Foster City, CA. BigDye Sequencing Kit version 3.1 or 1.1) and ran in an ABI 3130 DNA sequencer. Both strands of DNA were sequenced. Analysis of the repeat sequences around the breaking point was done online by using the program Repeat Masker [8].

MLPA analysis of the patient's DNA detected no duplication but a loss of exon 1 to 4 of *PLP1* gene extending upstream to the 5′ end conserving the adjacent *GLRA4* gene (Fig. 1A). To establish the extension of the deletion we tried to amplify, using different sets of primers (Fig. 1B), different sequences along the *GLRA4-PLP1* intergenic region. Positive amplifications were obtained for amplicons #1 and #2 in the patient whereas amplification for amplicon #3 was negative compared to controls. This approach allowed us to determine that the deletion extend at least 5.1 Kb from the ATG of *PLP1* gene. Finally, by using primers for amplicon #4, located 9678 bp upstream the ATG (the forward primer) and in exon 5 of *PLP1* (the reverse primer), we could amplify a fragment of 840 bp that presumably includes the breakpoints of the deletion. PCR amplification of DNA control did not get any fragment. We sequenced the patient 840 bp fragment; DNA sequence of breakpoint region is shown in Fig. 1C. From this sequence we conclude that deletion expands 17,654 nucleotides, from exon 4 to 6695 nucleotides upstream of the initial ATG of *PLP1* gene (Fig. 1D). The breakpoint of the deletion include a MER90a#LTR/ERV1 sequence with a homology close to 80% in the 5′ region of the breakpoint as detected by the Repeat Masker program.

2. Discussion

About 60% of patients with PMD have a duplication in the Xq22.2 region that includes the *PLP1* gene, whereas the deletions of the *PLP1* gene are extremely rare in contrast to the duplications. Large genomic deletions result in a lack of PLP protein and its isoform, DM20. Patients with this type of mutation (as well as other types of mutations leading to a lack of these proteins) seem to show a unique phenotype [3–5], with symptoms less severe than that caused by point mutations or duplication of the gene. The pathogenic mechanisms explaining these differences have been postulated by Cailloux et al. [9]. In the present case, a great deletion, the disease can be seen as a classical form of PMD. Interestingly, the patient also have mild peripheral neuropathy that can be ignored since it is moderate and can be overshadowed by the central nervous system manifestations.

To date, several patients with great deletions of *PLP1* gene have been described in the scientific literature [1]. In those patients it was shown that the deletion appeared to be smaller than the majority of

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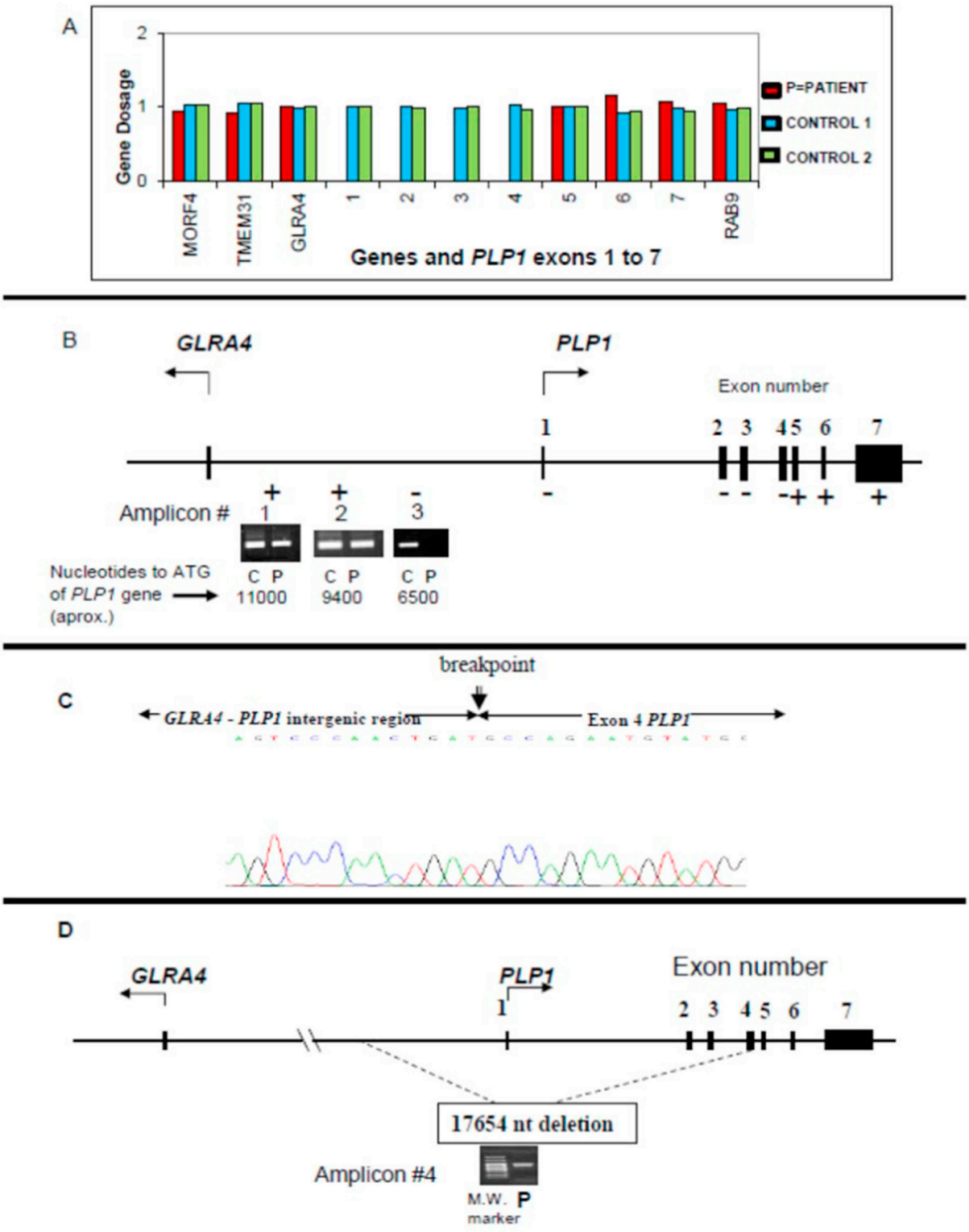

Fig. 1. A) Gene dosage in the PLP1 gene region as detected by Multiplex Ligation-dependent Probe Amplification (MLPA). It is shown that exons 1, 2, 3 and 4 of *PLP1* gene were lost in the patient. B) Experimental approach to define deletion's boundaries. Different oligonucleotide primers were designed to amplify short fragments of DNA along the PLP1-GLRA4 intergenic region. Signs + or – indicate positive or negative amplification as compare with a control DNA as it is shown in the pictures below. C = control, P = patient. C) DNA sequence at the breakpoint in the patient. It is shown that sequence of the GLR4A-PLP1 intergenic region is contiguous to the exon 4 of PLP1 gene. D) Scheme showing the extension of the deletion and the picture of the 840 bp fragment containing the breaking point of the deletion. P = patient.

PLP1 duplications. In those cases, two other genes (*RAB9B* and *TMSB15A*) are deleted, in addition to *PLP1*. It is possible that larger deletions are not frequent because they are lethal in the male sex. If the deletion/duplication mechanisms were based on an unequal crossing over, the frequency for both, deletions and duplications, would be roughly the same. Since duplication is a much more frequent event it has been hypothesized that probably large deletions result in embryonic lethality, whereas duplication of the same segment does not.

Deletion here described arises probably by a mechanism different of non-allelic homologous recombination (NAHR) since there were no homologous sequences between the regions around the distal and proximal breakpoint. Along with the fact that the breakpoint of the deletion includes a MER90a#LTR/ERV1 endogenous retrovirus sequence in the 5′ region of the breakpoint, with a homology close to 80%, we suggest that the more probable mechanism is a non-homologous end-joint mechanism (NHEJ). As previously reported [10], breakpoint of non-homologous large deletions are often located in or near these transposable elements (TEs).

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