Identification of a novel ABCD1 mutation in a family with Adrenoleukodistrophy

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Introduction

X-linked adrenoleukodistrophy (X-ALD) is the most common peroxisomal disorder. The disease is caused by mutations in the ABCD1 gene that encodes the peroxisomal membrane protein ALDP which is involved in the transmembrane transport of very long-chain fatty acids (VLCFA; >22). A defect in ALDP results in elevated levels of VLCFA in plasma and tissues. The clinical spectrum in males with X-ALD ranges from isolated adrenocortical insufficiency and slowly progressive myelopathy to devastating cerebral demyelination. The majority of heterozygous females will develop symptoms by the age of 60 years. We describe a family with six members carrying a novel heterozygous mutation IVS4+2T>A (c.1393+2T>A) of the ABCD1 gene highlighting the wide range of phenotypic manifestations of ALD.

Cases Presentation

Patient IV:3. In 2007, this 43-year-old patient developed progressive gait disturbance due to weakness of the extremities and dysarthria. Neurological examinations revealed a spastic paraparetic gait, bilaterally hyperreflexia of knee, ankle jerks, Hoffman and Babinki signs. Over the years, several brain and cervico-dorsal magnetic resonance were normal. In 2015 brain MRI become positive for ALD. Cerebrospinal fluid studies were also performed and it was within normal limits. The laboratory tests revealed normal serum cortisol but elevated adrenocorticotropic hormone, 426.2 pg/mL (normal 7.2-63.3). The 1 ug-synacthen test showed a peak cortisol response, confirming primary partial adrenal insufficiency. The fasting plasma levels of VLCFA, measured with gas chromatography-mass spectrometry, were: C22:0, 23.77 umol/L (normal 17.00-72.00), C24:0, 47.66 umol/L (normal 12.00-62.00); and C26:0 2.13 umol/L (normal 0.25-0.65). His C24:0/C22:0 and C26:0/C22:0 were significantly elevated at 2.01 (normal 0.50-1.10) and 0.09 (normal 0.01-0.03), respectively.

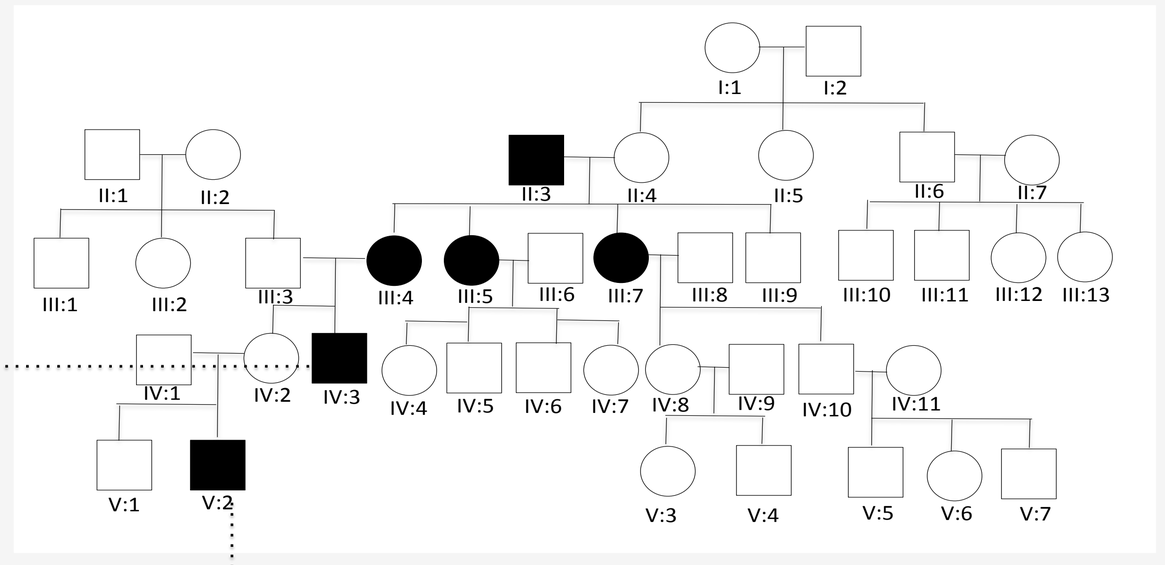

Fig. 1 *The cross inheritance of recessive X-linked adrenoleukodistrophy alleles in consanguineous pedigree*

Patient V:2. In 2015, this 9-year-old child referred to the pediatric endocrine clinic for obesity and raised TSH, his neurological examination was normal. Brain MRI revealed an unequivocal pattern of ALD. The 1 ug-synacthen test detected primary adrenal insufficiency. The fasting plasma levels of VLCFA were: C26:0 1.41 umol/L (normal 0.25-0.65). His C24:0/C22:0 and C26:0/C22:0 were 0.99 (normal 0.50-1.10) and 0.07 (normal 0.01-0.03), respectively.

Methods

Genomic DNA was extracted from peripheral whole blood samples collected from the patient and his family. All samples were taken after informed consent was obtained. The splice junctions and all coding regions of the ABCD1 gene were amplified by polymerase chain reaction using specific primers. The PCR products were directly sequenced using capillary electrophoresis sequencing ABI Prism 3130 XL.

Results

We obtained two products in the affected male (TM) and his family (PE) (Fig. 3): a low molecular weight product (estimated length c.a. 240 bp), which is also present in the normal control (SM), and a band of approximately 280 bp which was only present in the proband (TM) and his family (PE). The 280 bp fragment was cloned and sequenced and we identified a deletion of 121 bp encompassing part of exon 4 and the first two nucleotides of exon 5 (Fig. 4). The aberrant splicing results in a frame-shift in the coding sequence leading to a premature stop codon. This deletion should lead to the synthesis of a shorter protein (from 745 to 516 aa) lacking most of the ABC transporter domain (located from aa 474 to aa 700).

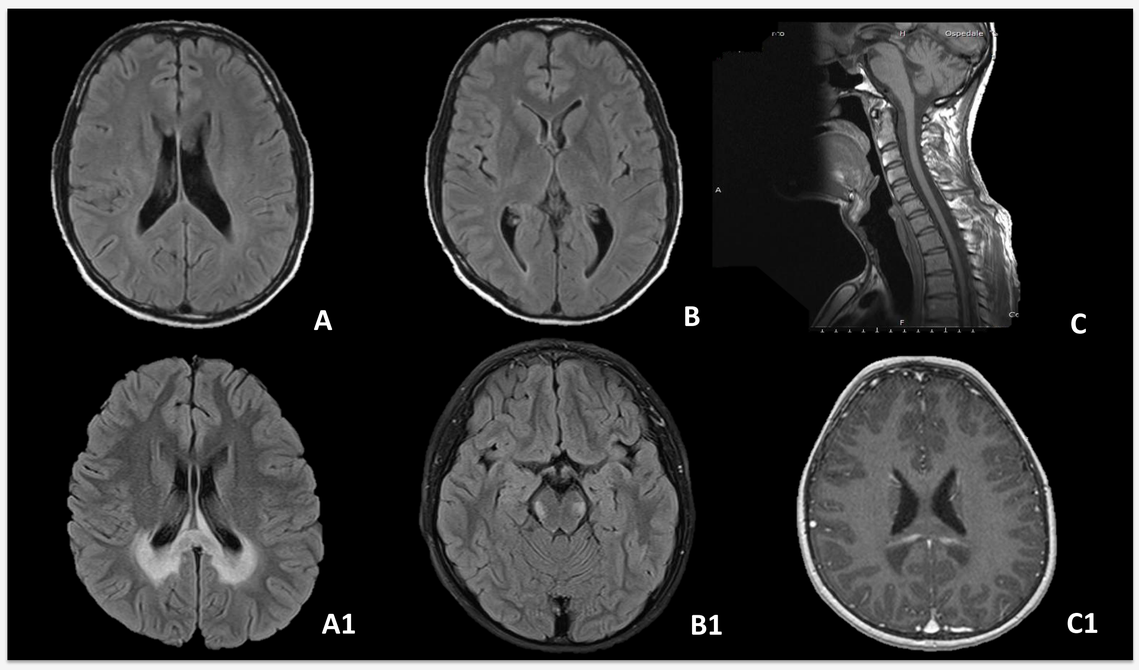

Fig.2 *Patient IV:3. Brain and cervico-dorsal MRI (A, B, C). Patient V:2. Brain MRI shows the typical pattern of ALD (A1, B1, C1).*

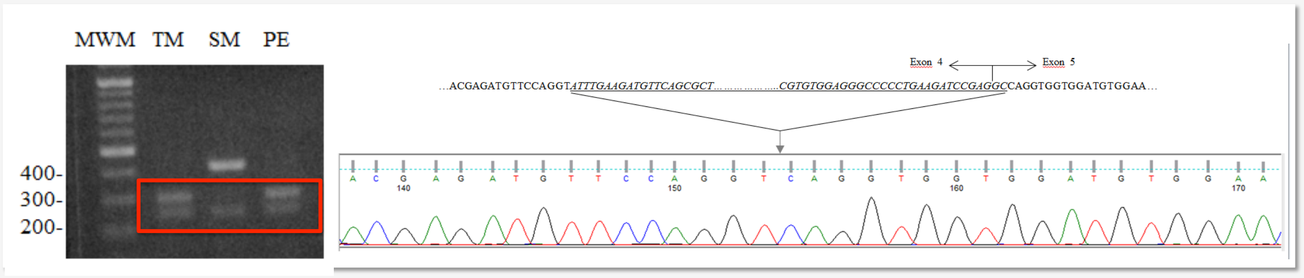

Fig. 3. *RT-PCR.*
Fig. 4. *Sequence analysis of cloned RT-PCR of the affected male. Sequence chromatogram showing a partial deletion of exon 4 and 5. The sequence corresponding to the normal splicing event is reported in the upper part of the figure. The underline italic sequence is absent in the patient's mature transcript.*

Discussion

This is the first published report of the IVS4+2 T>A mutation in intron 4 of the ABCD1 gene. Although there had been no systematic study conducted to support the predicted structure and function of ALDP, previous study showed that missense mutations in ABCD1 leading to decrease in ALDP levels may interfere with the peroxisomal targeting mechanism of the newly synthesized ALDP molecules, their correct membrane insertion and folding. It is plausible that our mutation, similar to other truncating mutations in ABCD1, can result to the same defects of peroxisomal targeting as reported. We showed that, within individual kindreds with the same mutation, different phenotypes of the entire clinical and radiological spectrum of X-ALD since asymptomatic patient, could be detected. Thus, early diagnosis is essential, since in some cases treatment is available, such as allogenic heterogeneous hallograft transplantation in the early stage and endocrine replacement therapy for adenocrotical insufficiency.