

## DATA REPORT

A novel *PEX1* mutation in a Moroccan family with Zellweger spectrum disordersAmale Bousfiha<sup>1</sup>, Amina Bakhchane<sup>1</sup>, Hicham Charoute<sup>1</sup>, Zied Riahi<sup>2,3</sup>, Khalid Snoussi<sup>1</sup>, Hassan Rouba<sup>1</sup>, Crystel Bonnet<sup>2,3</sup>, Christine Petit<sup>2,3,4,5</sup> and Abdelhamid Barakat<sup>1</sup>

Mutations in the *PEX1* gene are usually associated with recessive inherited diseases including Zellweger spectrum disorders. In this work, we identified a new pathogenic missense homozygous *PEX1* mutation (p.Leu1026Pro, c.3077T>C) in two Moroccan syndromic deaf siblings from consanguineous parents. This variation is located in the P-loop containing nucleoside triphosphate hydrolase of protein domain and probably causes an alteration in the hydrolysis of ATP.

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Peroxisomal biogenesis factor 1 (*PEX1*) gene, located on 7q21.2,<sup>1</sup> is the most involved gene associated with Zellweger spectrum disorders (ZSDs) accounting for nearly 70% of Zellweger syndrome (ZS) patients, showing the importance of the ATPase (Pex1p) encoded by this gene in peroxisome function.<sup>2</sup> Moreover, this hydrophilic protein plays an important role by interacting with Pex6p in an ATP-dependent manner forming a unique double-ring structure<sup>3</sup> that is responsible in the mobile cytosolic receptor Pex5p shuttling from the peroxisomal membrane to the cytosol via their recruiter, the Pex26p, at the terminal translocation machinery step.<sup>4</sup> Indeed, Pex1p is part of a group of 34 proteins named peroxins, which are involved in the biogenesis and in the maintenance of peroxisomes functions. These peroxins allow the importation of proteins and matrices into peroxisomes that harbor > 50 enzymatic activities essential in many metabolic processes such as catabolism of fatty acids and amino acids, detoxification of certain toxic compounds (glyoxylate and H<sub>2</sub>O<sub>2</sub>), and anabolism of bile acids and ether phospholipids like plasmalogens.<sup>5</sup>

Therefore, a change in one of the 15 human *PEX* genes is capable to alter the normal operation and/or peroxisome biogenesis, and generate severe metabolic abnormalities in many tissues engendering the onset of several autosomal recessive inherited diseases known as peroxisomal biogenesis disorders (PBDs).<sup>6</sup> These inborn errors of metabolism have an incidence of about 1/12,191 in Quebec, 1/50,000 newborn in North America and 1/500,000 in Japan.<sup>7–9</sup> Moreover, PBDs are highly clinically and genetically heterogeneous. They are subdivided in four groups with decreasing degree of severity and earliness relatively to the occurrence of death: group 1: ZS, the most severe neonatal form, death usually occurs in the first year; group 2: neonatal adrenoleukodystrophy (NALD), the infantile presentation, death often occurs in the first decade; group 3: infantile refsum disease, adult form, death occurs generally at the third decade; and recently, group 4: Heimler syndrome (HS), the less severe adult forms with prolonged survival.<sup>10</sup> The peroxisomals' total or partial functions loss explains the gradual onset of the disease. These variable phenotypes are included within the ZSDs clinically characterized by hypotonia, development delay, intellectual

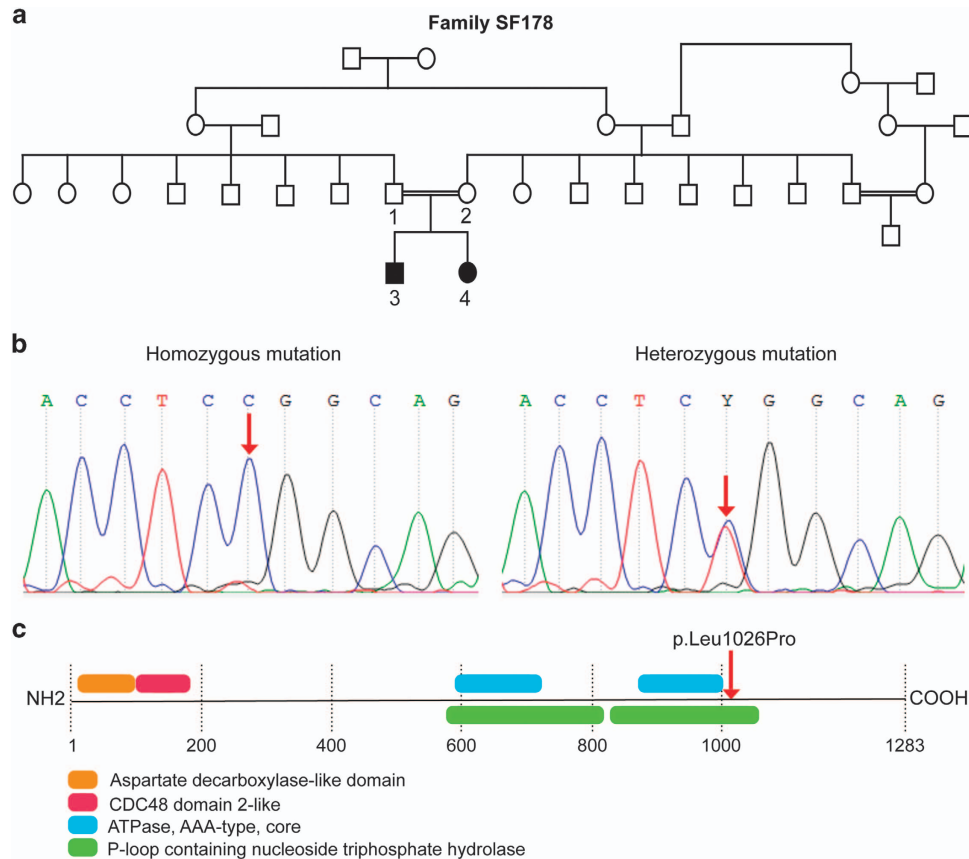
deficiency, craniofacial dysmorphism, hearing impairment, vision abnormalities, liver and renal dysfunction, and sometimes epileptic seizures.<sup>11,12</sup>

In this work, a novel homozygous *PEX1* mutation, the p.Leu1026Pro (c.3077T>C), was identified in two deaf siblings (6 and 12.5 years old) from a consanguineous Moroccan family. The personal interview with parents revealed for the older sibling—development delay, seizures, blindness occurring between 18 months and 2 years after birth, loss of walking at the age of 4 years, and he passed away during the study on 12 October 2015 following liver complications with jaundice and anemia. However, for his sister, a bilateral prelingual profound deafness was diagnosed at 2 years old by brainstem auditory-evoked potentials on top of very low vision, persistent nystagmus and a slight mental retardation. Her brain computed tomography (CT) performed at 9 months of age showed a bilateral frontoparietal and cortical atrophies, while the electroencephalography at 18 months indicated a normal recording with no electrical abnormalities. Her temporal bone CT and her cerebral magnetic resonance imaging performed at the age of 3 were found normal. The two siblings have no apparent craniofacial abnormalities and there is no information as to the degree of hypotonia. Additional clinical and laboratory tests are not available. An informed written consent was signed by parents of patients to perform genetic testing before taking the blood of all the family members. Genomic DNA extraction was obtained by using the standard phenol chloroform method. Using Sanger sequencing technique, the DNA of the younger affected child was tested negative for the mostly involved mutations in Moroccan family. Subsequently, the patient DNA was analyzed by whole-exome sequencing (WES) performed as previously described<sup>13</sup> with minor modifications, using the SureSelect V5 capture kit (Agilent, Santa Clara, CA, USA) and HiSeq 2000 sequencer (Illumina, Santa Clara, CA, USA). Bioinformatics analysis of sequence data was based on the pipeline provided by Illumina (CASAVA 1.8), CASAVA aligns reads to the human reference genome (hg19) and the algorithm ELANDv2e (Malony alignment and multiseed mismatch reducing artifact) was used for sequence

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**Figure 1.** Clinical and molecular findings in family SF178. (a) Pedigree of the family with two affected siblings. (b) Chromatogram of the homozygous mutation in SF178.03 and SF178.04, and the heterozygous mutation in their parents. (c) Schematic representation of the Pex1p structure showing the localization of a novel homozygous *PEX1* mutation, the p.Leu1026Pro (c.3077T>C).

alignment. Genetic variation was annotated with the IntegraGen (Evry, France) in-house pipeline. We achieved an average percentage of 98% of covered consensus coding DNA sequence at 4×, 96% at 10× and 89% at 25×, providing sufficient depth to analyze variants. Candidate pathogenic variants were defined as missense, nonsense, frameshift and splice site mutations with a minor allele frequency < 0.01, using the 1000 Genomes Project database and dbSNP132. Then the mutation identified was confirmed by Sanger sequencing to validate the family segregation. To predict the effect of amino acid substitution on protein function and structure, we used Polyphen2, and other prediction programs like SIFT, PROVEN, MUTATION TASTER, CUPSAT and MAESTRO web. Furthermore, the molecular modeling was performed to predict the structural impact of the amino acid substitution. The tridimensional structure of PEX1p was predicted using the protein homology modeling server SWISS-MODEL. FOLD-X software (Centre for Genomic Regulation, Barcelona, Spain) was used to generate mutated structure and for energy minimization. Protein structures were visualized using YASARA program.

Referring to the data filtering approach, we detected a novel causative missense variant in homozygous state in *PEX1* gene (Figure 1a). The mutation corresponds to a single base change c.3077T>C and causes a leucine to proline substitution at position 1026 of the protein. Sanger sequencing results confirmed segregation and revealed that the two parents are heterozygous, while the affected died brother carried the same homozygous variant (Figure 1b).

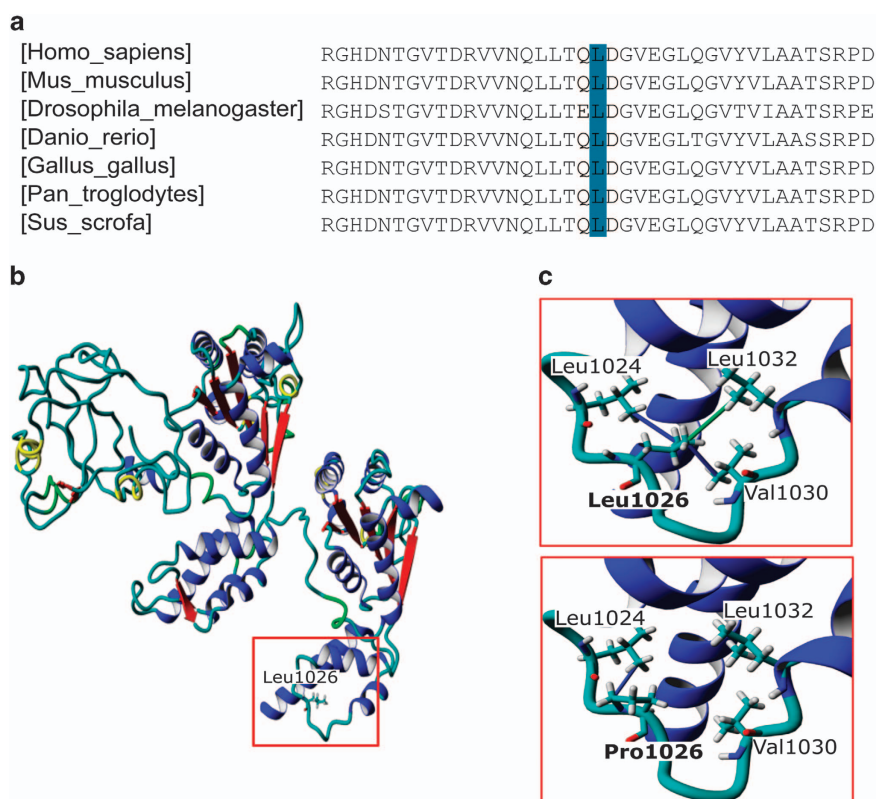
The p.Leu1026Pro is predicted to be probably damaging by Polyphen2 and SIFT programs. Using PROVEN and MUTATION TASTER, the variation was found respectively deleterious and

disease causing. The MAESTRO and CUPSAT tools predicted the amino acid change destabilizing this protein. A three-dimensional structure was constructed based on the crystal structure of the N-terminal domain of PEX1 AAA-ATPase (PDB ID: 1WLF) to evaluate the structural impact of the p.Leu1026Pro mutation (Figure 1c). The substitution of the highly conserved amino acid (leucine) (Figure 2a) may have led to loss of hydrogen bonds, thus altering the interactions between residues Leu1026 and Val1030. Moreover, it is likely to disrupt hydrophobic interactions between Leu1026 and its neighboring residue Leu1032 (Figure 2b,c).

The Pex1p contains two nucleotide-binding domains (D1 and D2) preceded by two N-terminal domains (N1 and N2).<sup>3,14</sup> The D2 domain is highly conserved and contains an essential substrate-binding loop, and two arginine finger residues probably participate in ATP hydrolysis and support oligomerization.<sup>14</sup> Thereby, the new variant p.Leu1026Pro probably leads to impaired ATP hydrolysis since it is located in P-loop containing nucleoside triphosphate hydrolase (Figure 1c).

Furthermore, the great diversity in ZSDs and the high number of genes involved makes their differentiation very difficult, and cannot be based solely on specialized biochemical tests or clinical presentation. It requires the use of powerful molecular tools such as WES for an accurate diagnosis, less expensive and faster than traditional sequencing techniques. It was only after the identification of the *PEX1* gene mutation from this family that the symptoms described clinically appear consistent with the milder form of the ZSDs, especially the NALD since the death of the affected boy was at the age of 12.5 years.

Until now, more than 114 variants in the *PEX1* gene have been identified in person suffering from ZSDs (<https://ghr.nlm.nih.gov/gene/PEX1#conditions>). The two most common *PEX1* gene



**Figure 2.** Structural and functional impacts of p.Leu1026Pro missense mutations predicted by molecular modeling and amino acid conservation. **(a)** Alignment of the amino acid sequences of Pex1 protein from different species. **(b)** Three-dimensional structure of the N-terminal domain of PEX1 AAA-ATPase. **(c)** Hydrogen bonds and hydrophobic interactions predicted by Yasara software. Blue lines represent hydrogen bonds and green lines represent hydrophobic interactions.

mutations are the p.Gly843Asp leading to the less severe form with long survivals<sup>15</sup> and the p.Pro970\* mutation engendering the severe form with shorter survival because of the formation of a nonfunctional Pex1p.<sup>16,17</sup> Effectively, Preuss *et al.*<sup>18</sup> found that missense mutations cause a milder phenotype than nonsense, insertion and deletion mutations. Likewise, Yik *et al.*<sup>19</sup> sequenced all *PEX1* coding regions and splice sites in a cohort of 58 ZSD patients. They identified 26 deleterious *PEX1* alleles including 12 new pathogenic mutations inducing a truncated protein and three novel missense variants affecting residues conserved in many species.<sup>19</sup> Similarly, in our case, the missense mutation identified was responsible for an intermediate severity phenotype: NALD. Contrariwise, in Morocco, the first two homozygous reported *PEX1* mutations were a nonsense variant p.Trp1250\* and a missense variant p.Leu1047Pro found in patients with the least severe forms: HS.<sup>10,20</sup>

In conclusion, this paper reports the case of one consanguineous Moroccan family having two affected individuals with many symptoms including profound deafness, impairment vision and development delay. The WES performed reveals a novel deleterious homozygous *PEX1* mutation consistent with the moderate form of ZSDs and NALD

#### HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.967>.

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#### COMPETING INTERESTS

The authors declare no conflict of interest.

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