Variability of endocrinological dysfunction in 55 patients with X-linked adrenoleucodystrophy: clinical, laboratory and genetic findings

G Christoph Korenke, Christian Roth, Ernst Krasemann1, Michael Hüfner2, Donald H Hunneman and Folker Hanefeld

Department of Paediatrics and Neuropaediatrics, University of Göttingen, Germany, 1Institute of Human Genetics, University of Göttingen, Germany and 2Medical Department, Division of Gastroenterology and Endocrinological Division, University of Göttingen, Germany

(Correspondence should be addressed to G Christoph Korenke, Department of Paediatrics and Neuropaediatrics, University of Göttingen, Robert-Koch-Straße 40, D-37075 Göttingen, Germany)

Abstract

X-linked adrenoleucodystrophy (ALD) has been shown to be one of the most frequent causes of Addison’s disease in men. It is characterized by an impaired peroxisomal β-oxidation of very long chain fatty acids and is associated with mutations of the ALD gene resulting in a defective peroxisomal membrane transport protein. There is a striking variability of endocrinological and neurological symptoms in patients with ALD, with no clearly evident correlation between mutations of the ALD gene and the different neurological phenotypes. No data on endocrinological symptoms and the ALD genotype have been published so far.

We report endocrinological, clinical, laboratory and molecular genetic data from 55 patients with ALD from 34 families. Endocrinological symptoms of adrenal insufficiency were observed in 33 patients, 20 of whom showed additional neurological symptoms of cerebral ALD or adrenomyelo-neuropathy. Isolated neurological symptoms were seen in 12 patients; in nine patients there were neither endocrinological nor neurological symptoms.

Mutations of the ALD gene (n = 28) were detected in 50 patients (including nine sets of brothers) from 32 families. No correlation was found between the ALD gene mutation and endocrinological dysfunction. However, we found that all sets of brothers were concordant for the endocrinological phenotype (cortisol synthesis was reduced in two sets and normal in seven sets), whereas four sets showed a discordant neurological phenotype. As yet unknown hereditary factors other than mutations within the ALD gene may interfere with the endocrinological phenotype more strongly than with the neurological phenotype of ALD.

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Introduction

X-linked adrenoleucodystrophy (ALD) has been shown to be one of the most frequent causes of Addison’s disease in men, with frequencies between 13% and 62% in countries without widespread tuberculosis (1–4). ALD is associated with demyelination of the central or peripheral nervous system, with or without adrenal insufficiency, and is characterized biochemically by an impaired peroxisomal β-oxidation of very long chain fatty acids (VLCFA). Mutations have been found in the ALD gene encoding a membrane transport protein which has been suggested to be involved in the import of VLCFA coenzyme A synthetase into the peroxisome (5).

There is a striking variability of endocrinological and especially neurological symptoms in patients with ALD, even within the same kindred. The most frequent neurological phenotypes are cerebral ALD (cALD, 45%) and adrenomyelo-neuropathy (AMN, 35%) (6). Demyelination of cerebral white matter in patients with cALD usually begins in childhood and is characterized by a rapid neurological deterioration. AMN develops in adolescence and adulthood as a slowly progressive spastic paraparesis because of spinal and peripheral demyelination. No clearly evident correlation between mutations of the ALD gene and the different neurological phenotypes has been found (7–11).

Cerebral ALD and AMN are frequently associated with Addison’s disease, but the primary adrenal insufficiency may precede, coexist or develop after neurological dysfunction (6). Adrenal insufficiency was reported to be present before onset of neurological symptoms in 39% of patients with ALD (12) and in 61% of patients with AMN at the time of diagnosis (13), and may remain the only clinical expression of ALD (8% of cases) (14). Hypogonadism as a further...
endocrinological manifestation of ALD has been observed in adult men (15). Examinations of a correlation between the ALD genotype and the endocrinological phenotype have not been reported previously.

Subjects and methods

Patients

We studied 55 male patients (median age 11 years, range 2–59 years) from 34 families. They were diagnosed as having ALD because of their clinical presentation (n = 33) or by biochemical examination of relatives of index cases (n = 22). The classification of the different ALD phenotypes (cALD/AMN with or without endocrinological symptoms, isolated endocrinological symptoms, and asymptomatic patients) was made on the basis of clinical, laboratory, electro-physiological and neuroimaging findings at the time of the first endocrinological examination, except for three initially neurologically asymptomatic boys, who developed cALD within the period of observation and whose classification was changed correspondingly. The relatively high proportion of children (age <14 years, n = 33) and adolescents (age 14–18 years, n = 6) included in the study is explained by the fact that the diagnostic laboratory is located in a children’s hospital. VLCFA levels were increased significantly in all patients: 26:0 – 2.46 ± 0.66 nmol/ml (control 0.83 ± 0.46 nmol/ml), 24:0/22:0 – 1.43 ± 0.19 nmol/ml (control 0.84 ± 0.08 nmol/ml) and 26:0/22:0 – 0.062 ± 0.018 nmol/ml (control 0.013 ± 0.009 nmol/ml). (All control data are from reference 16.)

The patients were asked specifically about symptoms of adrenal insufficiency, including exhaustion, exercise intolerance, weakness, drowsiness, nausea, vomiting, abdominal pain, dehydration, anorexia, weight loss or salt craving. Adults were asked in addition about symptoms of androgen deficiency such as loss of potency or libido. The examinations included a complete physical and neurological examination with determination of blood pressure, laboratory investigations, magnetic resonance imaging, nerve conduction velocity and evoked potentials. In all patients, routine laboratory variables (including blood count, sodium, potassium, calcium and transaminases) were measured. The patients were examined every 6 months in our hospital for a period between 6 and 60 months (median 29 months).

This study formed part of a therapy study of dietary treatment of ALD with glyceroltrioleate and glyceroltrierucate and was approved by the local ethics committee.

Endocrinological investigations

After placement of an i.v. access catheter in the forearm, blood samples for morning basal cortisol, ACTH, aldosterone, renin, testosterone, dehydroepiandrosterone (DHEA) sulphate, FSH and LH were obtained with the patient in the supine position. Immediately thereafter, ACTH (1–24 Synacthen, Ciba–Geigy, Wehr, Germany) was administered as an i.v. bolus injection of 0.25 mg/m², maximum 0.25 mg. Serum for measurement of cortisol was obtained at 30 and 60 min. Endocrinological examinations were repeated in most patients every 6 months and showed a good reproducibility.

Endocrinological assays

Serum samples were analysed routinely by an automatic analyser (Immulite, Biermann GmbH, Bad Neuenheim, Germany) for cortisol, testosterone, DHEA sulphate, FSH and LH. Serum aldosterone concentrations were determined using a commercially available RIA kit (Serono, Freiburg, Germany). Plasma ACTH concentrations were measured by immunoradiometric assay (Nichols, Bad Neuenheim, Germany). Plasma renin concentrations were determined by RIA (Sorin Biomedica, Düsseldorf, Germany). Adrenocortical antibodies were examined by a histochemical microanalysis method (17).

Metabolic and molecular genetic examinations

Examinations of plasma VLCFA were performed as described earlier (18). Genomic DNA was amplified by standard PCR methods, including the coding region of the entire ALD gene (EMBL database, no. z178569) and additional intron sequences of 20 (for example 5’ncr) to 120 bases (e.g. 3’ncr). Details have been described previously (11). Amplified fragments were subsequently sequenced on an automated sequencer (Applied Biosystems, model 373A).

Statistical methods

Pearson correlation coefficients were determined by statistical analysis system (SAS).

Results

Patients

Clinical symptoms of all patients are summarized in Table 1. In total, 46 of 55 patients developed endocrinological or neurological symptoms, or both. Endocrinological symptoms were observed in 34 patients, combined with neurological symptoms in 21 patients. Isolated neurological symptoms were seen in 12 patients (cALD n = 7, AMN n = 5). Cerebral ALD was found in 22 patients (median age at neurological presentation 8 years, range 4–15 years; median age at examination 9 years 6 months, range 5–26 years), the AMN phenotype was seen in 11 patients (median age at neurological presentation 30 years, range 18–50 years; median age at examination 35 years, range 24–59...
years). Figure 1 shows the individual sequence and course of endocrinological and neurological symptoms in each of the 46 affected patients. Endocrinological symptoms preceded neurological symptoms in 92% of the youngest children with clinical onset before the age of 6 years. In comparison, in 80% of older patients with clinical onset after the age of 20 years, neurological symptoms preceded the endocrinological symptoms. The higher proportion of endocrinological symptoms in children and adolescents than in adults is reflected by the fact that nearly 67% of the patients with cALD, but only 50% of the patients with AMN, had additional endocrinological symptoms. The interval between the onset of neurological and endocrinological symptoms may be longer than 10 years, independent of whether neurological or endocrinological symptoms occur first, and independent of the neurological phenotype. Nine patients (median age at examination 13 years, range 2–21 years) who showed neither endocrinological nor neurological symptoms were classified as asymptomatic.

A male-pattern baldness was a consistent finding in all patients with AMN (n = 11). They showed grades III to IV according to the baldness evaluation scheme of Norwood (19), independent of other endocrinological symptoms, and all had normal body hair. Male-pattern baldness was found in two patients with AMN (aged 35 and 59 years) with low normal testosterone values and in one patient (aged 51 years) without any clinical or laboratory evidence of disturbed endocrinological function. Sparse hair with the beginnings of alopecia was found additionally in three patients with cALD with Addison’s disease (ages 12 years 6 months, 18 years and 21 years). First signs of balding in the youngest patient were first noticed at the age of about 8 years.

**Glucocorticoids**

Low basal serum cortisol levels (<140 nmol/l, 0800 h) providing evidence for adrenal insufficiency were found in 21 of the 55 patients. Cortisol values between 140 and 280 nmol/l, strongly suggestive of this diagnosis, were observed in an additional 11 patients.

The i.v. ACTH loading test was performed in all patients, with calculation of the cortisol stimulation after 30 and 60 min. The stimulation was defined as normal when the cortisol levels increased at either time

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**Table 1 Clinical endocrinological and neurological symptoms of 55 patients with ALD.**

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Hyper-pigmentation</th>
<th>Exercise intolerance</th>
<th>Vomiting</th>
<th>Addison crisis</th>
<th>cALD</th>
<th>AMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrinological</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Endocrinological and neurological</td>
<td>21</td>
<td>19</td>
<td>6</td>
<td>12</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Neurological</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>No symptoms</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>30</td>
<td>13</td>
<td>19</td>
<td>5</td>
<td>22</td>
</tr>
</tbody>
</table>

cALD, Cerebral adrenoleucodystrophy; AMN, adrenomyeloneuropathy.

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**Figure 1** Clinical course of the asymptomatic and symptomatic periods of each of the 46 endocrinological and/or neurological symptomatic patients. Endocrinological symptoms are more frequent in younger than in older patients.
by more than 190 nmol/l or to more than 550 nmol/l (13, 20). A reduced cortisol response was found in 36 patients and among these the basal cortisol value was <140 nmol/l in 21 patients, 140–280 nmol/l in 10 patients and >280 nmol/l in five patients. A diminished cortisol response 30 min after i.v. ACTH which normalized after 60 min was found in two patients. No patient with a normal cortisol response 30 min after i.v. ACTH had a diminished response after 60 min.

In 48 of 55 patients with ALD, increased basal plasma ACTH (normal 2–11 pmol/l) was shown to be the most frequent pathological endocrinological parameter. The increase in ACTH was slight in three of them (11–22 pmol/l), moderate in 15 (22–66 pmol/l) and high in 30 (>66 pmol/l) (Table 2). There was no difference in frequency of increased ACTH between neurologically symptomatic (28 of 33) and neurologically asymptomatic patients (20 of 22). Increased ACTH levels were measured in 90% of the children and adolescent patients (36 of 40) and 80% of the adults (12 of 15). In our patients with normal plasma ACTH levels (n = 7) who had been followed between 6 and 60 months (mean 54 months), no time-dependent progression of ACTH values was observed. There was a significant negative correlation between basal ACTH levels and the cortisol level 60 min after i.v. ACTH (r = −0.84, P < 0.001). There was also a significant negative correlation between basal ACTH levels and the increase in cortisol in response to i.v. ACTH within 60 min (r = −0.80, P < 0.001, Fig. 2). All patients with a reduced cortisol response to i.v. ACTH (n = 36) had increased ACTH levels (Table 2). Among 30 patients with ACTH levels >66 pmol/l, 29 showed a pathological cortisol stimulation in response to i.v. ACTH. However, there was one patient (age 4 years 9 months) with a basal ACTH level of 181 pmol/l and a normal cortisol stimulation in response to i.v. ACTH only after 60 min (cortisol values 340 nmol/l at 0 min, 392 nmol/l at 30 min and 596 nmol/l at 60 min).

Thirty-three of the 36 patients with a reduced cortisol response to i.v. ACTH also showed clinical signs of adrenal insufficiency, such as discrete hyperpigmentation or exercise intolerance. The pathological ACTH stimulation test showed a better correlation than the basal ACTH level with the occurrence of endocrinological symptoms (Table 2), therefore hydrocortisone treatment was initiated independent of the basal plasma concentration of ACTH in all patients with a reduced cortisol response to i.v. ACTH. There were two exceptional patients with reduced cortisol response without clinical signs of adrenal insufficiency. They were brothers: the older (patient 8b, Table 3) had slightly increased ACTH (14 pmol/l) and a borderline increase in ACTH-stimulated cortisol response (cortisol values 348 nmol/l at 0 min, 483 nmol/l at 30 min and 497 nmol/l at 60 min), and the younger (patient 8a, Table 3) had a moderately increased basal ACTH level (33 pmol/l) and an inadequate ACTH-stimulated cortisol response (cortisol values 287 nmol/l at 0 min, 375 nmol/l at 30 min and 381 nmol/l at 60 min). ACTH-stimulation testing was repeated in both patients, and gave nearly the same results. Hydrocortisone therapy was refused by the parents. Both patients show no clinical symptoms of adrenal insufficiency and are active rowers, carrying first-aid kits with hydrocortisone during training.

There was no correlation between VLCFA concentrations and ACTH (r = −0.05) or between VLCFA levels and clinical endocrinological symptoms.

### Table 2 Relation between ACTH level (normal ≤ 11 pmol/l), pathological ACTH test and endocrine symptoms. A pathological ACTH test has a greater correlation to endocrine symptoms than pathologically increased ACTH levels.

<table>
<thead>
<tr>
<th>ACTH (pmol/l)</th>
<th>Patients</th>
<th>Pathological cortisol stimulation after i.v. ACTH</th>
<th>Endocrine symptoms</th>
<th>Hydrocortisone therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤11</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11–22</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22–66</td>
<td>15</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>&gt;66</td>
<td>30</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>35</td>
<td>33</td>
<td>33</td>
</tr>
</tbody>
</table>

**Figure 2** Semi-logarithmic graph showing a correlation between the basal plasma ACTH level and increase in cortisol 60 min after stimulation by i.v. ACTH in the Synacthen loading test (r = −0.80, P < 0.001, determined by statistical analysis system).
Mineralocorticoids

Aldosterone and renin were determined in all patients, but data before the start of hydrocortisone therapy with or without fludrocortisone therapy were available for only 35 patients. In 16 of these patients, hydrocortisone therapy was initiated because of increased ACTH levels and pathological i.v. ACTH-stimulated cortisol response. Serum aldosterone concentrations were not diminished in any patient, with or without hydrocortisone therapy. Plasma renin levels were slightly to moderately increased (7.8–28 μg/l per h, normal range 2–7) in seven patients. There was no correlation between renin and electrolyte concentration or blood pressure in these patients. In four of these seven patients, aldosterone levels were increased (1057–2644 nmol/l, normal range 40–970 nmol/l), but in the other three, aldosterone was normal. Increased aldosterone levels were observed in two more patients (1295 and 3018 nmol/l), resulting in a total of six patients with increased aldosterone levels. All patients with aldosterone concentrations greater than 1400 nmol/l showed an additional increase in ACTH (23–352 pmol/l).

Androgens

Indication of reduced androgen synthesis was seen in 12 of the 16 adult patients. The most frequent pathological parameter in ten of 14 adult patients examined was a decreased DHEA sulphate serum level (normal 1.9–13.7 μmol/l). DHEA sulphate levels were clearly decreased (0.3–1.7 μmol/l, median 0.8 μmol/l) in seven patients, and were in the lower normal range (1.9–2.4 μmol/l) in three other patients. Only one of the ten patients with a decrease in DHEA sulphate serum concentration had a low normal testosterone level (10.7 nmol/l, normal range 10.1–39.9 nmol/l).

An increase in LH indicating a Leydig cell insufficiency was found in five patients (12.4–19.1 IU/l, normal 0.8–8.3 IU/l), accompanied by borderline decreased testosterone values in two patients (10.4 and 10.7 nmol/l). A decrease in testosterone was not found in any other patients. Increase in FSH indicating a disturbed function of the tubuli seminiferi was seen in four patients (10.4–17.3 IU/l, normal range 1.2–10.1 IU/l), in three of them in combination with increased LH.

Most of the adult patients with diminished androgen synthesis also showed a diminished glucocorticoid synthesis (n = 11). Isolated disturbances of androgen synthesis were found in only one patient, compared with isolated diminished glucocorticoid synthesis seen in two patients. In only two patients were glucocorticoid and androgen synthesis completely normal.

Adrenocortical antibodies

All patients were examined for adrenocortical antibodies and proved negative.

Genetic examinations

In 50 patients from 32 families, 28 different mutations of the ALD gene were detected: missense mutations in seven patients, and were in the lower normal range (1.9–2.4 μmol/l) in three other patients. Only one of the ten patients with a decrease in DHEA sulphate serum concentration had a low normal testosterone level (10.7 nmol/l, normal range 10.1–39.9 nmol/l).

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(n = 18), frame-shift mutations (n = 5), in-frame deletions (n = 2), in-frame insertion (n = 1), nonsense mutation (n = 1), splice site mutation (n = 1). Sixteen of these mutations have been published before (11, 21); the remaining 12 mutations comprise nine missense mutations (A141T, Y281H, R389H, G512S, P543L, R554H, Y559H, R617H, R679R), two frame-shift mutations (del 740, del 2132) and one splice site mutation (ins 8 bp 2252). There was no correlation between the loci or kind of mutation and ACTH concentration (Fig. 3). In two families, no mutation was found in the entire coding region of the ALD gene, including flanking intron sequences.

Within the total group of 55 patients, 19 patients were brothers: eight pairs of two brothers and one family with three brothers (Table 3). All siblings had the same genotype of the ALD gene and were concordant for the endocrinological phenotype. There was evidence of laboratory adrenal dysfunction in seven sets, whereas cortisol synthesis was completely normal in two pairs. ACTH-stimulated cortisol response and basal ACTH values were very similar in eight of the nine sets. In comparison, four sets showed a discordant neurological phenotype. One of these sets with neurological discordance and endocrinological concordance were monozygotic twins (patients 3a and 3b), who have been reported previously (21).

Discussion

The as yet not understood variability in neurological symptoms (7, 22) has led to a keen interest in the neurology of ALD. However, endocrinological abnormalities are as frequent in patients with ALD as neurological symptoms are. The younger the patient at manifestation of disease, the more frequently do endocrinological symptoms occur as initial symptoms. On the other hand, the proportion of patients in whom Addison’s disease is attributable to ALD is age-dependent, and is highest when the adrenal insufficiency manifests before age 15 years (23). In light of the high and early prevalence of endocrinological symptoms in ALD, it is remarkable that these symptoms led to the diagnosis of ALD in only 11 of our 55 patients.

Adrenocortical dysfunction is defined by clinical symptoms of adrenal insufficiency or an impaired cortisol response in the conventional ACTH stimulation test, which is the standard for evaluating the adequacy of adrenal cortical function (24). In contrast to observations reported by others (2), we found significantly greater cortisol concentrations after 60 min than after 30 min in two patients. We therefore feel that it is important always to determine cortisol after 60 min.

We found plasma ACTH to be the most sensitive laboratory marker for adrenocortical dysfunction in infantile, adolescent and adult patients with ALD, as described recently for adults with AMN (13). In patients with Addison’s disease due to an adrenal autoimmune process, an abnormal ACTH stimulation test could be observed before an increase in plasma ACTH and clinical symptoms developed (25). In patients with ALD, serial measurement of plasma ACTH every 6 months may be sufficient to identify those patients with subclinical adrenal involvement, who then may be more closely monitored to permit early detection of clinical and biochemical evidence of overt adrenal insufficiency.

Mineralocorticoid deficiency in ALD is a rare phenomenon. A decrease in serum aldosterone concentration was not detectable in our patients. Similar results

Figure 3 ACTH concentration and the corresponding mutation found in the ALD gene (5) of each patient. The type of mutation and the location on ALD cDNA are shown. There is correlation neither of the kind nor of the locus of mutation and its corresponding domain with the level of ACTH. Mi, missense; No, nonsense; SM, splicing mutation; Fs, frameshift; ID, in-frame deletion, Il, in-frame insertion.
were reported by Cappa et al. (26) in eight of nine patients with ALD. Increased aldosterone levels together with increased ACTH, found in four patients, may be a consequence of overstimulation of the zona glomerulosa by increased ACTH. Increased plasma renin activity, found in seven patients, indicates latent mineralocorticoid deficiency and renal salt loss compensated by higher renin levels. In all patients, renin and aldosterone should be determined before the start of therapy. During hydrocortisone therapy, renin should be measured regularly to establish whether hydrocortisone therapy alone is sufficient or should be accompanied by fludrocortisone.

A reduced adrenal androgen synthesis, reflected by the low DHEA sulphate levels found in nearly all adult patients examined, seems to be much more frequent than testicular insufficiency with diminished testosterone levels. In contrast to findings in other male patients with non-ALD/AMN Addison’s disease, all patients with AMN presented here showed a ‘male-pattern baldness’. This term has now been replaced by ‘androgenetic alopecia’, which more precisely characterizes this androgen-driven hereditary disorder (27). The prevalence in healthy male controls is 47% between the third and fifth decade (28). Men with androgenetic alopecia show increased DHEA sulphate levels (29) and increased plasma cortisol concentrations (30), whereas in patients with AMN, reduced adrenal androgen and cortisol synthesis are found. We therefore suggest the term ‘pseudo-androgenetic alopecia’ for AMN patients with baldness, the pathomechanism of which remains to be elucidated.

It has been postulated that adrenocortical insufficiency in ALD is directly attributable to the accumulation of VLCFA (31, 32). However, there was no correlation between the plasma levels of VLCFA and ACTH in our patients. Blevins et al. (13) described a time-dependent progression of ACTH levels in patients with AMN after a median follow up of 3 years. In our patients with normal plasma ACTH levels (n = 7) who were followed between 6 and 60 months (mean 54 months), no time-dependent progression of ACTH levels could be observed. In addition, in our patients, there was no increasing incidence of endocrine symptoms with age. In contrast, children with ALD have endocrinological symptoms more often than adults.

We found no correlation between the kind and the location of ALD gene mutation and the endocrinological phenotype. The finding of normal adrenal function in patients with a splice site mutation of the ALD gene suggests that, besides ALD gene mutation and the increase in VLCFA, additional factors should be responsible for development of endocrinological symptoms in X-linked ALD. The concordance of endocrinological phenotype in brothers, and more frequent discordance of the neurological phenotype, that we observed in our patients, have been described recently in a pair of monozygotic twins with ALD (33). This phenomenon suggests that hereditary factors other than the ALD gene influence the endocrinological phenotype more than the neurological phenotype.

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