

# Differing clinical presentations of two unrelated cases of X-linked adrenoleukodystrophy with identical mutation Y296C in the ABCD1 gene

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## Abstract

**OBJECTIVES:** X-linked adrenoleukodystrophy is a genetically determined disorder that causes varying degrees of malfunction of the adrenal cortex and central nervous system. Our aim was to investigate the occurrence of known, or new, mutations in the ABCD1 gene in two unrelated patients with clinical suspicion of the adrenoleukodystrophy.

**METHODS:** Two unrelated patients – the first with behavioral changes, the second with progressive cognitive deficit – underwent a clinical and genetic examination in order to establish a diagnosis and discover a possible mutation.

**RESULTS:** In the first patient, a 47 year old man, the clinical examination showed dementia of the frontal type and spastic quadriparesis. The patient also suffered from adrenal insufficiency for 6 years. An MRI showed confluent hyperintensive lesions in FLAIR images in the frontal lobe of both hemispheres. The second patient, a 16 year old boy, suffered also from Addison's disease since the age of 9, and developed cognitive deficit in the course of one year. The MRI showed posterior atrophy and hyperintensive lesions in parietal and occipital lobes in T2WI. In both cases, genetic analyses showed a missense mutation at the codon 887 (A>G) in exon 1 of the ABCD1 gene, predicting the substitution Y296C in the ALD protein.

**CONCLUSION:** We detected the same mutation of the ABCD1 gene in two unrelated patients with ALD. In the first case there was frontal lobe involvement, in the second case parieto-occipital involvement. Both pathologic involvement and clinical presentation differed in two cases of the same mutation.

## Abbreviations:

X-ALD - X-linked adrenoleukodystrophy

VLCFAs - very long chain fatty acids

ABCD1 - adenosine triphosphate-binding cassette D1

ALDP - adrenoleukodystrophy protein

ACALD - adult onset cerebral form of X-ALD

AdoICALD - adolescent cerebral form of X-ALD

CCALD - childhood cerebral form of X-ALD

## INTRODUCTION

X-linked adrenoleukodystrophy (X-ALD) is a severe, inherited neurodegenerative peroxisomal disorder with a wide spectrum of phenotypes. It is characterized by progressive demyelination, adrenocortical insufficiency and accumulation of saturated, very long chain fatty acids (VLCFAs) in tissues and body fluids, due to the impairment of peroxisomal  $\beta$ -oxidation. The incidence of males with X-ALD is estimated at approximately 1:8500 of the total population, and appears to be the same in most ethnic groups (Moser *et al.* 2007). The gene responsible for X-ALD, *ABCD1*, has been mapped on chromosome Xq28. It comprises 10 exons spanning approximately 21kb of genomic DNA. It encodes for ALDP (adrenoleukodystrophy protein) a 75-kDa peroxisomal membrane protein that belongs to the ATP-binding cassette half-transporters superfamily. More than 700 different mutations have been reported (<http://www.x-ald.nl>) but no correlation between genotype and phenotype has been found (Mosser *et al.* 2007). The probable function of ALDP is the transport of the very long chain acyl-CoA synthase (VLCs) through a membrane of peroxisome to the peroxisomal matrix (Mosser *et al.* 1993). When the ALDP is defective, the metabolism of VLCFAs is impaired, particularly hexacosanoate (C26:0), which accumulates in brain lipids, mainly in cholesterol esters and gangliosides. There is a postulate that lipids containing an abnormally high proportion of

VLCFAs can act as triggers that initiate the cascade of inflammatory demyelination (Beutler *et al.* 1988, Moser *et al.* 2007).

X-linked adrenoleukodystrophy has various phenotypic presentations. Children most often develop a progressive cerebral disease rapidly; whereas adults most often have adrenomyeloneuropathy. These two phenotypes account for more than 75% of all X-ALD cases. Adult cerebral disease is the least frequent phenotype, accounting for only 2–3% of X-ALD cases. The phenotypic presentation of the adult onset cerebral form of X-ALD (ACALD) is characterized mainly by a gradual development of global cognitive deficit psychiatric symptomatology, such as euphoria, carelessness, emotional imbalance, and memory disturbance, and has a progressive course. In the course of the disease motoric symptoms were presented, such as pyramidal lesion or aphasia affiliate (Moser *et al.* 2001).

## METHODS

### Neuropsychological tests used

**Patient 1:** Mini Mental State Examination (MMSE), ADAS-Cog, Frontal Assessment Battery (FAB test), **Patient 2:** Raven Progressive Matricess, Rey-Osterrieth complex figure test, Trial making test.

### Magnetic resonance

The MRI equipment used operated at 3 T.

### Biochemical analysis of VLCFAs

All serum lipid fractions were extracted by dichloromethane/methanol and derivatised with acetylchloride (Lepage and Roy 1986). Methyl esters analysis was performed by Gas Chromatography-Mass Spectrometry (ITQ900, Thermo, USA).

### Analysis of the *ABCD1* gene

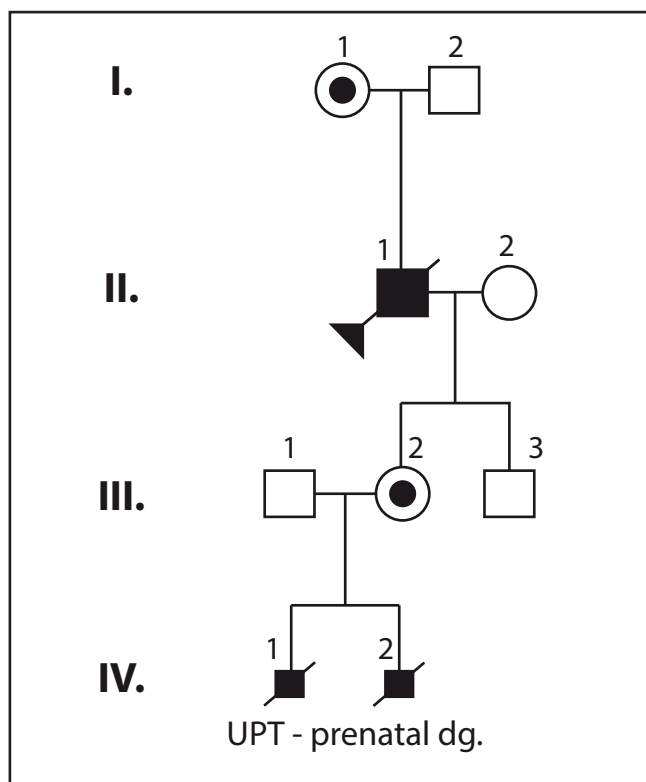
Genomic DNA was extracted from peripheral blood using the QIAamp blood kit (Qiagen, Germany) in accordance with the manufacturer's protocol. Exon 1 was amplified by polymerase chain reaction using primer 5' CCACGCCTACCGCCTCTACTT and 5' AGACTGTCCCCACCGCTC. The PCR products were directly sequenced using the Big-Dye™ Terminator cycle sequencing kit (Life Technologies, USA).

## RESULTS

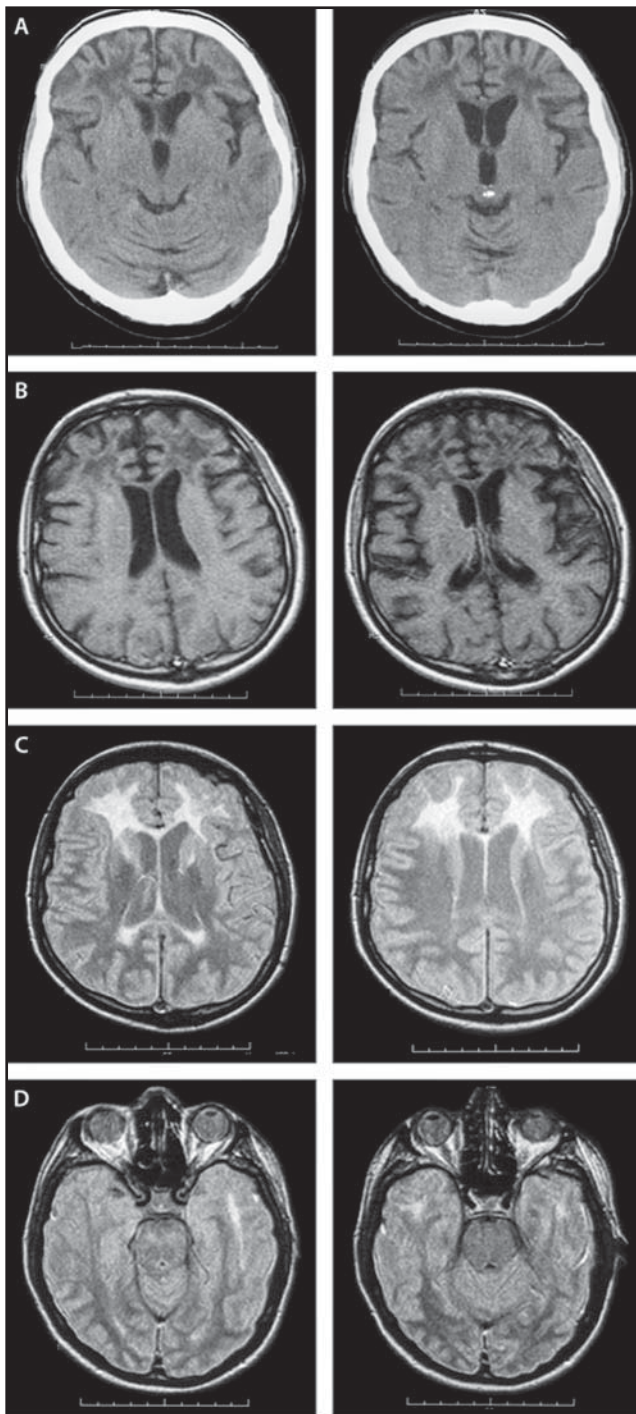
### First case

**Case description (Figure 1, case IV-2):** A 47 year-old man with a gradual development of psychiatric symptomatology with prefrontal syndrome, stepwise deterioration of the intellect and affiliated motoric symptoms with genetically confirmed X-ALD, with a known mutation Y296C in the *ABCD1* gene.

The patient was an only child. No ancestor on his mother's side is known to have had a similar disease.

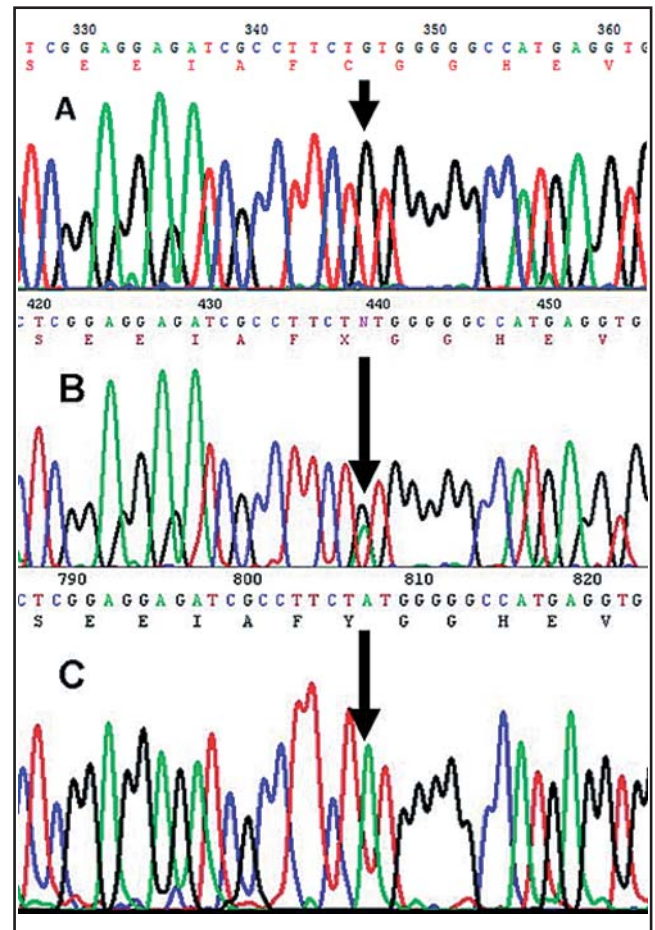


**Fig. 1.** Pedigree of the family. The index case is marked with an arrow.



**Fig. 2 Proband, (case IV-2)** (A). CT scan of the brain revealed mild frontal atrophy and hypodense signal in frontal lobe B,C,D. Transverse FLAIR MRI series (1 year after first clinical symptoms) showed confluent lesions in both frontal lobes, hypointense in T1WI (B) and hyperintense in FLAIR, representing severe demyelination including U fibers. Crus anterior of capsula interna also affected with demyelination (C). Incipient demyelination is also seen in both temporal lobes (D).

Neither his mother nor his daughter show clinical signs of CNS or PNS impairment. At the age of 45 behavioral changes developed, including carelessness, euphoria, problems keeping a job, and memory disturbances. These problems progressed during the four-



**Fig. 3** Sequence determination of c.887A>G, (Tyr296Cys) mutation of the *ABCD1* gene. The arrow indicates the involved nucleotide. (A) proband mutation in hemizygous state, (B) mother mutation in heterozygous state, (C) healthy control without mutation

teen months before he was admitted to our clinic. A neurological examination revealed an elevated mood, disorientation in time and space, confabulation, loss of self-insight, mild spasticity, slightly elevated tendon reflexes, the Babinski sign, no paresis, and no neuropathy on EMG. Neuropsychological assessment showed a generalized intellectual loss with impairment of language and memory, and psychological signs of prefrontal syndrome: the MMSE was 14; ADAS Cog was 27; the FAB test sensitive to frontal lobe functions was 9 points. Brain imaging showed extensive white matter lesions in both frontal lobes with a low density signal in CT. An MRI confirmed confluent hyperintense lesions in T2WI and FLAIR in the frontal lobe of both hemispheres, and also in the crus anterior of the internal capsule. U fibers were also involved, Loes score was 9, (Figure 2). Analysis of the X-ADL gene (*ABCD1*) showed a missense mutation at position 887 (c.887A>G) in exon 1, predicting the substitution Y296C in the ALD protein (Figure 3). Due to stage of the disease and the poor prognosis, the patient was not considered for blood marrow transplantation. He deteriorated gradually, developing severe dementia, apathy,

urinary incontinence and upper motor neuron signs. The patient died two years after the first clinical symptoms appeared.

**Other cases:** The same mutation was found in the patient's mother (Figure 1, I.1). She also has a slightly elevated level of VLCFAs (Tab.1). However, her cognitive functions have been preserved and there have been no symptoms of frontal lobe impairment or focal neurological signs: the MMSE was 27; ADAS Cog 8; the FAB test 16 points. The patient's daughter also carries this mutation (Figure 1, III.2). She also has an elevated level of VLCFAs (Tab.1). She completed university education, and does not show any signs of intellectual or other neurologic impairment. She has been pregnant twice, and in both gravidities the prenatal diagnostics revealed a male gender with mutation inheritance. In each instance she subsequently underwent an abortion (Figure 1, IV.1, IV.2).

### Second case

**Case description (Figure 4, case II-1):** A 16 year old boy who was in endocrinology care since the age of 9

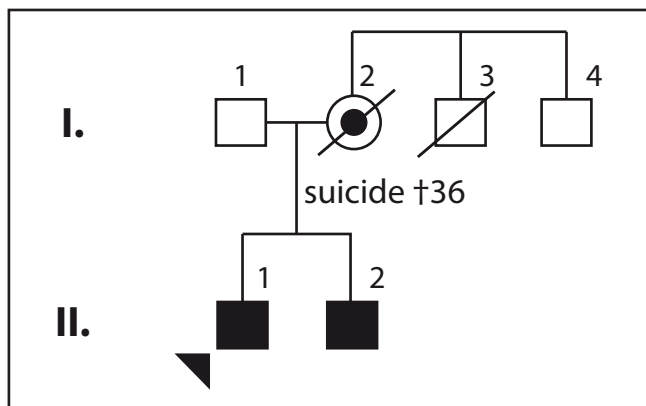


Fig. 4. Pedigree of the family. The index case is marked with an arrow.

for Addison's disease, treated with hydrocortisone with good compensation. Neurological investigations began at the age of 15 for problems with concentration and memory. Based on laboratory findings and genetic testing, the patient was confirmed as having an adolescent cerebral form of X-ALD with mutation c.887A >G in the *ABCD1* gene.

The patient is from the mother's second physiological gravidity, with a negative pre- and perinatal case history, and appropriate psychomotor development. Examinations began due to problems with memory and concentration, with deterioration over the course of a single year. The neurological findings were normal. Neuropsychological assessment showed a below-average intellectual functioning in both verbal and nonverbal areas. There were further signs of impairment in visuospatial organization, verbal memory and learning, overall logical thinking, verbal fluency, attention-span, and graphomotoric deficit. Patient achieved IQ 77; 8 months later this dropped to the value of 72.

MRI showed an increased signal intensity on T2WI and FLAIR sequences in the parietal and occipital areas, in the frontal peri- and paraventricular area of white mater bilaterally, as well as in the genu and splenium of corpus callosum. Signal of the white matter was reduced on T1WI and there was a streaky enhancement as an active zone of demyelination. Findings were typical for the cerebral form of X-ALD (Figure 5 A,B,C). The Loes score of our patient was 8.5 points. Results of further investigations: EEG with appropriate findings for the patient's age; with alpha activity; without specific epileptic signs; EMG examination with normal conduction velocity; VEP with prolonged P100 wave latency bilaterally and BAEP with normal values. The levels of VLCFAs were pathologically increased (Table 2). A DNA analysis revealed the *ABCD1* gene mutation c.887A>G (Y296C). We considered therapeutic intervention – bone marrow transplantation (BMT). Follow-

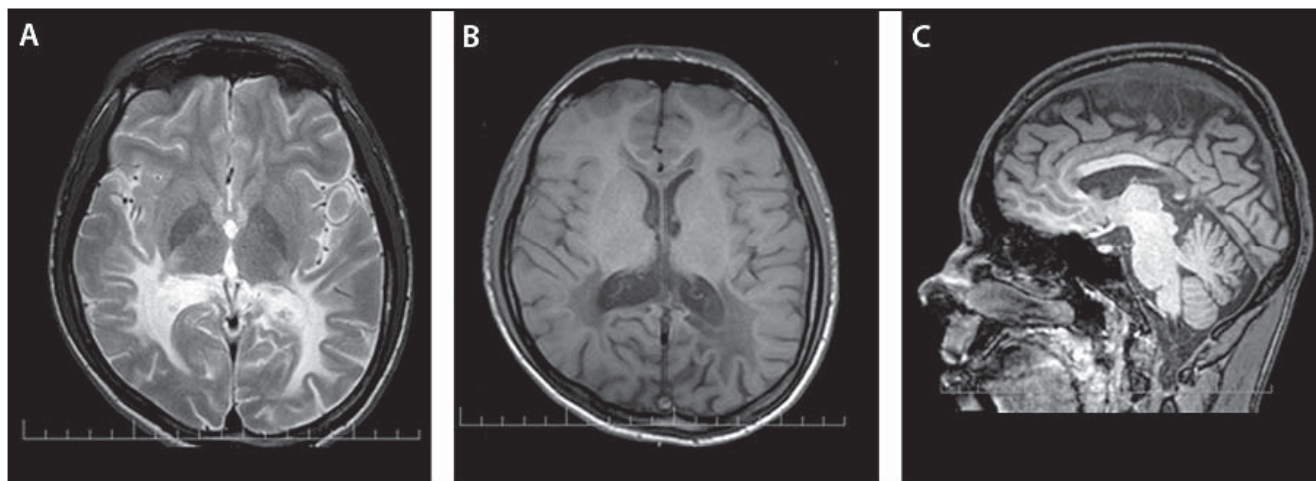
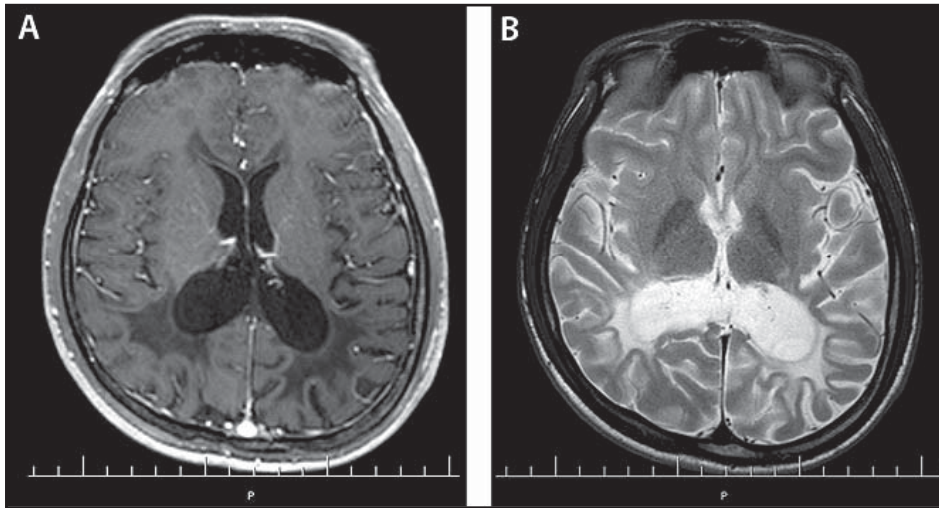


Fig. 5. MRI adolescent cerebral form of X-ALD, 17 years old patient with confluent symmetrical lesions of white mater, hyperintense on T2WI (A), hypointense in T1WI (B), predominantly in the parietal and occipital areas. Sagittal section, with affected genua and splenium of the corpus callosum (C).



**Fig. 6.** The same patient, 12 months after BMT, without progression of the findings on MRI T1WI, with gadolinium: the lesions do not show activity (A), T2WI also without progression (B).

ing a consultation with Professor Raymond (Kenedy Krieger Institute in Baltimore, USA) regarding our therapeutic options, we performed the bone marrow transplant in April 2012.

The patient is currently 24 months after the allogeneic BMT. MRI findings of the brain do not show progression, and only marginal activity of the process is observed (Figure 6). The laboratory values of VLCFAs are slightly lower (Table 2). Neurological findings are without significant alterations. In IQ testing, 6 months after BMT patient achieved IQ 65, and has not changed since. In the foreground of current clinical features there are signs of overall mental deterioration, slow-thinking, defect of logical thinking, problems of mental and visual integration, visual and motor coordination. The overall condition of the patient seems to be stable.

The case history of the patient's family members is incomplete: his mother committed suicide before his disease was diagnosed, and no health impairments were reported (Figure 4). Further information about the mother's family history is not known. Patient's older brother has also elevated level of VLCFAs, but he subsequently refused further investigation.

## DISCUSSION

The first patient showed signs of a pure cerebral form of X-ALD. The frontal lobes were the most affected; demyelination started also in the temporal lobes. Clinical presentation has been dementia of frontal type, which is a relatively frequent phenotype of adult cerebral form. In the literature there are several reports of dementia of frontal type as a prominent manifestation of ACALD (Dziewas *et al.* 2001; Larner *et al.* 2003; Luda *et al.* 2001; Mukherjee *et al.* 2006). In the past we also reported the case of a 37 year old man with ACALD, with dementia of frontal type and with a new mutation in ABCD1 gene (Sutovsky *et al.* 2006). There are also several reports concerning the Y296C mutation in the

literature. All of these cases had cerebral forms of ALD, mainly childhood CALD (Asheuer *et al.* 2005; Kemp *et al.* 2001; Pan *et al.* 2005; Shimozawa *et al.* 2011; Takano *et al.* 1999). This patient is the first reported adult cerebral form of ALD in a Y296C mutation carrier. Likewise, dementia of frontal type had not previously been reported in carriers of this mutation.

The second patient had an adolescent cerebral form of ALD (AdolCALD) with the global cognitive deficit in the front. The pattern of cerebral impairment was different from that of the first patient. Frontal lobes were not affected with demyelination. Demyelination affected mainly parietal and occipital white matter and corpus callosum. Blood marrow transplantation had a positive effect on the course of the disease, in the sense of a significant slowing of the progression. Early diagnostics and early BMT in indicated cases can significantly slow the progress of the disease and preserve cerebral function. In this regard the search for early stages of X-ALD seems to be crucial. Every male patient with Addison's disease should be examined for VLCFA level, in order to detect ALD in the preclinical cerebral stage. BMT in such cases can deliver a significant deferment of cerebral affection.

## ACKNOWLEDGEMENTS

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