

## X-linked adrenoleukodystrophy diagnosed in three brothers

Adrenoleukodystrofia sprzężona z chromosomem X zdiagnozowana u trzech braci

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### Abstract

Adrenoleukodystrofia is a genetic disease classified in the group of peroxisomal disorders caused by mutations in *ABCD1*, gene located on the X chromosome (Xq28). It demonstrates X-linked recessive inheritance. There is an accumulation of very long-chain fatty acids (VLCFA) in plasma and all tissues in the process of ALD. No causal treatment for ALD is known, although hematopoietic stem cell transplantation (HSCT) and gene therapy are allowed for early diagnosis in childhood cerebral form. The case report describes three of the brothers suffering from ALD, diagnosed in different stages of disease. The eldest brother was diagnosed by the age of nine, with the first symptom of hearing loss. After that, test was also conducted in boy's younger brother – he was then asymptomatic, however diagnosed with ALD at age 7. The mutation in the *ABCD1* gene was also detected in the mother's blood. Due to the significant severity of the disease, the eldest boy was not qualified for HSCT. Nowadays, 2 years after, the health condition of the eldest brother is severe. The middle brother underwent HSCT. Currently, he is in a good general condition. The youngest brother had genetic testing performed shortly after birth. The mutation in the *ABCD1* gene also was confirmed, but in this moment there is no regarding to HSCT. His development is adequately to age, but he has adrenal insufficiency. All of brothers are treated hydrocortisone. Parental awareness and early genetic tests (including prenatal tests) are very important because ALD is associated with severe course and high mortality, and its symptoms are non-specific.

### Key words:

adrenoleukodystrofia, adrenal insufficiency hematopoietic stem cells transplantation.

### Streszczenie

Adrenoleukodystrofia (ALD) jest chorobą genetyczną sklasyfikowaną w grupie zaburzeń peroksyosomalnych spowodowanych mutacjami w genie *ABCD1*, zlokalizowanym na chromosomie X (Xq28), dziedziczną recesywnie sprzężoną z chromosomem X. W przebiegu choroby dochodzi do akumulacji bardzo długołańcuchowych kwasów tłuszczowych (VLCFA) w osoczu i wszystkich tkankach. Nie jest znane leczenie przyczynowe ALD, jednakże przeszczep krwiotwórczych komórek macierzystych (HSCT) dozwolony jest w leczeniu wczesnej postaci mózgowej u dzieci. W pracy przedstawiono opis rodzinnego występowania ALD – przypadków zdiagnozowanych w różnych stadiach choroby. Najstarszy brat został zdiagnozowany w wieku 9 lat, pierwszym objawem choroby była utrata słuchu. Przeprowadzono badania u młodszego brata chłopca, który wówczas nie miał objawów, i również wykryto ALD. Został on poddany HSCT. Jego rozwój przebiega prawidłowo. Mutację genu *ABCD1* stwierdzono u matki. Z powodu znacznego nasilenia choroby najstarszy chłopiec nie został zakwalifikowany do HSCT. Obecnie jego stan zdrowia jest ciężki i systematycznie się pogarsza. U najmłodszego z braci testy genetyczne przeprowadzono wkrótce po urodzeniu. Potwierdzono mutację genu *ABCD1*. Nie podjęto decyzji o zastosowaniu w leczeniu HSCT. Rozwój chłopca przebiega prawidłowo, ale stwierdzono niedoczynność nadnerczy. Wszyscy trzej bracia leczeni są hydrokortyzonem w dawkach podzielonych. Świadomość rodziców i wczesne testy genetyczne (w tym badania prenatalne) są bardzo istotne, ponieważ ALD związana jest z ciężkim przebiegiem oraz dużą śmiertelnością, a jej objawy są niespecyficzne.

### Słowa kluczowe:

adrenoleukodystrofia, niedoczynność kory nadnerczy, przeszczep komórek macierzystych.

## Introduction

In 1970, Blaw [1] introduced the term “adrenoleukodystrophy” (ALD) as a disease entity with X-linked inheritance. ALD is a genetic disease classified in the group of peroxisomal disorders caused by mutations in *ABCD1*, gene located on the X chromosome (Xq28). X-linked adrenoleukodystrophy (X-ALD) is the most common inherited peroxisomal disorder with incidence of hemizygotes (all phenotypes) plus heterozygous female carriers is 1 : 16,800 newborns [2]. It demonstrates X-linked recessive inheritance. X-linked adrenoleukodystrophy is a metabolic disorder characterized by impaired peroxisomal beta-oxidation of very long-chain fatty acids (VLCFA;  $\geq C22$ ). Consequently, there is an accumulation of VLCFA in plasma and all tissues, including the white matter of the brain, the spinal cord, adrenal cortex and the Leydig cells in the testes. Mutations in this gene cause the absence or dysfunction of ALDP, a peroxisomal transmembrane protein. Adrenoleukodystrophy could have different onset and severity. Main adrenoleukodystrophy phenotypes include: cerebral demyelination, adrenomyeloneuropathy, adrenal insufficiency and asymptomatic. Clinically, ALD is a heterogeneous disorder. Symptoms may also vary depending on the phenotype of the disease. The most common initial symptoms of ALD include: alterations in behaviour, auditory impairment, memory loss, diminished visual acuity, speech difficulties and signs of adrenal failure. Then the symptoms associated with demyelination can occur. The full-blown X-ALD usually affects men, although many heterozygous women may display different levels of symptom advancement. Adrenal insufficiency is characterized by inadequate glucocorticoid production owing to destruction of the adrenal cortex. The clinical presentation of adrenal insufficiency depends on extent of the loss of adrenal function. Common features of adrenal insufficiency include weight loss, low blood pressure, anorexia, nausea, vomiting, lethargy and fatigue. If there is also mineralocorticoid insufficiency the symptoms as postural hypotension, muscle cramps, abdominal discomfort and salt craving occurs. Skin pigmentation is present in most of people with adrenal failure. Hypoglycemia may also be a manifestation of the disease. The goal of glucocorticoid replacement in adrenally insufficient patients is to abolish symptoms of glucocorticoid deficiency and prevent adrenal crisis. The glucocorticoid replacement is based on oral administration of hydrocortisone in divided doses. Almost all males with X-ALD develop adrenocortical insufficiency during life, about 80% before adulthood [3]. Due to the diverse ALD phenotype, the diagnosis is complicated. When ALD is suspected based on clinical symptoms, the initial testing usually includes plasma VLCFA determination. Then, if it occurs positive, diagnostic involves molecular genetic analysis of *ABCD1* gene. No causal treatment for ALD is known, although hematopoietic stem cell transplantation (HSCT) and gene therapy are allowed for early diagnosis in childhood cerebral form. HSCT is the transplantation of multipotent hematopoietic stem cells. In qualification for HSCT, the Loes scale is used, which allows to assess the severity of demyelination brain in magnetic resonance images [4]. Lorenzo’s oil is used in the treatment of

asymptomatic patients. It is a preparation intended to prevent the formation of VLCFA. Adrenal insufficiency in ALD patients can be successfully treated, usually with hydrocortisone.

## The family adrenoleukodystrophy case report

The eldest brother (brother A) was born in 2006 with the birth weight 4300 g and birth length 56 cm from uncomplicated pregnancy. In 1<sup>st</sup> minute he received 6 points in Apgar score, in the 5<sup>th</sup> 2 points (skin color 1, pulse rate 1) and in the 8<sup>th</sup> 8 points. The delivery period was complicated by a 30 second apnea, during which the boy was artificially ventilated. The postnatal period was complicated by infection (high levels of CRP) treated with antibiotics both in the mother and in the boy. By the age of nine, the boy was developing properly. The first disturbing abnormality in the boy’s health was sudden hearing loss. The disease was initially associated with nasopharyngeal infection, and later with thickening of mucous membranes as a result of allergies. In the meantime, the boy was diagnosed with an external squint which had not occurred before. In addition, the parents noticed a high degree of distraction and disturbance in the boy – the child turned over on his bike, stumbled. In a retrospective assessment, the onset of the disease could give the first symptoms much earlier. The child experienced difficulties in reading and writing- he attended a special therapeutic school. The boy did not have episodes of aggression, but he became more and more withdrawn. He was no longer willing to take part in family trips, he preferred to be alone. The boy was consulted many times by a laryngologist and ophthalmologist, with no specific results. In the fundus examination no changes were shown. The reason for the occurrence of strabismus was the weakening of the eye muscles. The tympanometry and audiogram were performed, which showed no pathological changes. The boy was referred to the clinic for a trial with a hearing aid set to 100 decibels. No reaction from the child was observed. The obtained clinical picture disturbed one of the clinicians who recommended brain imaging. Brain tomography was performed urgently. The study revealed lesions of a leukodystrophy character. In order to widen the diagnosis, a magnetic resonance imaging of the brain was performed, which enabled the initial diagnosis of adrenoleukodystrophy. Because adrenoleukodystrophy belongs to genetic diseases, the boy’s younger brother (brother B) was called to hospital urgently. Blood was collected in children to determine the level of VLCFA and mutation in the *ABCD1* gene- both tests were positive for adrenoleukodystrophy diagnosis in both brothers. The mutation in the *ABCD1* gene was also detected in the mother’s blood (asymptomatic carrier). Due to the significant severity of the disease, the eldest boy was not qualified for HSCT. The Loes scale rating was 9 points. However, stem cell infusions were used. The initial dose of hydrocortisone was 5 mg – 2.5 mg. During the first endocrinological consultation, the results were: ACTH 249 pg/ml, morning cortisol 8.1  $\mu$ g/dl. The boy’s condition was already severe at that time – he understood single words, his speech was silent, there were squint,

a lack of quick understanding and shaky gait observed. Physical examination showed a weak response to the environment, the onset of puberty and darkening of the scrotum. After the consultation, the dose of hydrocortisone was increased as follows: 5 mg – 2.5 mg – 2.5 mg. In addition, the boy took Lorenzo's oil for a short period of time. Nowadays, 2 years after, the health condition of the eldest brother is now severe. It requires constant care. The last test results of ACTH was 188 pg/ml and the doses of hydrocortisone were: 12.5 mg – 5 mg – 5 mg.

The middle brother (brother B) was born in 2009 with birth weight 3990 g and length of 56 cm with an Apgar score 10. He was diagnosed with adrenoleukodystrophy, by detection of increased level of VLCFA and mutation in *ABCD1* gene, at the age of 7 when it was detected in an older brother. At the time of diagnosis he was asymptomatic, although the initial phase of adrenocortical insufficiency has been recognized due to increased level of ACTH. The boy started taking Lorenzo's oil and hydrocortisone. His BMI was over 50 percentile – 15.8 kg/m<sup>2</sup> (23.5 kg weight, 122 cm height). No abnormalities in CNS were detected in brain's 1 MRI and he was qualified for transplantation of hematopoietic stem cells (HSCT). HSCT from the bone marrow of an unrelated donor was conducted in Department of Bone Marrow Transplantation, Pediatric Oncology and Hematology, Wrocław Medical University. After the HSCT he discontinued taking Lorenzo's oil. At the age of 8 during routine control, due to asymptomatic hypertension, captopril treatment was added. In the same year, the patient presented urinary symptoms – at the end of the micturition the urine was lightly tinted with blood. BKV viruria was detected and acyclovir therapy was conducted for 2 weeks. At the age of 9, after discontinuation of acyclovir, which was administrated as a prophylaxis, he underwent shingles of upper right half of chest and the area of the right shoulder joint. Patient had a fracture of right collarbone and left forearm he was treated conservatively. Densitometry test was performed (total T-score –4,5/PR60%, z-score – 1,0/92%), and osteoporosis was diagnosed. Due to diagnosis patient is taking vitamin D<sub>3</sub>, vitamin K<sub>2</sub> and calcium. Currently, the patient is in good general condition, he goes to the 3<sup>rd</sup> grade of primary school, trains on Physical Education, goes to the pool and tolerates the effort well. His development is similar to his peers. His BMI increased to 18.1 kg/m<sup>2</sup>, still over 50 percentile (32 kg weight, 133 cm height). In the recent brain NMR examination no obvious changes typical of adrenoleukodystrophy were shown. Level of VLCFA is kept elevated. Because of adrenal insufficiency (recent examinations: ACTH 81.70 pg/ml (–46), cortisol in a 24-hour urine sample 164 nmol/24 h (normal range 38–208), he takes hydrocortisone (5 mg – 5 mg – 2.5 mg).

The youngest brother (brother C) was born in 2017 as the third child with birth weight 3950 g and birth length 56 cm. His Apgar score was 10. The pregnancy went without any deviations. Due to the family genetic load of X-adrenoleukodystrophy, genetic testing was performed shortly after birth. The mutation in the *ABCD1* gene was confirmed. A VLCFA level test carried out earlier in the hospital showed their high concentration in the blood. At the age of 3 months, he was diagnosed with early adrenal insufficiency (ACTH 166 pg/ml) without

any symptoms from central nervous system. The boy started therapy with a hydrocortisone in doses 1.25 mg – 1.25 mg – 1.25 mg. Physical examination revealed darkening of the scrotum and nipples. After consultation in the Department of Bone Marrow Transplantation, Oncology and Hematology, the HSCT was initially planned at the age of 2–3 years. The boy is currently developing properly. Periodic check-ups of ACTH level are performed. In the last performed MRI, no pathological changes were found. During the last endocrinological consultation there were no deviations from the norm and features of adrenocortical dysfunction in the physical examination. No dark staining of the nipples and scrotum was observed. Last ACTH value results is 421 pg/ml. The boy is taking hydrocortisone at doses of 2.5 mg – 1.25 mg – 1.25 mg. Currently it is difficult to predict what form of adrenoleukodystrophy the youngest brother will develop, therefore there is no official decision regarding to HSCT. The boy requires multidisciplinary care, control of the adrenal functions and imaging MRI of the central nervous system. At the moment, the boy does not take Lorenzo's oil. His possible supplementation is considered after the child reaches the age of 2 years.

## Discussion

X-linked adrenoleukodystrophy is a complex disorder that is still incompletely understood and has very limited treatment options. The disorder is associated with severe morbidity and mortality in the majority of affected patients. X-linked adrenoleukodystrophy is a peroxisomal metabolic disorder with a highly complex clinical presentation. It is characterized by surprising and fascinating heterogeneity in the clinical manifestations: from rapid cerebral demyelination in childhood to myelopathy in late adulthood. However, one cannot identify these individuals until the early changes are seen using brain imaging (MRI).

The eldest brother (brother A) and the middle one (brother B), were diagnosed with cerebral form of ALD associated with adrenal insufficiency. Due to late diagnosis and severe symptoms of ALD, the brother A was not qualified for HSCT. Unfortunately, it significantly reduced his chances of survival and condemned him to the deteriorating quality of life. The boy is currently in a vegetative state and requires advanced medical care. His treatment is only symptomatic. The brother B was early diagnosed because of the knowledge of his brother's disease. Early diagnosis and low severity of the symptoms allowed the boy to be qualified for HSCT. Currently, two years after cell transplantation, he is treated symptomatically, and the progress of ALD has been significantly slowed down. The boy develops well, however, he did not avoid complications after HSCT, such as osteoporosis. The youngest brother is developing properly to his age. He is under the control of the Department of Bone Marrow Transplantation, Oncology and Hematology, however it is still unclear what form of ALD he will develop in the future. HSCT carries a high risk and many complications, so unless he is diagnosed with a specific phenotype of ALD, HSCT is not recommended.

Within a family, there could be several different phenotypes, despite the presence of the same mutation. Due to the diverse phenotype and multisystem symptomatology of the disease, the patient should be under continuous specialist medical care, including: a pediatrician (in adulthood general practitioner), an endocrinologist and a neurologist. Recognition of X-ALD is highly important, since in some cases treatment is available. In the case of the brain form, when the Loes scale is 9 or less, it is possible to perform HSCT. One of the available treatment options is also gene therapy, but it is not yet a common and available method. Adrenocortical insufficiency could be without much trouble treated with endocrine replacement therapy. HSCT is, for now, the only treatment that can stop demyelination, although it carries significant risks. There are limitations to allogeneic transplantation, including the risks of graft failure and graft-versus-host disease (GVHD) [5]. Most patients with cerebral adrenoleukodystrophy die within a decade after they receive the diagnosis if they are not treated with HSCT [6]. The most successful outcomes to date have been achieved with the use of cells from HLA-identical, unaffected related donors [7]. Adrenal insufficiency does not resolve with successful transplant; most patients still require hormone replacement [8]. Nutritional therapy based on restriction of VLCFA intake by the child plays currently minor role. Limiting the consumption of VLCFA does not solve the ALD problem, because dietary intake is not the only source for VLCFA in the body, as they are also synthesized endogenously. In therapy of ALD an important element plays Lorenzo's oil, which is a mixture of unsaturated fatty acids (glycerol trioleate and glyceryl trierucate in a 4 : 1 ratio) that inhibits elongation of saturated fatty acids in the body [9]. Supplementation with Lorenzo's oil has been found to normalize the VLCFA concentrations in the body, although it does not stop the neurological degradation in

symptomatic patients and does not improve adrenal function [9]. One of the options to improve the prognosis in adrenoleukodystrophy is the initiation of prenatal tests or screening of newborns. Due to the lack of symptoms of ALD in children after birth, introducing screening for newborns seems to be a good solution [10]. Prenatal tests are based on the determination of the level of VLCFA or testing the *ABCD1* gene mutations in the material collected during chorionic villus sampling or from the amniotic fluid (depends on gestational age). Neonatal screening tests are not yet available in Poland. However, they are based on the measurement of C26:0 lysophosphatidylcholine in a dry drop of blood.

The case of described family shows, how important for development of the child and his future life is early diagnosis of ALD. All of the brothers are under multi-specialist care, they all have the same *ABCD1* gene mutation, yet they significantly differ in prognosis. Their quality of life also varies considerably. Therefore raising awareness among parents and clinicians about the diagnosis and the possibility of ALD are so important.

## Conclusions

Adrenoleukodystrophy is associated with the severe morbidity and mortality and the symptoms are significantly unspecific. Adrenoleukodystrophy occurs mostly among young boys and progresses rapidly. In early stages HSCT gives the best chances of slowing down the progression of disease and allows to alleviate the subsequent consequences of the disease. Therefore, parents' awareness and early genetic testing (including prenatal testing) are very significant for the progress of ALD. The introduction of prenatal tests in Poland seems to be a very important idea.

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