Clinica Chimica Acta 453 (2016) 141–146

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Phenotypic variability in a Tunisian family with X-linked adrenoleukodystrophy caused by the p.Gln316Pro novel mutation

Fakhri Kallabi [a,*], Emna Ellouz [b], Mouna Tabebi [a], Ghada Ben Salah [c], Naziha Kaabechi [d], Leila Keskes [a], Chahnez Triki [b], Hassen Kamoun [a,e,*]

[a] Laboratory of Human Molecular Genetics, Faculty of Medicine of Sfax, University of Sfax, Tunisia
[b] Service of Neuropaediatrics, C.H.U. Hedi Chaker, Sfax, Tunisia
[c] Uniazah Pharmacy College, Qassim University, Alqassim, Saudi Arabia
[d] Service of biochemistry, C.H.U. La Rabta, Tunis, Tunisia
[e] Service of Medical Genetics, C.H.U Hedi Chaker, Sfax, Tunisia

ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form 10 December 2015
Accepted 11 December 2015
Available online 12 December 2015

Keywords:
ABCD1 gene
Addison disease
Childhood cerebral ALD
Novel mutation
Phenotypic variability
VLCFA
X-linked adrenoleukodystrophy

ABSTRACT

Introduction: X-linked adrenoleukodystrophy is a neurodegenerative recessive disorder that affects the brain white matter and associated with adrenal insufficiency. It is characterized by an abnormal function of the peroxisomes, which leads to an accumulation of the Very Long Chain Fatty Acids (VLCFA) in plasma and tissues, especially in the cortex of the adrenal glands and the white matter of the central nervous system. Mutations in the *ABCD1* gene affect the function of the encoded protein ALDP, an ATP-binding cassette transporter located in the peroxisomal membrane protein.
Patients and methods: The present study reports the clinical, biochemical and molecular investigation in a Tunisian family with two affected males with childhood cerebral adrenoleukodystrophy.
Results: The *ABCD1* gene sequencing indicated a novel hemizygous missense mutation c.947A > C (p.Gln316Pro) in the exon 2 of the *ABCD1* gene in the patients, their mother and their sisters. This missense variation was predicted to be possibly damaging by the PolyPhen and SIFT prediction software. Although presence of the same mutation c.947A > C in both siblings, they present different clinical signs.
Conclusions: Based on the disease's progress, the clinical signs and biochemical aspects between the two siblings, we demonstrate that there is no correlation genotype–phenotype.

1. Introduction

X-linked adrenoleukodystrophy (X-ALD — MIM #300100) is a peroxisomal, progressive and neurodegenerative disorder that affects the cortex of adrenal glands and the white matter of the nervous system [1]. Biochemically, this disease is characterized by the accumulation of the Very Long Chain Fatty Acids (VLCFA) in the tissues and the body fluids [2,3]. It is characterized by an abnormal function of the peroxisomes, which leads to an accumulation of the VLCFA in plasma and tissues, especially in the cortex of the adrenal glands and the white matter of the central nervous system, causing demyelinating disease and adrenocortical insufficiency [2]. Several phenotypes are recognized in males according to the age of onset, affected organs and rate of the progression of neurologic symptoms. The X-ALD clinical spectrum ranges from the rapidly progressive childhood cerebral form (ccALD), which typically leads to severe disability and death during the first decade, to the milder adrenomyeloneuropathy (AMN) that usually manifests between the ages of 20 and 30 and may be compatible with survival into the eighth decade to pure Addison's disease [4]. The major phenotypes include ccALD, AMN, adolescent cerebral adrenoleukodystrophy, Addison only, olivo-ponto-cerebellar, and asymptomatic [3,5]. Cerebral adrenoleukodystrophy results from inflammatory demyelination of the brain whereas AMN results from a noninflammatory demyelination of the spinal cord and peripheral nerves [3]. The disease most commonly affects boys during early childhood, but between 20% and 50% of female carriers may also present with late-onset phenotypes similar to AMN, and rarely with adrenal insufficiency [6]. This disease is related to mutations in the ATP-binding cassette, subfamily D, member 1 gene (*ABCD1* — MIM #300371) located on chromosome Xq28 and consisting on 10 exons [7]. It encodes adrenoleukodystrophy protein (ALDP) which is a transporter protein of the adenosine triphosphate-binding cassette family [8], and consisting of 745 amino acids. The ALDP protein is located in the peroxisome membrane and consists of two important domains: the transmembrane domain (TMD) and ATP-binding cassette domain (ABC) [9].

The most asked question in the genetic diseases it has a correlation between genotype and phenotype. In the monogenetic and

* Corresponding authors at: Laboratoire de Génétique Moléculaire Humaine, Faculté de Médecine de Sfax, Avenue Majida Boulila. 3029 Sfax, Tunisia.
E-mail addresses: fakhrikallabi@yahoo.fr (F. Kallabi), hassen.kamoun@yahoo.fr (H. Kamoun).

http://dx.doi.org/10.1016/j.cca.2015.12.014
0009-8981/

multifactorial diseases, the environmental factors and modifying genes appear to determine the clinical manifestation.

In the present report, we describe the clinical and genetic analyzes of two siblings belonging to a Tunisian family affected by ccALD form of X-ALD. The two brothers have different clinical signs: before this case, what can we say about the correlation genotype_phenotype?

2. Patients and methods

2.1. Patients

Two-affected boy belonging to a Tunisian family was born to unrelated parents. Two additional healthy siblings and the parents were also recruited. Informed consent was obtained from patients and control individuals in accordance with the ethics committee of CHU Hedi Chaker Hospital (Sfax, Tunisia). The diagnosis of X-ALD was made on the basis of clinical manifestation, the biochemical values, the cerebral magnetic resonance imaging (MRI). The extent and severity of cerebral demyelination was determined by T2 MRI analysis and graded per the method described by Loes [10].

2.2. Genomic DNA extraction

Blood samples (10 mL) were collected from six family members and healthy individuals. Genomic DNA was extracted from the whole blood following a standard phenol–chloroform method [11].

2.3. PCR and sequencing

In total, 10 PCR amplifications were performed to obtain complete coverage of all coding regions and exon–intron junctions of the *ABCD1* gene using primers as published by Boehm et al. [12]. All exons were amplified in a thermal cycler (Applied Biosystem 2720) in a final volume of 50 μl containing 100 ng of genomic DNA, 0.2 μM of each primer, 1× PCR buffer (Promega), 1.2 mM $MgCl_2$, 0.2 mM each dNTP, and 1 U Taq DNA polymerase (Promega). The polymerase chain reaction conditions were as follows: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 63–71 °C (depending upon the primer sets used) for 30 s and extension at 72 °C for 45 s, and final extension at 72 °C for 10 min. Each PCR product was then purified by enzyme reaction (Exonucléase I; 20 units/μl; Fermentas), and directly sequenced using a Big-Dye di-deoxy-terminator cycle sequencing kit and an ABI-PRISM 3100 automated sequencer (Applied Biosystems).

2.4. Mutation screening and PCR- restriction fragment length polymorphism (RFLP)

Sequence analysis of the *ABCD1* mutant allele in the two sibling revealed a novel mutation c.947A > C predicting the substitution Q316P. Since the mutation c.947A > C has no RFLP associated, a restriction site-generating PCR strategy was employed using ALDe2-F primer modified. The modified forward primer (5′CTACCAGGACCTGGCCTCTC3′) generates a specific restriction site for the enzyme DdeI for the wild type allele. This novel variation abolished a DdeI restriction site and the mutant allele was distinguishable by PCR-RFLP. Restriction enzyme digestions were performed using 10 μl of PCR products of exon 2 and were incubated at 37 °C overnight with 10 units of DdeI restriction enzyme (Fermentas, EU). The restriction fragments were separated by 3% agarose gel electrophoresis stained with ethidium bromide.

2.5. Controls

To confirm that alteration found in this work is novel mutation and not merely polymorphism, 100 Tunisian healthy individuals from the same ethno cultural group were tested as controls. These controls did not have any personal or family history of adrenoleukodystrophy or any other disorder. These controls were screened using PCR-RFLP analysis and direct sequencing. All individuals (patient and controls) signed informed consent.

2.6. Bioinformatic tools

The access to *ABCD1* gene was performed using Ensembl (http://www.ensembl.org) or GenBank Database (www.ncbi.nlm.nih.gov/gene/). In addition, there is an adrenoleukodystrophy database, which contains all news of this disease (http://www.x-ald.nl). A blast homology search was performed using the progam BLAST2SEQ available at the National Center for Biotechnology Information Website (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq). The multiple alignment of the ALDP peptide sequences were performed using the ClustalW program (http://www.ebi.ac.uk/Tools/msa/clustalw2/), and the sequences of the different species were obtained from the NCBI database. The choice of restriction enzyme was performed using the program NEBcutter (http://nc2.neb.com/NEBcutter2/).

To predict the functional consequences of the tested variants, we used two computational analysis softwares: SIFT and PolyPhen-2. The Sorting Intolerant from Tolerant tool (http://sift.jcvi.org/) predicts whether an amino acid substitution affects protein function. A SIFT score with normalized probabilities less than 0.05 are predicted to be deleterious, those greater than or equal to 0.05 are predicted to be tolerated [13]. The Polymorphism Phenotyping v2 (http://genetics.bwh.harvard.edu/pph2/) is a software tool which predicts possible impact of amino acid substitutions on the structure and function. The PolyPhen score represents the probability that a substitution is damaging, so values nearer 1 are more confidently predicted to be deleterious [14].

Table 1
Difference of clinical signs between the two siblings.

	Sibling II.2	Sibling II.3
Age (years)	12	Died at the age of 7 years and 9 months
Age of onset of the disease (years)	7	6
Clinical signs	–Decline in school performance –Gait difficulties –Decrease in visual and auditory acuity –Progressive cognitive decline –Weakness of the 4 limbs –Motor axonal neuropathy	–Decrease in visual and auditory acuity –Focal epilepsy –Progressive cognitive decline –Language regression –Behavior disturbance –Spasticity in the lower limbs and patient become wheelchair –Died after 9 months
MRI	–Hyper signal on T2 weighted images involving the pyramidal tract in the brain stem and the internal capsule	–Peri ventricular demyelinating leukodystrophy especially in the posterior area with peripheral gadolinium enhancing