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ORIGINAL ARTICLE

A Distinct Clinical Phenotype in Two Siblings with X-linked Adrenoleukodystrophy

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Abstract

OBJECTIVES: X-linked adrenoleukodystrophy(X-ALD) is a rare X-linked recessive metabolic disorder. The mutations in the ATP Binding Cassette Subfamily D Member 1 (ABCD1) gene account for the underlying molecular mechanism. Herein, we present two siblings with X-ALD due to a missense, presumably identical, ABCD1 mutation, who had extremely distinct clinical phenotypes.

MATERIAL AND METHODS: Patient 1 (6y/o) was admitted with primary adrenal insufficiency (PAI). His VLCFA analysis and cranial MRI suggested the diagnosis of X-ALD with no cranial involvement. Although the PAI was successfully managed using hydrocortisone replacement therapy, during follow-up he was admitted with the complaints of perception impairment, seizures, loss of vision and deafness suggesting cranial involvement which was not able to be recovered despite intensive supportive therapies; in the end patient died. Patient 2 (21y/o) had mild symptoms of PAI with no organ manifestation. He was undertaken to a molecular genetics analysis for ABCD1 gene due to history of his brother. His VLCFA analysis revealed mildly elevated C26, C22 and C26/C22 ratio suggesting ALD diagnosis. However, his cranial imaging and other results were within normal limits.

CONCLUSION: Two siblings with X-ALD due to presumably an identical, missense ABCD1 mutation and distinct clinical phenotype have confirmed the lack of phenotype-genotype correlation and proved the essential role of molecular genetics analysis in the early diagnosis. It is crucial to follow up for the development of cranial involvement and decide a bone marrow transplantation which is the only option that can prevent the progression of the disease, thus extend the lifespan.

INTRODUCTION

X-linked adrenoleukodystrophy (X-ALD) is a rare (1: 17 000) X-linked recessive metabolic disorder caused by the defect in the ATP-Binding Cassette Subfamily D, Member 1 (*ABCD1*) mapped to Xq28 and encodes the peroxisomal transmembrane adrenoleukodystrophy protein (ALDP) which plays a critical role in the transmembrane transport of very long-chain fatty acids (VLCFA) (Engelen *et al.* 2012). About 600 mutations have been identified so far. Peroxisomal transmembrane protein mediates the transport of VLCFA-CoA esters from cytosol into peroxisome (Van Roermund *et al.* 2008). The lack of this protein causes hexacosanoic (C: 26) and tetracosanoic (C: 24) acid accumulation mainly in the brain, adrenal glands and testicles (Muranjan *et al.* 2018).

The three forms frequently seen in males are cerebral, isolated primary adrenal insufficiency (PAI) and adrenomyeloneuropathy (AMN). Heterozygous females present with AMN at a rate of 20% or rarely as PAI, while some intermediate types are also available (Apaydin *et al.* 2005).

Patients with ALD can first manifest with either PAI or another organ involvement. Thus, close follow up of male cases with the diagnosis of X-ALD is essential for the prompt diagnosis of adrenocortical insufficiency thereby an early diagnosis of cranial involvement for considering allogenic hematopoietic stem cell transplantation (HCT) if an HLA-compatible donor is available. Despite a high mortality risk, allogenic HCT remains to be the only therapeutic option to prevent the progression of the disease in cases with early stage of cranial involvement (Shapiro *et al.* 2000).

Here, we present two siblings with X-ALD who had extremely distinct clinical manifestations as well as prognosis due to a hemizygous, presumably identical, missense mutation in the *ABCD1* gene.

PATIENT 1 (P1)

A 6-year-old male was admitted to the pediatric endocrinology unit with short stature. He was born to non-consanguineous parents after an uneventful gestation via spontaneous vaginal delivery. His family and past medical history was unremarkable. At the time of the presentation his weight was 18.6kg (-0.95 SDS) and height was 107cm (-1.92 SDS). He had skin hyperpigmentation and otherwise normal physical examination findings. In the laboratory examination he had an elevated adrenocorticotropic hormone (ACTH) and inappropriately low cortisol level suggesting PAI which was confirmed by ACTH stimulation test (Laboratory investigations and ACTH stimulation test results are displayed in Table 1). The hydrocortisone replacement therapy was commenced. Further investigations for the evaluation of underlying etiology of PAI in the male case revealed mildly elevated plasma VLCFA suggesting the diagnosis of ALD (Table 2). The cranial magnetic resonance imaging (MRI) did not show any sign of cranial involvement.

Although his PAI was successfully managed with hydrocortisone replacement therapy, he was admitted with the complaints of seizure, visual and hearing loss about 1.5 years later following the diagnosis of PAI. He was hospitalized in the critical care unit for supportive care and further investigations. Heterogeneous hypodense lesions were detected in cranial MRI in

Tab. 1. The laboratory investigation results of Patient 1 and Patient 2

	Patient 1	Patient 2
Glucose (mg/dL)	107	97
Na (mmol/L, N: 136-145)	136	137
K (mmol/L, N: 3.5-5.1)	3.9	4.29
Total testosterone (mmol/L, N: 4–5.5)	0.025	8,73
FSH (mIU/mL, N:0.95-11.95)	1.31	12.92
LH (mIU/mL, N:0.57-12.07)	0.565	20,32
FreeT4 (ng/dL, N:0.93-1.70)	1.1	1.41
FreeT3 (pg/mL, N:2.00-4.40)	4.3	4.63
TSH (µIU/mL, N:0.270-4.20)	2.9	2.32
Serum cortisol (µg/dl, N:6.2-19.4)	8.28	15.92
ACTH (pg/mL, N:7.2-63.6)	705	152.8
Peak cortisol level at 250 µg ACTH stimulation test (µg/dl)	8.98	22,64

Na: sodium, K: potassium, FSH: Follicle Stimulating Hormone LH: Luteinizing hormone, T4: Thyroxine hormone T3: Triiodothyronine, TSH: Thyroid-stimulating hormone, ACTH: Adrenocorticotropic hormone

Tab. 2. The results of very long-chain fatty acids analysis of Patient 1 and Patient 2.

Test	Patient 1	Patient 2
C26:0 (µmol/L)	4.76 (N: 1.3-4.1)	0.42 (N: 0-1.3)
C24:0 (µmol/L)	58.47 (N: 31.3-98.2)	3.03 (N: 0-91.4)
C22:0 (µmol/L)	47.37 (N: 22.9-100.2)	1.68 (N: 0-96.3)
C26:0/C22:0 ratio	**0.1 (N: 0.069-0.0058)**	**0.25 (N: 0-0.023)**
C24:0/C22:0 ratio	1.23 (N: 0.766-2.407)	**1.8 (N: 0-1.39)**

periventricular white matter at the level of the parieto-occipital lobes in the distal corpus callosum. In the diffusion MRI examination, iso-heterogeneous lesions were detected in temporo-parieto-occipital lobes periventricular white matter, corpus callosum distal corpus and splenium in B-1000 images, heterogeneous hyperintense diffusive signals were observed in ADC images, diffusive signals compatible with demyelination in the peritrigonal area, and hyperintense signals in the T2A sequences along the corticopontine tract to the brainstem in the corticospinal tract. These findings suggested the cranial involvement of ALD. In the MR spectroscopy examination, a decrease in creatine and NAA in both parietals, an increase in choline and a minimal lactate increase were observed. Based on the clinical, laboratory and radiological imaging findings, a diagnosis of X-ALD with cerebral involvement was considered. Nevertheless, the patient's clinical condition rapidly deteriorated and he died.

PATIENT 2 (P2)

A 21-year-old male patient, the elder brother of the first case, was examined genetically due to the history of his brother. DNA was extracted from peripheral blood leucocytes using standard methods. Sanger sequencing of the coding and flanking intronic regions for *ABCD1* gene was performed. The DNA sequencing results were analyzed using Mutation Surveyor Software® (SoftGenetics LLC. PA, USA). In mutation analysis, a hemizygous missense c.1534G>A(pGly512Ser) mutation was detected in the exon-6 of the *ABCD1* gene. This mutation was first described by Kok *et al.* in an 8-year-old male with the cerebral type X-ALD (Kok *et al.* 1995). The variant causes an amino acid change (glycine to serine) within the ATP-binding domain of ALDP and is classified as pathogenic (class 1) according to the American College of Medical Genetics and Genomics (ACMGS) guidelines.

Considering the mutation result and history of his brother, a diagnosis of X-ALD was considered and the patient was referred to our endocrinology and metabolism outpatient clinic for an endocrine assessment. At the time of the presentation, he had fatigue and weakness for 1 year and was otherwise healthy with an unremarkable past medical history. In the physical examination, his height was 163 cm, his weight was 55kg, systolic and diastolic blood pressure was 100/50 mmHg and his pulse rate was 96 beats per minute. Laboratory examination results are shown Table 1. In the VLCFA analysis he had elevated C26/C22 ratio (0.25) suggesting the diagnosis of ALD (Table 2). Contrast-enhanced MRI of the upper abdomen, scrotal USG and cranial MRI were normal.

DISCUSSION

X linked ALD has a broad range of clinical spectrum including cerebral ALD (37% childhood onset, 7% adolescent onset and 3% adult onset), adrenomyeloneuropathy (AMN) (32%), isolated Addison's disease (13%), presymptomatic disease (7%) and olivo-pontocerebellar atrophy in adolescents or adults (1–2%) (Loes *et al.* 2003). Here, we present two siblings with X-linked ALD, presumably carrying an identical missense *ABCD1* mutation, with a very distinct clinical manifestation as well as prognosis.

Adrenal cortex, central nervous system myelin and Leydig cells of testicles are the main sites for the involvement of ALD. Plasma levels of VLCFA is the most commonly used test in the diagnosis of ALD (Ono *et al.* 2014). Molecular genetics analysis of *ABCD1* gene is a useful tool for confirming the diagnosis, providing genetic counselling and screening of other family members for early diagnosis and appropriate management of asymptomatic cases. However, there is no strong phenotype-genotype correlation even within the individuals from the same family carrying an identical mutation. Likewise, our two cases, presumably with an identical *ABCD1* mutation, had a very distinct clinical phenotype. While P1 presented with isolated PAI and developed cranial ALD within a short time period at an early age, his elder brother was diagnosed with molecular genetics analysis of *ABCD1* gene which was undertaken due to the history of his brother (P1). However, he was still asymptomatic and showed no sign of system involvement.

The childhood cerebral type of ALD usually manifests between the ages of 4–8 years with attention deficiency and hyperactivity. It progresses with the impairment of cognition, behavior, vision, hearing and motor function. The AMN manifests as progressive paraparesis, sphincter and sexual dysfunction in the late twenties. Adrenal insufficiency usually occurs between

the ages of 5 and 10 years. Weakness, fatigue, unexplained vomiting and usually neurologic symptoms can be the presented complaints. Hyperpigmentation is observed due to excessive ACTH secretion. Cerebral involvement is typically characterized by inflammatory myelinopathy with symmetrical parieto-occipital white matter lesions (Karapanou *et al.* 2014). The abnormal VLCFA accumulation in the white matter leads to a myelin and axonal degeneration. The classification proposed by Loes in 1994 is the gold Standard scoring system that is derived from standard MRI findings. This system is useful for follow-up and predicting the progression of the disease and for the assessment of therapies (Loes *et al.* 1994). A 34-point severity scale for brain MRI features was developed based on location and the extent of involvement and on the presence of either focal or global atrophy. The temporo-anterior, frontal and parieto-occipital white matter, corpus callosum, visual and auditory tracts, cerebellum and basal ganglia are the sites which point to the severity and increase the score in the way of severity (5). In P1, those sites were affected. According to the Loes scoring system; a score <4 suggests a very early stage; a score between 4 and 8 suggests an early stage; a score between 9 and 14 suggests a late stage and a score >13 suggest an advanced disease (Ono *et al.* 2014). Loes scoring also helps making decision and timing for bone marrow transplantation (BMT), the only treatment opportunity for the regression of the demyelination process (Ono *et al.* 2014). Peters *et al.* recommend BMT for patients with a Loes score of <9 (Peters *et al.* 2004). Patients with Loes score of <4 are recommended as better candidates for BMT (Warren *et al.* 2007). The BMT treatment has been proven to extend life expectancy (Miller *et al.* 2011).

Present cases had an entirely distinct clinical phenotype in terms of organ system that was involved, and particularly in terms of severity and progression rate of the disease. The first case was admitted with PAI at the age of 6 years and developed cranial involvement within 1.5 years. Although at his first admission, he had no findings of cranial involvement and the PAI was successfully managed using hydrocortisone replacement therapy, during follow-up he was admitted with the complaints of perception impairment, seizures, loss of vision and deafness suggesting cranial involvement which was not able to be recovered despite intensive supportive therapies. The patient died in the first episodes of cranial manifestation. However, the second patient (P2) had mild symptoms of PAI with no organ manifestation at the age of 21 years. He was undertaken to a molecular genetics analysis for *ABCD1* gene due to the history of his brother (P1). His VLCFA analysis revealed mildly elevated C26, C22 and C26/C22 ratio suggesting ALD diagnosis. However, all his other investigations were within normal limits and he was under follow up with genetically proven diagnosis. Distinct clinical phenotypes of these male patients from the same family, presumably with identical *ABCD1* mutation further supported the phenotypical variability and severity of the ALD due to *ABCD1* gene mutations. Therefore, molecular genetics analysis of other male individuals from the families of X-ALD patients would provide a valuable insight into their prompt diagnosis as well as follow-up for the development of other organ involvement which is highly predictive for appropriate management and improving the prognosis.

A laboratory and clinical androgen resistance, attributed to the VLCFA accumulation in testosterone receptor and/or post-receptor levels, has been reported in X-ALD patients (Karapanou *et al.* 2014). However, although P2 had a relatively high gonadotropin and testosterone levels (Table 1), he had normal secondary sexual characteristics (He had no gynecomastia. Beard and axillary hair development was normal) suggesting an absence of a clinically relevant androgen resistance. Besides, his scrotal USG was normal with no sign of VLCFA accumulation.

The choice of treatment for X-ALD patients critically affects the disease progression, thus the overall prognosis. In the late cerebral involvement, a diet therapy (low-VLCFA and unsaturated fatty acids) has not proven to change the prognosis while it can be a supportive therapy in the early brain involvement in combination with BMT (Shapiro *et al.* 2000; McGuinness & Smith, 1999). An assessment with cranial MRI and Loes scoring periodically (every 6 months) is strongly recommended for the prompt diagnosis of cranial involvement and immediate BMT preferably at a Loes score lower than 6–7. For the cases with a slowly progressive disease like P2, the most appropriate strategies are prescribing diets containing low- VLCFA and unsaturated fatty acids and regular follow-up for the development of PAI and periodic cranial MRI for the detection of the cranial involvement at an early stage. Yet, the first case (P1) was not brought for a regular follow up visit; therefore, we were not able to assess the cranial involvement periodically and failed to detect the cranial involvement at an early stage. Besides, he had rapid clinical progression including cranial involvement and severe episodes of neurological deterioration leading to his dead, in the end.

In conclusion, the present two male siblings with X-ALD due to a presumably identical, missense *ABCD1* mutation and a distinct clinical phenotype have shown the lack of phenotype-genotype correlation and proved the essential role of molecular genetics analysis in the early diagnosis. It is crucial to follow up for the development of cranial involvement and decide a bone marrow transplantation which is the only option that can prevent the progression of the disease, thus extend the lifespan.

DISCLOSURE OF INTEREST

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