Evidence of Mutational Heterogeneity of the *ALDH7A1* Gene in Tunisian Families: Molecular and Insilico Investigations

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Abstract: Pyridoxine-dependent epilepsy (PDE) is a disorder of neonates or infants with autosomal recessive inheritance characterized by seizures, which responds to pharmacological dose of pyridoxine. Recently, the mutation p.Leu455Pro has been identified in the *ALDH7A1* gene in Tunisian families with PDE. We investigated two unrelated patients affected with PDE. Diagnosis as having PDE was confirmed by pyridoxine-withdrawal test. To confirm linkage to *ALDH7A1* gene, genotyping and direct sequencing of *ALDH7A1* gene were performed. Genotyping analysis revealed linkage with *ALDH7A1* locus and suggested the segregation of different mutations in these two affected families. Mutation screening revealed the presence of two mutations c.1279G>C (p.Glu427Gln) and c.1292C>T (p.Pro431Leu) in the *ALDH7A1* gene. The molecular modeling and bioinformatics studies of these two variations suggest that irregular structures of the mutated *ALDH7A1* proteins are likely to cause the functional defect that contributes to the pathology of PDE. On the other hand, our findings indicate an allelic heterogeneity of PDE in Tunisian population.

Key words: *ALDH7A1* gene, allelic heterogeneity, linkage analysis, mutation analysis, pyridoxine-dependent epilepsy (PDE), p.Glu427Gln and p.Pro431Leu mutations, molecular modelling.

1. Introduction

Pyridoxine dependent epilepsy (PDE) is a disorder of neonates or infants with autosomal recessive inheritance (MIM#266100) characterized by a combination of various seizure types. Affected individuals are unresponsive to standard anticonvulsants but they can be controlled by pharmacological doses of pyridoxine [1, 2]. In 2006, mutations in *ALDH7A1* gene have been identified to cause PDE [3]. The *ALDH7A1* gene has 53,550 bp, including 4953 bp of coding sequence, and spans 18 exons (GenBank NM_001201377.1). It encodes antiquitin (also known as *ALDH7A1*, α -AASA or ATQ), an α -aminoadipic-semialdehyde

dehydrogenase in the pipecolic acid pathway of lysine catabolism. This enzyme catalyzes the conversion of α -aminoadipic semialdehyde (α AASA) to α -aminoadipic acid. The reaction requires NAD⁺ as cofactor [4]. Antiquitin deficiency causes epilepsies, because it leads to severe secondary deficiency of pyridoxal 5'-phosphate, which is an essential enzyme cofactor in the metabolism of several amino acids and neurotransmitters [3, 5].

Up to now, more than 70 *ALDH7A1* different pathogenic mutations within the 18 exons of the *ALDH7A1* gene have been reported in the literature associated with PDE phenotypic spectrum in patients from diverse ethnic backgrounds [6]. The majorities of *ALDH7A1* mutations are single-base changes with 38 are missense, 7 are nonsense, and 15 are splice site

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mutations. Two previous studies on seven Tunisian families affected with PDE, revealed the presence of a common founder missense mutation c.1364T>C (p.Leu455Pro) [7].

In this study, we have enlarged the mutational spectrum of the *ALDH7A1* gene in the Tunisian population, with the identification of two additional gene alterations. We also performed an in silico investigation to predict the potential effect of the identified mutations on structure of the mutated proteins.

2. Experimental Setup

2.1 Patients

In this study we investigated two Tunisian consanguineous families transmitting an autosomal recessive PDE (Fig. 1).

The two patients were suspected to be affected with PDE on the basis of seizures resistant to anticonvulsants, good response to pyridoxine and pyridoxine withdrawal test.

Peripheral blood samples were collected from unaffected parents and all families' members after informed consent obtained in accordance with the ethics committee of Hedi Chaker Hospital (Sfax, Tunisia).

2.2 DNA Extraction

DNA extraction was performed following standard phenol-chloroform method [8].

2.3 Microsatellite Typing

For the genotypic analysis, we screened the microsatellite marker D5S2059, located approximately 1 megabase upstream of the *ALDH7A1* gene. We used the True Allele PCR Premix (Applied Biosystems, Foster City, CA) in the presence of 2.5 pmol of 5-propargylamino-dCTP-6-FAM (Jena Bioscience) for PCR reactions. Fluorescently-labeled alleles were analyzed on an ABI PRISM 3100-Avant automated Genetic Analyser (Applied Biosystems, Foster City, CA). Genotypes were determined using the Gene

Mapper software (Applied Biosystems, Foster City, CA).

*2.4 Mutation Analysis of *ALDH7A1* Gene*

The 18 exons and flanking intron regions of the *ALDH7A1* gene were tested for mutations in the PDE patients by sequence analysis. The coding regions and intron-exon boundaries were amplified using specific primers. PCR products were purified and sequenced as previously described [6].

The sequence alignment and the pathogenicity prediction

The sequence alignment of the *ALDH7A1* gene was performed using the Clustal W program (<http://www.ebi.ac.uk/Tools/msa/clustalw2>). To predict whether an amino acid substitution affects the *ALDH7A1* protein function SIFT (<http://sift.jcvi.org/>) and PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>) were used.

*2.5 Molecular Modeling of *ALDH7A1* Protein*

To investigate the eventual effect of the *ALDH7A1* mutations, we modeled and compared the wild type and mutated proteins. Protein crystal structures were taken from (PDB) by 3D-JIGSAW. The models were evaluated on the basis of geometrical and stereo-chemical constraints using: ProSA-Web (<https://prosa.services.came.sbg.ac.at/prosa.php>) and Verify 3D (http://nihserver.mbi.ucla.edu/Verify_3D/). Deep View/Swiss-PDB viewer 3.7 was used to display and compare models.

3. Experimental Results

Two Tunisian unrelated consanguineous families including two patients with PDE, were investigated (Fig. 1). We first screened in the two studied patients the founder mutation c.1364T>C previously described in the Tunisian population. Since this gene alteration was absent in the patients, linkage analysis to the *ALDH7A1* gene was performed. Six individuals belonging to the two studied families were genotyped using the informative microsatellite marker D5S2059. Genotyping analysis revealed that in the first family,

Fig. 1 Genotyping and sequencing analysis in the two affected families. A) c.1279G normal allele; B) c.1279 C mutant allele; C) c.1292 C > T normal allele and D) c.1292 T mutant allele.

the 183 bp allele of D5S2059 marker segregates with the disease whereas in the second family, segregation was found with another allele of 191 bp (Fig. 1). Result of genotyping analysis suggested the presence of two different mutations in the tested families. So, mutation screening of all *ALDH7A1* coding region and intron-exon boundaries was performed and revealed the segregation of the c.1279G>C mutation in first family and the segregation of the c.1292C>T mutation in the second family (Fig. 1). We found that parents were carriers, whereas the variations c.1279G>C and c.1292 C>T were not found in 100 control alleles.

To predict whether these two mutations (Glu427Gln) and (Pro431Leu) could potentially affect the protein function, Polyphen-2 and SIFT were used. As a result, we found that these amino acids are highly conserved and their substitutions are predicted to be “damaging” (data not shown).

The Glu427Gln and Pro431Leu mutations were also modeled using Deep View/Swiss-PDB viewer 3.7 software. The comparison between the normal and the mutant structures showed that in both situations the root mean squares (RMS) were very significant (19.11 Å and 18.61 Å, respectively). Interestingly, we noticed

Fig. 2 Effect of Gln427Glu (a) and Pro 431Leu (b) mutations on ALDH7A1 protein structure as shown by “Deep View/Swiss-PDB viewer 3.7” software. Both mutations resulted in gain of a hydrogen band between Thr428 and Ala430, causing conformational change that negatively affect cofactor binding.

also that these two substitutions add one hydrogen bond between (Thr428-Ala430) (Fig. 2). These findings suggest that the overall architectures of the mutant proteins were different to that of wild-type protein.

4. Discussion

We report two Tunisian consanguineous families affected with PDE. The affected patients had an early diagnosis of PDE and received appropriate pyridoxine therapy. In 2000, Cormier et al. localized a PDE disease causing gene to chromosome 5 locus q31.2 [9]. In 2006, Mills et al. identified the *ALDH7A1* gene as responsible for PDE since they detected mutations in affected PDE persons [3]. So far, several mutations in the antiquitin (*ALDH7A1*) gene have been described [6]. In the absence of the unique mutation c.1364T>C, previously reported in the Tunisian population [6], genotyping analysis showed that both patients are homozygous for two different alleles of the closest marker to *ALDH7A1* gene, D5S2059. Sequencing and mutation analysis confirmed the presence of the two different mutations, c.1279G>C and c.1292 C>T. The

first mutation, c.1279G>C has been reported to be prevalent in Caucasian patients [5], it substitutes the Glutamic amino acid at position 427 to a Glutamin residu. Whereas, the c.1292C>T transition was reported once, in a heterozygous form in trans with an intronic splicing mutation [10]. It is a missense mutation which substitutes a Proline at position 431 to a Leucine residu. Both Sift and Polyphen predict that these mutations showed to have a damaging effect on the protein function. Interestingly, modeling results showed that these two different mutations are predicted to have the same impact. In fact, both Glu427Gln and Pro431Leu mutations induce the addition of one hydrogen bond between amino acids Thr428 and Ala430. This extra-bond makes the surrounding region tighter, which might affect the shape and the conformation of the corresponding domain. Previous studies suggested that these two amino acids are located in the NAD⁺ cofactor binding site [11]. The interaction between this site and the NAD⁺ cofactor is crucial to make the substrate site accessible and allows formation of the ternary enzyme-cofactor (NAD⁺)-substrate (α AASA)

complex [12]. Consequently, it is possible that the mutated proteins in our situations are incapable of interacting with the NAD⁺ cofactor. If so, these mutations may cause a defect in substrate recognition, which would impact catalytic function.

5. Conclusions

Our result takes the number of published disease causing mutations in *ALDH7A1* gene in the Tunisian population to three and confirmed the mutational heterogeneity of PDE in Tunisia. In addition, our findings suggest that the Glu427Gln and Pro431Leu mutations might indirectly affect the *ALDH7A1* catalytic function by reducing or inhibiting the substrate (α AASA) - enzyme (*ALDH7A1*) interaction.

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