DATA REPORT

A novel PEX1 mutation in a Moroccan family with Zellweger spectrum disorders

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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>G) 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.

Human Genome Variation (2017) 4, 17009; doi:10.1038/hgv.2017.9; published online 13 April 2017
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). 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. 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
mutations are the p.Gly843Asp leading to the less severe form with long survivals15 and the p.Pro970* mutation engendering the severe form with shorter survival because of the formation of a nonfunctional Pex1p.16,17 Effectively, Preuss et al.18 found that missense mutations cause a milder phenotype than nonsense, insertion and deletion mutations. Likewise, Yik et al.19 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.19 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.10,20

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.

ACKNOWLEDGEMENTS
We thank all the patients and their families for their collaboration. This study was approved by the Ethics Committee of the Morocco Pasteur Institute.

COMPETING INTERESTS
The authors declare no conflict of interest.

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