Reproductive function in men affected by X-linked adrenoleukodystrophy/adrenomyeloneuropathy

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Abstract
Background: X-linked adrenoleukodystrophy (X-ALD) is the most frequent, severely neurodegenerative, clinically heterogeneous peroxisomal disorder, the signs of which are a consequence of myelin, adrenal cortex, and testes impairment.

Objective: We studied testosterone, LH, and FSH levels in X-ALD/adrenomyeloneuropathy (AMN) patients. We evaluate the ability to procreate of these patients by analysis of pedigree and family screening by detection of very long-chain fatty acid (VLCFA) levels.

Subject and methods: Seventeen patients with X-ALD/AMN (16 with AMN and one asymptomatic) aged 24–48 (mean ± s.d., 34.7 ± 5.9) years, were identified based on the clinical picture, magnetic resonance imaging, and the presence of increased serum VLCFA levels. Nine X-ALD/AMN patients’ daughters, mean ages ± s.d. = 7.7 ± 3.8 years, were identified as heterozygote by elevated VLCFA levels.

Serum VLCFA levels were determined as ester derivatives by a gas chromatography method. Serum testosterone, LH, and FSH levels in X-ALD/AMN patients were detected by IRMAs.

Results: Serum testosterone levels were at the lowest levels of normal range but serum LH and FSH concentrations were increased in 57.1 and in 42.9% of X-ALD/AMN patients respectively. Among the 11 investigated of X-ALD/AMN married adult men, nine had produced offspring, a total of 13 children.

Conclusion: In this study, we report that in a group of X-ALD/AMN married adult men, we did not find a significant decrease in fertility compared with the Polish population (18.2 vs 15%).

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Introduction
X-linked adrenoleukodystrophy (X-ALD; MIM 300100), is a neurodegenerative disorder characterized by progressive demyelination within the central and peripheral nervous systems. Biochemically, it is characterized by the accumulation of very long-chain fatty acids (VLCFAs), particularly lignoceric (C24:0) and cerotic (C26:0) acids in blood and tissues, a cause of disturbance in the peroxisomal β-oxidation process. X-ALD results from mutations in the ABCD1 gene (300371) encoding an integral peroxisomal membrane protein (ALDP), belonging to the ATP-binding cassette-transporter superfamily (1). Accumulation of VLCFA contributes to X-ALD pathogenesis, although the mechanism is unclear (2). X-ALD is clinically heterogeneous, and can present at a variety of ages. The resulting symptoms include effects on the nervous systems, adrenal gland, and testes. Hemizygotes present progressive demyelination of the central and peripheral nervous systems, and adrenal insufficiency (3). Patients with adrenomyeloneuropathy (AMN) also present hypogonadism, impairment of Leydig cells, and a decrease in testosterone level (4, 5). The aim of this study was to evaluate the procreative ability of adult males affected by X-ALD/AMN.

Subjects and methods

Subjects
Seventeen patients with X-ALD/AMN (16 with AMN and one asymptomatic) aged 24–48 (mean ± s.d., 34.7 ± 5.9) years, were identified based on the clinical picture, magnetic resonance imaging, and the presence of increased serum VLCFA levels or family screening (6). The median duration of neurological symptoms was 6 years, range <1 to 18.
Among the patients, 11 men, aged 29–48 years (mean ± s.d., 35.5 ± 5.4), were married. Nine of these (eight with AMN and one asymptomatic) had produced offspring, a total of 13 children (nine girls and four boys). The date of conception of the children was 1–12 years before the primary onset of the disease. In spite of the fact that the other two marriages were open to having children, they did not have them, the reasons for this being unknown.

Nine X-ALD/AMN patients’ daughters, mean ages 7.7 ± 3.8 years, were obligatory heterozygotes.

Blood sampling
Blood (0.5 ml) was drawn after an overnight (12 h) fast. The serum was separated and stored frozen at below −20 °C before analysis.

Analysis of VLCFA by gas chromatography method
Serum VLCFA levels were determined as ester derivatives by a gas chromatography technique (GC), according to a previously described method (7).

Analysis of testosterone
Serum testosterone level was assayed using a CIS Bio International (Gif sur Yvette Cedex, France) TESTO-CT2 RIA kit. The intra-assay coefficient of variation (CV%) was 7.5% and inter-assay 7.0%. Reference values for men ranging from 8.2 to 34.6 nmol/l were used.

Analysis of LH and FSH
IRMA kits for the in vitro quantitative determination of human LH and human FSH in serum or plasma were purchased from DIAsource ImmunoAssays S.A., Nivelles, Belgium. Detection limit for the LH-IRMA was 0.2 mIU/ml and intra- and inter-assay CV were 3.9 and 8.0%. The respective values for the FSH-IRMA were 0.1 mIU/ml and 2.0 and 4.4% respectively.

Reference values for men ranging from 1.0 to 5.3 IU/l for LH and 1.3 to 8.1 IU/l for FSH were used.

Table 1 Serum very long-chain fatty acid ratios in hemi- and heterozygotes X-linked adrenoleukodystrophy/adrenomyeloneuropathy (X-ALD/AMN) (mean ± s.d.).

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Age at diagnosis (years)</th>
<th>C24:0/C22:0</th>
<th>C26:0/C22:0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemizygotes X-ALD/AMN</td>
<td>17</td>
<td>34.7 ± 5.9</td>
<td>1.634 ± 0.089</td>
<td>0.056 ± 0.013</td>
</tr>
<tr>
<td>Daughters of X-ALD/AMN patients</td>
<td>9</td>
<td>7.7 ± 3.8</td>
<td>1.098 ± 0.110</td>
<td>0.019 ± 0.017</td>
</tr>
<tr>
<td>Heterozygotes* X-ALD/AMN</td>
<td>30</td>
<td>(–)</td>
<td>1.150 ± 0.120</td>
<td>0.028 ± 0.012</td>
</tr>
<tr>
<td>Control</td>
<td>35</td>
<td>(–)</td>
<td>0.782 ± 0.054</td>
<td>0.008 ± 0.003</td>
</tr>
</tbody>
</table>

*Heterozygotes X-ALD/AMN – 30 mothers X-ALD/AMN patients, aged 22–50 years (21).
*bControl – 35 healthy subjects (17 women and 18 men, aged 14–50 years (21).

Results
In patients with AMN the VLCFA ratios (mean ± s.d.) were: C24:0/C22:0 = 1.634 ± 0.089 and C26:0/C22:0 = 0.056 ± 0.013. In the patients’ daughters, the presence of increased VLCFA was proven (C24:0/C22:0 = 1.098 ± 0.110 and C26:0/C22:0 = 0.019 ± 0.017 mg/ml: Table 1). Mean VLCFA levels in patients’ daughters were elevated at heterozygote levels.

The serum concentration of testosterone was from 10.5 to 26.5 nmol/l for all X-ALD/AMN patients and from 10.5 to 23.1 nmol/l for the group of married men, and was within the normal range. Mean ± s.d. testosterone values were 18.5 ± 5.5 and 16.1 ± 4.9 nmol/l respectively. Three patients from those who were fathers had the lowest testosterone levels (P < 0.0012 vs all patients). The mean testosterone level of this group constituted only about 60% of the mean testosterone for all patients.

The patients’ serum concentration of LH and FSH was (mean ± s.d.) 7.2 ± 3.2 (range 2.9–11.8) IU/l and 7.5 ± 7.9 (range 1.7–14.8) IU/l respectively. LH concentration was increased in 57.1% and FSH in 42.9% of patients compared with reference values.

An analysis of family history and screening by detection of VLCFA as a biomarker for X-ALD/AMN shows that nine out of 11 married men had produced offspring (nine girls and four boys), being 81.8% (9/11).

Discussion
The first published data in 1986, by Libber et al. (8), found normal plasma testosterone and minor testicular deficiency in X-ALD/AMN patients. Moser et al. (9) in 1991 found that serum testosterone levels were low in 11% and at the lower levels of normal in an additional 11% of 69 AMN patients. Brennemann et al. (4) (49 patients, mean age 36 years) showed mean testosterone did not differ significantly between patients and healthy controls, with two results at upper normal values, but the mean free testosterone concentration was significantly lower in comparison with controls. In retrospective studies, Assies et al. (10) (26 patients, with
median age 34 years) found basal testosterone below the normal value in 12% (3/26) of subjects.

Our studies showed similar results, i.e. low serum testosterone levels but in the normal range. Interestingly, the lowest testosterone levels (P<0.0012) were noted in three patients, who were in the advanced stage of disease, and descended further 1 to 1.5 years later. This observation confirmed that disease progression is connected with relapsed testicular hypofunction.

The above data shows that testosterone levels in X-ALD/AMN hemizygotes are in the lower part of the normal range. However, the testosterone increase after human chorionic gonadotropin stimulation was estimated to be insufficient in 88%, indicating deficient testicular steroidogenesis (10).

It was claimed earlier that VLCFA cholesterol ester accumulates intercellularly in X-ALD/AMN, probably leading to a shortage in the cholesterol pool (11) as a basal substrate of steroid hormone synthesis and difficulties in their production. On the other hand, incorporation of VLCFAs in cell membrane lipids influences membrane rigidity and permeability, thus interfering with receptor binding of ACTH (12).

Biochemical studies by measurement of LH levels (for a description of the endocrine function of the Leydig cell) and FSH levels (the sensitive endocrine parameter of the quality of spermatogenesis (13)) confirm to various degrees lesions of the Leydig and Sertoli cells in X-ALD/AMN patients.

A comparison of LH and FSH results in X-ALD/AMN patients presented in different studies is summarized in Table 2. The highest values, increased to 63.3% for LH levels and 53.1% for FSH, were detected by Brennemann et al. (4). According to Moser et al. (9), the respective values were lower: 42 and 30%. Assies et al. (10) referred to 16 and 32% increased results for these parameters. Our results for LH were similar to those of Brennemann et al. (4), and for FSH was located in the area referred to earlier (9, 10, and 4). Dysfunction of Leydig cells and/or of the Sertoli cells was reported to be as much as 81.6% of 49 AMN patients (4). In our finding it concerned 71% of males affected by X-ALD/AMN. These results are consistent with defective testicular hormonogenesis and compatible with observation of pathological changes in the Leydig cell and seminiferous tubules (5, 11). Assies et al. (10) found that 77% of 26 patients with X-ALD/AMN had clinical or subclinical testicular insufficiency, even in the absence of neurological defects and/or adrenocortical insufficiency.

In patients with AMN impairment of Leydig cells, adrenocortical and testicular interstitial cells, a decrease in testosterone level resulting in infertility and impotence (5) has been found. Powers & Schaumburg (14) described damaged sexual function as the first symptom of AMN in five out of six men.

Results of recent investigations agree with Powers’ previous observations (5, 11). By detection, peroxisomal marker proteins showed the presence of peroxisomes in most cell types in the testes (except for mature spermatozoa), and during the course of spermatogenesis. The highest levels of peroxisomal protein–lipid transporters, i.e. ABCD1, were found in Sertoli cells (15). The above results claimed that peroxisomal β-oxidation is essential for lipid homeostasis in the testes and for male fertility. Inactivation of this process in a study done on mice, by knocking out the multifunctional protein-2, and ACOX1 (which take part in three of the four stages of the β-oxidation process), causes full degeneration of the testes and infertility (16, 17). However, it is known that the X-ALD/AMN defect concerns another, preliminary stage of the β-oxidation process (transport substrate).

Actually, our results were based on the determination of testosterone, LH, and FSH levels in AMN patients at the time of diagnosis, which was about 6 years after the presumed onset of the disease.

However, an analysis of family history and screening by detection of VLCFA as a biomarker for X-ALD/AMN showed that nine out of 11 married men had produced offspring at a time before the occurrence of clinical manifestations.

This evidence could cause some surprise compared with earlier morphological and biochemical results showing testicular dysfunctions (4, 5). After all, the evaluation of fertility on the basis of clinical and biochemical parameters is only approximate. The final efficient positive proof is one or more offspring. The mechanism leading to demyelination, spinal cord axonal loss and adrenal as well as steroid hormone insufficiency in X-ALD/AMN is still unknown. Increased VLCFA levels have been detected in infants (18, 19), at least a few years before any onset occurs. However, in our present state of knowledge, it is impossible to predict the form and course of the disease. Among different phenotypes of X-ALD we could only examine the reproductive function in those with a late onset. It seems that the amount of reduction in fertility due to impairment of spermatogenesis is connected with the degree of progressive disorders (20). Certainly, it is a complex problem and requires detailed investigation.

It must be noted that the date of conception of children was 1–12 years before the primary onset of the disease. Fathers did not know about the risk of the disease and transmission of the mutant gene to all their daughters. Obviously, it is necessary to highlight that

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**Table 2** Increased LH and FSH levels in X-linked adreoleukodystrophy/adrenomyeloneuropathy patients in different studies.

<table>
<thead>
<tr>
<th>LH (%)</th>
<th>FSH (%)</th>
<th>Age (years)</th>
<th>Number of patients</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>30</td>
<td>(21–53)</td>
<td>69</td>
<td>(9)</td>
</tr>
<tr>
<td>63.3</td>
<td>53.1</td>
<td>36.2 ± 1.5</td>
<td>49</td>
<td>(4)</td>
</tr>
<tr>
<td>16</td>
<td>32</td>
<td>34 (18–78)</td>
<td>26</td>
<td>(10)</td>
</tr>
<tr>
<td>57.1</td>
<td>42.9</td>
<td>34.7 ± 5.9</td>
<td>17</td>
<td>Our results</td>
</tr>
</tbody>
</table>
in all daughters, obligatory heterozygotes showed an increase in VLCFA parameters at heterozygote levels (Table 1).

The above results allow us to conclude that nine out of 11 AMN-hemizygotes had a normal ability to procreate in the pre-symptomatic period. Currently, infertility of couples in Poland is estimated at about 10–15% (22). Dysfunction in the ability to procreate in our investigated group of AMN patients was evaluated at 18.2% (2/11). The level is slightly higher than the index for the whole Polish population.

In X-ALD/AMN patients with late onset of the disease, the possibility of procreation and the resulting inheritance by their daughters should be taken into consideration. All families should have genetic counseling concerning the inheritance of X-ALD.

In our investigated group of X-ALD/AMN patients, significantly decreased fertility was not observed.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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