Short Report

**PLP1** duplication at the breakpoint regions of an apparently balanced t(X;22) translocation causes Pelizaeus–Merzbacher disease in a girl


**PLP1** (proteolipid protein1 gene) mutations cause Pelizaeus–Merzbacher disease (PMD), characterized by hypomyelination of the central nervous system, and affecting almost exclusively males. We report on a girl with classical PMD who carries an apparently balanced translocation t(X;22)(q22;q13). By applying array-based comparative genomic hybridization (a-CGH), we detected duplications at 22q13 and Xq22, encompassing 487–546 kb and 543–611 kb, respectively. The additional copies were mapped by fluorescent in situ hybridization to the breakpoint regions, on the derivative X chromosome (22q13 duplicated segment) and on the derivative 22 chromosome (Xq22 duplicated segment). One of the 14 duplicated X-chromosome genes was **PLP1**. The normal X chromosome was the inactive one in the majority of peripheral blood leukocytes, a pattern of inactivation that makes cells functionally balanced for the translocated segments. However, a copy of the **PLP1** gene on the derivative chromosome 22, in addition to those on the X and der(X) chromosomes, resulted in two active copies of the gene, irrespective of the X-inactivation pattern, thus causing PMD. This t(X;22) is the first constitutional human apparently balanced translocation with duplications from both involved chromosomes detected at the breakpoint regions.

Conflict of interest

None of the authors have any conflict of interest to disclose.

Pelizaeus–Merzbacher disease (PMD, MIM 312080) is a rare recessive X-linked hypomyelinating disorder of the central nervous system (CNS), characterized by pendular nystagmus, hypotonia, spastic paraplegia, ataxia and mild developmental delay (1). A great majority of patients are males. PMD is caused by alterations of the **PLP1** gene that encodes the proteolipid protein 1, abundant in CNS myelin (2). **PLP1** duplications are responsible for 60–70% of the cases, and **PLP1** intragenic mutations are detected in 15–20% of the patients (3). Deletions of **PLP1** have been reported (4), and altered expression of the gene due to position effect might be the mechanism responsible for those cases with no detectable **PLP1** alterations (5, 6). Patients with **PLP1** duplications typically present with the classic form of PMD (7), whereas carriers of loss-of-function mutations or **PLP1** deletions have a milder form of PMD or are affected by spastic paraplegia type 2 (SPG2, MIM...
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312920) (8, 9). Three or more copies of PLP1 (10) and nonsense PLP1 mutations result in a more severe form of PMD (11).

We report on a girl with classic PMD, who carries an apparently balanced t(X;22) translocation. Micro duplications of chromosomes X and 22 were detected on each derivative chromosome, and PLP1 was one of the X-chromosome duplicated genes. Two female carriers of a PLP1 duplication presenting with classic PMD phenotype have been described (12, 13), but this is the first time the PMD phenotype has been associated with a reciprocal translocation. This constitutional translocation is peculiar because of the presence of duplicated segments of both involved chromosomes at the breakpoint regions.

Patient and methods

This study was approved by the Ethics Committee at the Biosciences Institute, University of São Paulo.

Patient

The girl, the first child of unrelated and healthy parents, was delivered after an uneventful pregnancy, with height and weight below the third centile. At birth, nystagmus was noticed. Her psychomotor development was severely delayed, and at 12 months of age, delayed myelination was suggested by brain magnetic resonance imaging (MRI) that showed diffuse increased T2 signal throughout the white matter of cerebral hemispheres. At 5 years, she presented seizures and since then she has been on antiepileptic drugs. She was evaluated at our Genetic Counseling Service, at 6 years of age, because her chromosomal examination revealed a t(X;22)(q22,q13) translocation. Her weight and length were below the third centile; she was able to speak a few words, to sit up without support and to crawl dragging her body. She was hypotonic and presented rotational head movements and choreoathetosis, as well as pendular nystagmus. Our documentation of the PLP1 duplication in association with the clinical features allowed the diagnosis of PMD. A subsequent MRI, at age 8 years, revealed severe diffuse hypomyelination (Fig. 1).

Methods

GTG-banding analysis was performed on metaphases from cultured peripheral blood lymphocytes. Fluorescent in situ hybridization (FISH) was performed with BACs and PACs (Table S1) selected on the University of California, Santa Cruz, Genome Browser (http://genome.ucsc.edu; hg 18). Probes were labeled with biotin-14-dATP or digoxigenin-11-dUTP by nick translation (Bio/Dig-Nick Translation kits; Roche, Mannheim, Germany) and visualized with fluorescein isothiocyanate- (FITC) or rhodamine-conjugated

**Fig. 1.** Brain magnetic resonance imaging (MRI) of patient at age 8 years. (a) Axial T2-weighted image shows homogeneous and diffuse hyperintensity of the cerebral white matter with moderate cortical and subcortical atrophy. (b) Axial T1-weighted image displays isointensity of the cerebral white matter with relative higher signal of the posterior limbs of the internal capsules, the anterolateral part of the thalamus (white arrow) and the optic radiations (black arrow), reflecting profound lack of myelination.
antibodies; chromosomes were counterstained with 4,6-diamidine-2-phenylindole.

The X-inactivation pattern was determined after 5-Brdu incorporation (200 μg/ml) for the last 7 h of the lymphocyte culture and acidine orange staining. The methylation status of the androgen receptor gene (AR) was evaluated as described previously (14), with modifications. The polymerase chain reaction products were separated by capillary electrophoresis on ABI Prism 310 Genetic Analyzer, and products were analyzed using Gene Mapper Software v4.0 (Applied Biosystems, Foster City).

Array-based comparative genomic hybridization (a-CGH) was performed using the Human Genome 105A CGH Microarray (Agilent Technologies Inc., Santa Clara, CA). Microarray images were processed by Feature Extraction 9.5, and data were analyzed with DNA Analytics 3.4 (both softwares from Agilent), using the following parameters: statistical algorithm ADM-2, sensitivity threshold 6.7 and at least three consecutive probes with aberrant log2 ratios.

Results

G-banded chromosome analysis confirmed the apparently balanced translocation t(X;22)(q22;q13). The proband’s parents had normal karyotypes.

a-CGH revealed the duplication of oligonucleotides mapped to an approximately 487-kb segment at 2q13.32-q13.33 (Chr22:47,732,501-48,219,751; Human GRCh36 Assembly, hg18); the proximal breakpoint was mapped to an approximately 21-kb interval (chr22:47,711,644-47,732,501), and the distal breakpoint to an approximately 38-kb segment (chr22:48,219,751-48,257,835) (Fig. 2a). FISH probes RP5-925J7 (chr22:47,740,384-47,838,754) and CTA-722E9 (chr22:48,181,791-48,314,060) hybridized to the normal chromosome 22 and to both derivative chromosomes, der(22) and der(X) (Fig. 2b). The similar intensities of RP5-925J7 signals on the normal chromosome 22 and on the derivative chromosomes pointed to the presence of two copies of this clone, one at each translocation breakpoint region. The weaker signal of CTA-722E9 on the der(22) indicated that this clone contained the distal duplication breakpoint. The signal on the der(X), however, was similar to that on the normal chromosome 22, thus pointing to its partial duplication, in agreement with the array data. On the X chromosome, oligonucleotides mapped to an approximately 543-kb segment at Xq22.1-q22.1 (chrX:102,437,622-102,980,861) were found to be duplicated; the proximal breakpoint was mapped to an approximately 26-kb interval (chrX:102,411,735-102,437,622), and the distal breakpoint to an approximately 42-kb segment (chrX:102,980,861-103,022,577) (Fig. 3a). FISH probes RP11-265I15 (chrX:102,270,516-102,453,744), RP3-421I20 (chrX:102,569,986-102,622,582), RP11-142J15 (chrX:102,785,715-102,935,799,) and RP4-540A13 (chrX:102,953,929-103,039,303) produced FISH signals on the normal X chromosome and on the der(22) and der(X) (Fig. 3b). The signals of similar intensity yielded by both RP3-421I20 and RP11-142J15 clones on the normal X and on the der(22) showed that they were duplicated at the translocation breakpoint regions. The weaker signals of clones RP11-265I15 and RP4-540A13 on the der(22) and the der(X), respectively, indicated that they contained the X-chromosome breakpoints. In turn, the signals of RP11-265I15 on the der(X) and RP4-540A13 on the der(22) were similar to those on the normal chromosomes, as expected if these clones were partially duplicated at the breakpoint regions, as pointed out by the a-CGH analysis.

No genes were mapped to the duplicated segment on chromosome 22. PLP1 was among the 14 genes mapped to the X chromosome duplicated segment.

X-chromosome replication analysis after 5-Brdu incorporation showed that the structurally normal X was late replicating in all 60 metaphases analyzed (Fig. S1). A 82:18 inactivation ratio was estimated based on the methylation pattern of the AR locus, with the maternally inherited X chromosome being predominantly inactivated (Fig. S2). As the translocated X was shown cytologically to be the active one, the X chromosome involved in the translocation was of paternal origin.

Discussion

We studied an apparently balanced de novo translocation t(X;22) in a girl presenting with classical PMD. By combining a-CGH and FISH, duplicated segments were identified at the translocation breakpoint regions, an X-chromosome extra segment on the der(22) and a chromosome 22 additional segment on the der(X). The X chromosome duplication encompassed the PLP1 gene. Thus, the patient had three PLP1 copies, one on the normal X and the other two copies on the der(X) and on the der(22) chromosomes. As a consequence of X-inactivation, she always had two functional copies of the PLP1 gene and manifested PMD.

The majority of heterozygotes for a PLP1 duplication are asymptomatic. Only two females who carried a PLP1 duplication and manifested the PMD classical phenotype have been reported: a girl carrying a 23–29 Mb duplication at Xq21.32-q24 (12), and another with an extra copy of PLP1 inserted into 1p36 (13).

Heterozygous carriers of a PLP1 duplication usually show an extremely skewed pattern of X inactivation in peripheral blood cells, probably the result of selection against cells with an active duplication-bearing X chromosome (15). This would explain their normal phenotype. Duplication carriers manifesting PMD features with an extra copy of PLP1 located on the X-chromosome have been described who showed random or moderately skewed X-inactivation in blood cells (12, 16, 17); the absence of significant selection in brain against cells bearing the PLP1 duplication on the active X might have contributed to their symptoms. In the t(X;22) reported here, as in the rearrangement described by Yue et al.(13), the extra PLP1 copy is located on an autosome, thus guaranteeing two
Fig. 2. Analysis of the t(X;22) breakpoint region on chromosome 22. (a) The Agilent 105A a-CGH profile of chromosome 22 shows a duplication at 22q13.32-33. (b) No genes are mapped to the duplicated segment of about 487 kb (solid blue line) or to the possibly duplicated proximal and distal flanking regions (dashed blue line). The duplicated RP5-925I7 and partially duplicated CTA-722E9 clones are depicted (based on UCSC Genome Bioinformatics, hg18). These clones yield fluorescent in situ hybridization (FISH) signals on the normal chromosome 22 and on both the der(22) and der(X). The similar intensities of the RP5-925I7 hybridization signals show that both the der(22) and the der(X) contain a copy of this clone. The weaker signal of the CTA-722E9 clone on chromosome der(22) indicates that it contains the distal breakpoint of the chromosome 22 duplication. The signal yielded by this clone on the der(X) is comparable to that on the normal chromosome 22, as expected if this clone is partially duplicated.

Our patient presents seizures, as well as persistence of hypotonia at 6 years of age, which are uncommon in classical PMD. A higher PLP1 expression on the der(22) or the effect of other duplicated X-chromosome genes might account for these atypical features.

This is the first report of PMD associated with a reciprocal translocation. The applying of a-CGH has shown that a proportion of balanced chromosomal rearrangements have crypt deletions/duplications at or in cis to breakpoint regions (19). These imbalances are predominantly deletions and there is only one report of a duplication located at the breakpoint region of a constitutional reciprocal translocation in humans (20). In turn, in the t(X;22) described here, each derivative chromosome had a duplicated segment from the other participating chromosome. Recently, Howarth et al. (21) proposed that translocations with large duplications at breakpoints in breast cancer cells would arise from stalled replication bubbles. This could potentially functional copies of the gene, irrespective of the inactivated X chromosome. However, autosomal-located extra copies of PLP1 might not be properly expressed and have been detected in patients without PMD manifestations (4, 18). The PMD phenotype of our patient points to the relocation of PLP1 to a favorable chromatin environment, together with putative cis-acting regulatory elements (5, 6), thus assuring its expression on chromosome 22.
PLP1 duplication associated with t(X;22) in a girl

Fig. 3. Analysis of the t(X;22) breakpoint region on the X chromosome. (a) The Agilent 105A a-CGH profile of chromosome X shows a duplication at Xq22.1-22.2. (b) Fourteen genes are mapped to the duplicated segment of about 543 kb (solid blue line) and to the possibly duplicated proximal and distal flanking regions (dashed blue line). The PLP1 gene is indicated by an arrow. Duplicated RP3-421I20 and RP11-142J15 clones and partially duplicated RP11-265I15 and RP4-540A13 clones are depicted (based on UCSC Genome Bioinformatics, hg18). These clones produce fluorescent in situ hybridization (FISH) signals on the normal X and on both the der(X) and the der(22). The similar intensities of the RP3-421I20 and the RP11-142J15 signals on the normal X, on the der(X) and on the der(22) points to a copy of each of these clones on the translocated chromosomes. The weaker signals of clones RP11-265I15 and RP4-540A13 on the der(22) and the der(X), respectively, indicate that these clones contain the proximal and distal breakpoints of the X-chromosome duplication. The RP11-265I15 and RP4-540A13 signals on the der(X) and on the der(22) are similar to those on the normal chromosomes, in accordance with duplication of the proximal segment of these clones.

Fig. S2. X-inactivation assay based on the methylation status of the AR locus in peripheral blood cells: The alleles were amplified from genomic DNA, undigested (graphs A, C, E) and digested by the methylation-sensitive enzyme HpaII (graphs B, D, F). After digestion, only the methylated alleles, on the inactive X, are amplified. The inactivation ratio (82:18) shows that the same allele is inactive in 82% of the cells. The comparison of the patient’s alleles with those of her parents shows that the maternally inherited X chromosome is predominantly inactivated.

Supporting Information
The following Supporting information is available for this article: Fig. S1. X-inactivation analysis after 5-BrdU incorporation, in the t(X;22) carrier. The normal X chromosome was late replicating (inactive) in 60 metaphases from peripheral blood lymphocytes analyzed.

This report illustrates the effectiveness of characterizing chromosomal rearrangements by a-CGH, which revealed the duplication, and by FISH, which allowed the chromosomal localization of the duplicated sequences, for genotype–phenotype correlations to be shown.

Additional Supporting information may be found in the online version of this article. Please note: Wiley-Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.
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