INVITED REVIEW

Adrenoleukodystrophy: phenotype, genetics, pathogenesis and therapy*

Dedicated to the memory of Peter Moser

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Summary

The occasion of the presentation of the eighth Gordon Holmes Lecture left me feeling both honoured and awed, as a result of my review of the Selected Papers of Gordon Holmes (Phillips CG: Selected Papers of Gordon Holmes, compiled and edited for the Guarantors of Brain. Oxford University Press, 1979), kindly presented to me by the sponsors of the meeting. This volume lists 174 publications produced over a 55-year period, and contains reprints of contributions to neuroanatomy, neuropathology, and to disorders that affected the adrenal cortex, the spinal cord, the cerebellum and the cerebral cortex.

Yet I also feel a sense of sadness; the invitation to present the lecture came from the late Anita Harding who, such a short time before her illness, gave me personal guidance and encouragement. In this lecture I endeavour to follow the example of Gordon Holmes, namely the stepwise analysis of a clinical problem, first by observation of the patient, followed by the application of techniques that can clarify it, leading to new knowledge not only about the specific disorder, but also about the nervous system and human biology in general and, it is to be hoped, to more effective therapy.

Keywords: adrenoleukodystrophy; genetics; pathogenesis; phenotype; therapy

Abbreviations: ABC = ATP-binding cassette; AMN = adrenomyeloneuropathy; CCER = childhood cerebral form of X-ALD; GTE = glyceryl trierucate (oil); GTO = glyceryl trioleate (oil); TNF-α = tumour necrosis factor; VLCFA = very long chain fatty acids; VLCS = very long chain fatty acid coenzyme-A synthase; X-ALD = X-linked adrenoleukodystrophy

History of X-linked adrenoleukodystrophy

The history of adrenoleukodystrophy has taken many twists and turns, and is marked by serendipity and surprises; it is likely that Haberfeld and Spieler (1910) described the first patient with what is now called X-linked adrenoleukodystrophy (X-ALD). A previously normal boy developed disturbances in eye movement and vision at the age of 6 years, became apathetic, and his schoolwork deteriorated. Four months later his gait became spastic, and this progressed to an inability to walk. He was hospitalized at 7 years. Dark skin was noted, but otherwise not discussed. He had a spastic paraparesis, severe apathy alternating with irritability, did not speak, and was incontinent. He died 8 months later. An older brother had died of a similar illness at 8.5 years. The post-mortem brain was studied by Paul Schilder (1913) and reported as the second of three cases that he referred to as ‘encephalitis periaxialis diffusa,’ characterized by diffuse involvement of the cerebral hemispheres in children with severe loss of myelin, which resembled multiple sclerosis because of the relative preservation of axons and the accumulation of lymphocytes, fat-laden phagocytes and glial cells (Schilder, 1912 and 1924). The findings in the adrenal gland were not reported. Subsequent studies have shown that what had been referred to as Schilder’s disease is heterogeneous and includes a variety of entities in addition to X-ALD (Poser and van Bogaert, 1956; Poser et al., 1986). Siemerling and Creutzfeldt (1923) reported on a patient with a similar clinical history

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and neuropathological findings, except that involvement of the adrenal glands was documented. They named the disorder ‘Bronzekrankheit und Sklerosierende Encephalomyelitis (diffuse sklerose)’. The name adrenoleukodystrophy was coined by Blaw in 1970 (Blaw, 1970).

During the period 1923–1974 there was considerable speculation about the nature of X-ALD. The connection between the adrenal insufficiency and the demyelinative process remained a puzzle. In their classic paper on the pathology of the demyelinative diseases, Adams and Kubic (1952) placed multiple sclerosis and X-ALD in the same general category, but noted points of difference, such as the contiguous involvement of large portions of the cerebral hemispheres and the more severe degree of axon-cylinder degeneration in X-ALD. In their discussion of pathogenetic mechanisms they noted the points of resemblance to experimental allergic encephalomyelitis and suggested autoimmune mechanisms. They discounted a metabolic disorder or the direct action of an enzyme on the nervous system ‘because of the presence of the infiltrates of inflammatory cells which are not seen in any of the known metabolic disorders of the brain’. Even though there is now no doubt that X-ALD results from a metabolic disorder, recent studies of the pathogenesis of the brain inflammatory response in X-ALD indicate that the autoimmune mechanisms invoked by Adams and Kubic (1952) are still highly pertinent. Hoefnagel et al. (1962), Fanconi et al. (1963) and Blaw et al. (1964) noted that all patients had been male, and on the basis of pedigree analysis, suggested that X-ALD is a genetically determined disorder with an X-linked mode of inheritance.

The major insight into the nature of X-ALD took place at the Albert Einstein College of Medicine in New York in 1973, 50 years after the initial description of the disease. It was the consequence of the collaboration between a neurologist, Herbert Schaumburg, who had been attracted to the study of X-ALD by E. P. Richardson at the Massachusetts General Hospital, and an endocrine pathologist, James Powers. Powers and Schaumburg (1973) demonstrated unusual striations in adrenocortical cells which were shown, by electron microscopy, to have a characteristic lamellar structure. Igarashi et al. (1976a) later showed that these inclusions contained cholesterol esterified with saturated very long chain fatty acids (VLCFA). These findings stamped X-ALD as a lipid-storage disease and revolutionized the direction of research.

Singh et al. (1984a, b) in Baltimore demonstrated that X-ALD patients had an impaired capacity to degrade VLCFA, and that this reaction normally takes place in the peroxisome. This finding assigned X-ALD to the newly recognized category of peroxisomal disorders, along with the Zellweger cerebrohepatorenal syndrome, which had been characterized by Goldfischer et al. (1973), also at the Albert Einstein College of Medicine and in the same year as Schaumburg and Powers’ key observations about X-ALD. At that time, however, the relationship between the two disorders was not recognized. Follow-up studies by Singh and his associates (Lazo et al., 1988) and by Wanders et al. (1988) in Amsterdam pinpointed the VLCFA oxidation defect to the impaired capacity to form their coenzyme-A derivatives, leading to the plausible suggestion that the basic defect involved the enzyme ‘very long chain fatty acid coenzyme-A synthase’ (VLCS). Between 1978 and 1981 it was shown in Baltimore and in Japan that X-ALD can be diagnosed reliably by demonstrating abnormally high levels of VLCFA in cultured skin fibroblasts (Moser et al., 1980), red blood cells (Tsuij et al., 1981b) and plasma (Moser et al., 1981). These non-invasive assays have led to the identification of >3000 patients in the Kennedy Krieger Institute diagnostic programme alone. The ultrastructural and biochemical techniques also led to the recognition of milder X-ALD phenotypes in adults such as adrenomyeloneuropathy (AMN) by Budka et al. in Vienna in 1976 (Budka et al., 1976) and Griffin et al. at the National Institutes of Health in 1977 (Griffin et al., 1977). The era of dietary therapy was ushered in by the demonstration of Kishimoto et al. (1980) that the abnormal VLCFA in the brain of an X-ALD patient are, at least in part, of dietary origin and it was extended by the observation that mono-unsaturated fatty acids, such as oleic and erucic acid, reduce their endogenous synthesis (Rizzo et al., 1986, 1989). The demonstration that bone marrow-derived cells have the capacity to degrade VLCFA led to therapeutic trials of bone-marrow transplantation (Moser et al., 1984), which have proved successful in some instances (Aubourg et al., 1990; Krivit et al., 1995).

In 1981 the X-ALD gene was mapped to Xq28, the terminal segment of the long arm of the X-chromosome (Migeon et al., 1981). Twelve years later Mosser et al. (1993) isolated the gene by positional cloning. It came as a surprise that the gene codes for a peroxisomal membrane protein, referred to as ALD protein, that belongs to the ATP-binding cassette (ABC) protein family (Higgins, 1992). It has no homology to VLCS, which has been isolated recently (Uchiyama et al., 1996).

**X-ALD phenotypes**

**Clinical presentations**

Table 1 summarizes the main phenotypes of X-ALD, which have been described in detail elsewhere (Kaback, 1972; Schaumburg et al., 1975; Griffin et al., 1977; H. W. Moser et al., 1987, 1994; A. B. Moser et al., 1991; Sadeghi-Nejad and Senior, 1990). A wide range of phenotypic variability is evident. The disease ranges from the rapidly progressive childhood cerebral (CCER) form, which leads to total disability during the first decade, to the milder AMN, which is compatible with survival to the eighth decade. These two forms of the disease differ fundamentally with respect to age of onset (Fig. 1), length of survival (Fig. 2) and neuropathology. In CCER there is a diffuse demyelinative process that involves the cerebral hemispheres, beginning most commonly in the parieto-occipital region and is
Table 1 | Phenotypes in X-linked adrenoleukodystrophy hemizygotes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Childhood cerebral</td>
<td>Onset before 10 years of age, progressive behavioural and cognitive neurological deficits, inflammatory brain demyelination. Total disability often within 3 years.</td>
</tr>
<tr>
<td>Adolescent cerebral</td>
<td>Like childhood cerebral, but onset at 10–21 years of age.</td>
</tr>
<tr>
<td>AMN</td>
<td>Onset at 28 ± 9 years, paraparesis progressive over decades, distal axonopathy, inflammation mild or absent, mainly spinal cord involvement, cerebral involvement later in 45% of cases.</td>
</tr>
<tr>
<td>Adult cerebral</td>
<td>Rapid inflammatory cerebral progression resembling the childhood form, without preceding AMN, onset after 21 years of age.</td>
</tr>
<tr>
<td>Addison’s disease only</td>
<td>Primary adrenocortical insufficiency without neurological abnormalities.</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>ALD gene abnormality without neurological or endocrine abnormalities.</td>
</tr>
</tbody>
</table>

Fig. 1 Age at onset of neurological symptoms of cerebral forms of X-ALD (broken line) and AMN (solid line). Note that the cerebral forms begin most commonly in childhood; adolescent and adult onset occurs only occasionally. The earliest onset of AMN was at the age of 12 years. (With permission, from Moser et al., 1992.)

Fig. 2 Survival from time of first neurological symptom (untreated males only). Note the rapid rate of progression of cerebral forms irrespective of age at onset. Filled circles = AMN (n = 116); filled triangles = adult cerebral (n = 3); plus signs = adolescent cerebral (n = 60); asterisks = childhood cerebral (n = 257).

associated with a perivascular infiltration with lymphocytes and macrophages, resembling that seen in multiple sclerosis (Siermerling and Creutzfeldt, 1923; Schaumburg et al., 1975; Powers, 1985; Powers et al., 1992). MRI Studies show features that are often characteristic, with symmetrical demyelination in the parieto-occipital region and accumulation of contrast material at the advancing margin (Fig. 3) (Kumar et al., 1987). There are other less characteristic patterns, however, such as early frontal involvement (MacDonald et al., 1984; Castellote et al., 1995) in ~15% of patients, or presentation as an asymmetric mass lesion, which has been mistaken for a brain tumour (Young et al., 1982; Close et al., 1993; Afifi et al., 1996). Magnetic resonance spectroscopy shows a diminution in the N-acetylaspartate peak and an increase in the choline peak (Kruse et al., 1994; Confort-Gouny et al., 1995; Rajanayagam et al., 1996). The magnetic resonance changes precede those demonstrable by MRI. In contrast to CCER, AMN is a distal axonopathy that most severely involves the distal aspects of the long tracts in the spinal cord. The inflammatory reaction is mild or absent (Schaumburg et al., 1977; Powers, 1985; Van Geel et al., 1996). AMN may also present as a progressive cerebellar disorder, resembling olivopontocerebellar degeneration (Marsden et al., 1982; Kuroda et al., 1983; Ohno et al., 1984).

In a series of 112 AMN patients we found that in 54% the brain MRI was normal and demonstrable neurological involvement was confined to the spinal cord and peripheral nerves (Kumar et al., 1995). These patients, to whom we refer as cases of ‘pure AMN,’ have a better prognosis, and their neuropsychological function is normal except for mild deficits in psychomotor speed and visual memory (Edwin et al., 1996). Forty-six percent of the AMN patients had various degrees of MRI abnormalities in brain, which were associated with greater impairment of neuropsychological function (Edwin et al., 1996) and a less favourable prognosis (Fig. 4). We refer to these patients as AMN-cerebral. In some patients with AMN the MRI abnormality resembles that in CCER, and clinical progression may be as rapid. Thus, some patients with the slowly progressive AMN phenotype may develop the rapidly progressive inflammatory cerebral disease...
at a later age. The ‘adult cerebral’ patients are those who develop rapidly progressive cerebral symptoms (associated with an inflammatory response) without prior evidence of AMN. This distinction may not be absolute, because subtle signs pointing to spinal cord or peripheral nerve involvement may have been missed. Distinction between younger adolescent cerebral and CCER, and older adolescent cerebral and young AMN-cerebral patients may also be difficult.

Approximately 20% of women heterozygous for ALD develop neurological disability which resembles AMN, but it is of later onset (mean age 37.8 ± 14.6 years) and somewhat milder than in affected males. Dr Sakkubai Naidu has examined 165 heterozygous women who attended meetings of the United Leukodystrophy Foundation mainly for the sake of their affected sons. She noted that 53% of these women showed some degree of neurological involvement which varied in severity from mild hyper-reflexia and vibratory sense impairment with little or no functional disability, to paraparesis requiring a wheelchair in six patients. Signs of dementia, behavioural or visual disturbances occur in 1–3% of heterozygous women, and adrenal insufficiency in ~1% (H. W. Moser et al., 1991a).

Relative frequency of phenotypes and patterns of distribution within kindreds

Estimates of the relative frequency of X-ALD phenotypes may be hampered by ascertainment bias, as mildly involved patients or those with unusual phenotypes are more likely not to be diagnosed. We have used two approaches to reduce this bias. First, we have based our estimates on the 123 pedigrees in which we have the most complete information, thus reducing the likelihood of excluding undiagnosed X-ALD patients (Table 2). We have placed particular emphasis on the relative frequency of the CCER phenotype because, as discussed below, this value is important for the evaluation of therapeutic interventions. In this 123-kindred sample, 37% of the patients had the CCER phenotype, and this percentage was not altered significantly when the proband was excluded. Secondly, we analysed only those sibships in which the genotype and phenotype of all affected males had been ascertained (Table 3). In this somewhat smaller sample, 39% of the patients had the CCER phenotype. Exclusion of the proband reduced this figure to 33%. Our results are fairly comparable to those reported from the Netherlands (Van Geel et al., 1994) and France (Sereni et al., 1993), except that in the Dutch series AMN was the most common phenotype. Of note, nearly two-thirds of untreated X-ALD patients escape the most severe phenotype, CCER.

The various phenotypes co-occur frequently in the same kindred or nuclear family (Table 4). Indeed, it is more common for the severe and mild phenotypes to occur
Biochemical abnormalities

**Increased levels of saturated VLCFAs**

The abnormal accumulation of saturated unbranched VLCFAs is the principal and consistent biochemical abnormality in X-ALD (Igarashi et al., 1976a; Menkes and Corbo, 1977; Ramsey et al., 1979; Moser et al., 1980; Moser and Moser, 1991; Molzer et al., 1981; Tsuji et al., 1981b; Reinecke et al., 1985; Taketomi et al., 1987; Antoku et al., 1991; Theda et al., 1992). For the purpose of this discussion the abbreviation VLCFA refers to unbranched fatty acids with a chain length of 24 carbons or greater. VLCFAs are distributed widely in bacteria, plants, and normally also in mammalian tissues, such as the brain, retina, skin and hair (Rezanka, 1989; Poulos, 1995). In X-ALD tissues the excess is greatest in nervous tissue white matter, the adrenal cortex and testis, and in some lipid fractions, such as the cholesterol esters of the adrenal gland and brain, the excess over control values may approach 1000-fold.

In X-ALD patients, some degree of excess of saturated VLCFAs is present in all tissues and body fluids, and this has led to diagnostic assays with plasma VLCFA and cultured skin fibroblast, and also prenatal diagnosis (Moser et al., 1982; Verhoeven et al., 1995). We have recently re-examined the sensitivity and specificity of the plasma VLCFA assay by analysing data obtained in >1000 male X-ALD patients and 30,000 normal and non-peroxisome disease control subjects. We focused on three measurements, namely, the level of hexacosanoic acid (C26: 0), and its ratio to docosanoic (C22: 0) and tetracosanoic (C24: 0) acids. Although each was abnormal in X-ALD patients, some overlap with control subjects was observed. However, we were able to develop a discrimination function that provides virtually complete separation of X-ALD patients and control subjects (Fig. 5). With the aid of this function we have not experienced the false-negative or equivocal results that have been reported from other laboratories (Wanders et al., 1993; Kennedy et al., 1994). VLCFA are also increased in other peroxisomal disorders (Verhoeven et al., 1995), including the Zellweger syndrome, neonatal adrenoleukodystrophy and infantile Refsum disease, but these disorders have a completely different clinical presentation and are almost never confused with X-ALD. Approximately 85% of women

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**Table 2** Percentage of ALD phenotypes in the 123 kindreds with two or more affected males

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Distribution (%)</th>
<th>All hemizygotes (n = 637)</th>
<th>Hemizygote proband excluded (n = 530)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childhood cerebral</td>
<td>37</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Adolescent cerebral</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>AMN</td>
<td>32</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Adult cerebral</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Addison only</td>
<td>13</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

Our epidemiological survey of 826 kindreds currently includes 2196 X-ALD hemizygotes, 1712 heterozygotes, 1290 persons with diagnosis of X-ALD excluded by plasma VLCFA assay and 2128 persons without known neurological or adrenal dysfunction attributable to X-ALD, but with X-ALD status not determined by VLCFA assay. Since this last group includes undiagnosed mildly involved X-ALD patients, the data may underestimate the proportion of mildly involved phenotypes. In an attempt to reduce ascertainment bias, we include only the 123 kindreds for whom we have the most complete information.

**Table 3** Phenotype distribution among ALD hemizygotes in 253 sibships in which genotype and phenotype has been determined in all male members

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Distribution (%)</th>
<th>All affected males (n = 388)</th>
<th>Hemizygote proband excluded (n = 276)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childhood cerebral</td>
<td>39</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Adolescent cerebral</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>AMN</td>
<td>26</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Adult cerebral</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Addison only</td>
<td>14</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>13</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

This analysis of phenotype is based exclusively on sibships in which the genotype and phenotype of all males has been determined and it thus eliminates ascertainment bias introduced by persons whose X-ALD status is unknown.

within the same kindred than for a kindred to display only a single phenotype. Consequently, because of phenotypic heterogeneity within families, comparison of clinical course in treated and untreated sibs is of limited value in assessing the effectiveness of therapeutic interventions. Results of segregation analysis are discussed below.

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**Table 4** Phenotype distribution among multiplex kindreds and multiplex nuclear families

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Distribution (%)</th>
<th>Multiplex kindreds</th>
<th>CCER</th>
<th>CCER/AMN</th>
<th>AMN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>54</td>
<td>90</td>
<td>34</td>
<td>178</td>
</tr>
<tr>
<td>Multiplex nuclear families</td>
<td></td>
<td></td>
<td>90</td>
<td>35</td>
<td>33</td>
<td>134</td>
</tr>
</tbody>
</table>

*‘Nuclear families’ include sibships, and grandfather–grandson relationship. ‘Kindreds’ include all relatives. ‘CCER’ indicates that the childhood cerebral form was the only phenotype in the kindred or nuclear family. ‘AMN’ indicates that adrenomyeloneuropathy was the only phenotype. ‘CCER/AMN’ indicates that the childhood cerebral form and adrenomyeloneuropathy were both present. The rarer phenotypes in Tables 2 and 3 were present, but not included in this analysis.*
et al. and immunocytochemical studies of the gene product (ALD cytoplasmic face of the membrane and share considerable assay does not permit exclusion of heterozygote status. More membrane and they are believed to determine the specificity a in plasma and/or cultured skin fibroblasts (Moser et al.) would be found to involve the formation of VLCS synthase, to the peroxisomal membrane protein (Shani et al.).

The VLCFA excess is due to the impaired capacity to degrade as well as its relationship to the exons that code it and the Cause of the increased VLCFA levels

The VLCFA excess is due to the impaired capacity to degrade these substances (Singh et al., 1984a), a reaction that normally takes place in the peroxisome (Singh et al., 1984b). The defect in VLCFA oxidation has been pinpointed to the first step, i.e. the formation of the coenzyme-A derivative of VLCFA, a reaction that is catalysed by peroxisomal VLCS (Lazo et al., 1988). This enzyme has been purified and cloned only recently (Uchiyama et al., 1996). VLCS has been localized to the peroxisome, but there is dispute as to whether it is located on the cytosolic or luminal surface of the peroxisomal membrane (Lazo et al., 1990; Lageweg et al., 1991). It had been presumed that the gene defect in X-ALD would be found to involve the formation of VLCS synthase, but, as already mentioned and discussed below, the gene that is defective codes for a peroxisomal membrane protein, ALD protein. Since transfection of X-ALD cells with ALD protein restores their capacity to oxidize VLCFA (Cartier et al., 1995), it must be concluded that the protein facilitates this process in some way. Elucidation of the role of ALD protein in VLCFA oxidation is under intense investigation. Possibilities include a role in the transport of VLCS (Hettema et al., 1996) or their Co-A derivative into the peroxisome, or action as a platform for VLCS or its transport.

Genetics

The incidence of X-ALD is estimated to be between 1 : 20 000 and 1 : 100 000 (Moser et al., 1995). During the last 15 years we have identified 2238 hemizygotes in 740 kindreds. Patients have been identified in many races and geographic locations, including Afro-Americans and native Americans, Hispanics, Jews, Chinese, Japanese and Maoris, without apparent predilection for any one race. As already noted, the gene has been mapped to X-q28 and has been shown to be subject to X-inactivation (Migeon et al., 1981).

The gene was isolated by positional cloning (Moser et al., 1993). It contains 10 exons and spans 20 kb of genomic DNA. The protein product, ALD protein, contains 745 amino acids. It has been localized to the peroxisomal membrane (Moser et al., 1994). ALD protein appears to be a member of the ABC transporter superfamily (Higgins, 1992) a family of proteins that also includes the cystic fibrosis protein and the glycoproteins that impart multi-drug resistance. The typical ABC transporter consists of two related halves that together contain four domains. Two of these domains, one from each half, are highly hydrophobic and, in most instances, consist of six membrane-spanning segments. These form a channel through which the substrate crosses the membrane and they are believed to determine the specificity of the transporter. The other two domains are located on the cytoplasmic face of the membrane and share considerable sequence identity among ABC transporters. The ATP binding domain contains ~200 amino acids, and includes two short, highly conserved sequences, which are referred to as Walker A and B. Figure 6 shows the overall structure of the gene, as well as its relationship to the exons that code it and the location of the mutations that have been identified in X-ALD patients. Many of the ABC transporter genes encode a half-transporter which then dimerizes either with an identical molecule to form a homodimer or with another ABC half-transporter to form a heterodimer. Interestingly, peroxisomes have been shown recently to contain four ABC half-transporters, namely ALD protein, ‘ALD-related protein’, which has considerable homology to ALD protein but is not located on the X-chromosome (Lombard-Platet et al., 1996), 70-kDa peroxisomal membrane protein (PMP70) (Kamijo et al., 1990; Gartner et al., 1992), and another protein related to the peroxisomal membrane protein (Shani et al., 1995, 1996). Whether ALD protein dimerizes with itself or with

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**Fig. 5** Discrimination function for males based on the three plasma measurements evaluated in our VLCFA assay for the diagnosis of X-ALD and peroxisomal disorders (Moser and Moser, 1991). Dotted lines, values in 1097 patients with documented X-ALD; solid lines, values in 17 780 males who were normal or who had a variety of nonperoxisomal disorders. Postprandial samples are included, as VLCFA plasma levels do not change greatly after eating. All ages are represented; VLCFA levels in X-ALD patients do not vary with age (H.W. Moser, unpublished observation). Although there is some overlap between X-ALD and control when each of the three measurements are considered in isolation (data not shown), this is virtually eliminated by application of the discriminant function [function = 5.028(C24/22) – 2.539(C26/22) + 3.317(C26: 0)]. Diagnosis of those patients whose values fell within the small region of overlap is clarified by repeat analysis of a fasting plasma sample or by study of cultured skin fibroblasts. (With permission, from Moser et al., 1981.)

**Cause of the increased VLCFA levels**

The VLCFA excess is due to the impaired capacity to degrade these substances (Singh et al., 1984a), a reaction that normally takes place in the peroxisome (Singh et al., 1984b). The defect in VLCFA oxidation has been pinpointed to the first step, i.e. the formation of the coenzyme-A derivative of VLCFA, a reaction that is catalysed by peroxisomal VLCS (Lazo et al., 1988). This enzyme has been purified and cloned only recently (Uchiyama et al., 1996). VLCS has been localized to the peroxisome, but there is dispute as to whether it is located on the cytosolic or luminal surface of the peroxisomal membrane (Lazo et al., 1990; Lageweg et al., 1991). It had been presumed that the gene defect in X-ALD would be found to involve the formation of VLCS synthase, but, as already mentioned and discussed below, the gene that
Fig. 6 Distribution of X-ALD mutations in 81 ALD probands. Data are based upon results in our laboratory and patients reported in the literature. Top: postulated structure of ALD protein (only one half of the typical ABC transporter dimer). The transmembrane domain spans the membrane six times. The ATP-binding domain is peripherally located at the cytoplasmic face of the membrane. The typical ABC transporter contains two such domains with a total of 12 transmembrane spanning regions and two ATP binding domains. Middle: genomic organization of ALD protein (Sarde et al., 1994). The introns are not to scale. Bottom: location and relative frequencies of the mutations.

There is now convincing evidence that defects in ALD protein are indeed the cause of X-ALD. Mutations in the X-ALD gene have been demonstrated in all X-ALD patients who have been studied in sufficient detail, and these defects have not been found in control samples. Furthermore, transfection of X-ALD cells with the normal cDNA has been shown to correct the defect in VLCFA oxidation (Cartier et al., 1995). We have carried out mutational analyses in 35 unrelated X-ALD patients (Kok et al., 1995). Approximately 6% of patients have large deletions. Figure 6 shows the nature, location and frequency of mutations in 81 X-ALD patients based upon our data and those reported in the literature up to 1995. Except for an AG deletion in exon 5, which was identical in 13 (17%) of unrelated families, and thus represents a ‘hot spot,’ most mutations were ‘private,’ i.e. specific for a single kindred. Forty families (55%) had missense mutations, one of which occurred in three families, seven in two, and the remainder once. Frameshift mutations occurred in 23 families (30%), nonsense mutations occurred in six (8%), splice defects in four (5%), and an amino acid deletion was present in one family (1%). The exon 5 AG deletion has been associated with all phenotypes (Kemp et al., 1994). In one kindred, a missense mutation was associated with five different phenotypes (Berger et al., 1994). No correlation between the nature of the mutation and phenotype has been detected so far.

Immunocytochemical studies have shown that 70% of X-ALD patients lack ALD protein (Watkins et al., 1995b). No correlation was found between disease severity and the presence or absence of immunologically detectable
ALD protein. At the latest count (November, 1996), 105 separate mutations have been identified in 162 patients, with an overall pattern comparable to that cited above (S. Kemp, personal communication).

The basis of the phenotypic variability: search for a modifier gene
The severity of the biochemical defect (Boles et al., 1991), or as noted above, the nature of the mutation, does not account for the phenotypic variation in X-ALD. It has been suggested that a modifier gene might account for some of the phenotypic variability (H. W. Moser et al., 1991a, b). Environmental factors may also play a role, as suggested by two reports of phenotypic differences in two sets of identical twins; in one, two Japanese men with the AMN-cerebral phenotype show moderate differences in respect to the age of onset (32 versus 36 years) and severity of cerebral involvement and adrenal insufficiency (Sobue et al., 1994). In the other report, one 11-year-old boy developed typical childhood cerebral ALD at 10 years of age, while his twin remains asymptomatic and has a normal MRI at 11 years of age (Korenke et al., 1996). Further follow-up is required to determine if this fundamental difference in expression will persist.

Since the presence or absence of the brain inflammatory response is the main factor that differentiates the rapidly progressive cerebral forms of X-ALD from AMN and the milder variants, it is our working hypothesis that the postulated modifier gene acts by modulating the severity of this response. Because of the postulated role of tumour necrosis factor (TNF)-α (Powers et al., 1992), which is discussed below, we examined the possibility that variations in the inflammatory response might be related to variations in the TNF promoter, as has been shown for cerebral involvement in malaria (McGuire et al., 1994), or to allelic forms of the cytokine, as has been proposed for multiple sclerosis (Hauser, 1995). There was no correlation between either of these variables, however, and the severity of the inflammatory response in X-ALD (McGuinness et al., 1995).

Pathogenesis
Evaluation of the pathogenesis of X-ALD is in a state of flux. At this time, prime consideration is given to the role of abnormally high levels of VLCFA, the principal biochemical abnormality that has been demonstrated so far. It is possible that ALD protein has other functions unrelated to VLCFA degradation and that other, as yet unrecognized, pathogenetic mechanisms play a role.

Sources of VLCFA
C26: 0 has two sources: diet and endogenous synthesis. Kishimoto et al. (1980) showed that the VLCFA in X-ALD brain are of dietary origin, at least in part. These investigators administered 10 mg of deuterium-labelled C26: 0 ([3,3,5,5–2H]C26: 0) via a nasogastric tube to a terminally ill 9.5-year-old patient with CCER during the last 100 days of his life. At post-mortem examination, cholesterol esters were extracted from both mildly and severely involved white matter, and the percentage of deuterium-labelled C26: 0 was determined (Fig. 7). In the mildly involved area 96% of the C26: 0 was labelled. The pattern of label in the post-mortem brain samples was similar to that which had been administered by mouth. This finding leads to the conclusion that nearly all of the abnormal fatty acid in that region had been derived from what the patient had been fed during the last 100 days of his life.

Synthesis of fatty acids with chain length greater than 16 carbons is carried out by a fatty acid elongation system, which is present in both mitochondria and microsomes. The microsomal system appears to be more active and to have greater physiological significance (Murad and Kishimoto, 1978). The reaction has been demonstrated in rat sciatic nerve (Cassagne and Darriet, 1978) and in cultured skin fibroblasts (Tsuji et al., 1981a, 1985). Bourre et al. (1976) concluded that a single enzyme is responsible for the elongation of saturated fatty acids, such as behenic (C22: 0) and its mono-unsaturated counterpart, erucic acid (C22: 1), a finding that provided the rationale for the dietary therapy of X-ALD that is under current investigation. Studies in which deuterated water (2H2O) was administered to X-ALD patients have demonstrated substantial C26: 0 synthesis (Moser et al., 1983b, 1995). Indeed, Tsuji et al. (1981a) and Wilson et al. (1992) found that the microsomal elongating system was more active in X-ALD than in control cultured skin fibroblasts, which may contribute to the VLCFA accumulation in X-ALD. Dietary manipulation studies in X-ALD patients, which is discussed in the section on therapy (and illustrated in Figs 12 and 13), indicate that endogenous synthesis is quantitatively more important as a source of VLCFA in X-ALD patients than is the diet.

Effect of VLCFA on membrane structure and function
The greater length of the aliphatic chain causes VLCFA to be extremely insoluble and alters their physiological properties. Whereas albumin has six or more high- and low-affinity binding sites for fatty acids with 12–18 carbon chain length (Spector, 1975; Hamilton et al., 1991), it has only a single low affinity binding site for hexacosanoic acid (C26: 0) (Ho et al., 1995). Desorption of C26: 0 from phospholipid membranes is 10 000 times slower than that of fatty acids with a 14–18 carbon chain length (Fig. 8) (Ho et al., 1995). Microcalorimetric studies have shown that inclusion of C26: 0 in a model membrane disrupts membrane structure (Fig. 9). The microviscosity of red-cell membranes is increased in patients with X-ALD (Knazek et al., 1983). The
most direct evidence that abnormally high VLCFA levels can alter membrane function is provided by the study of Whitcomb et al. (1988), who assessed ACTH-stimulated cortisol release in cultured human adrenocortical cells. The addition of C26:0 or C24:0 to the culture medium in concentrations equivalent to those in X-ALD plasma increased the microviscosity of adrenocortical cell membranes and decreased ACTH-stimulated cortisol secretion. They concluded that the excess VLCFA altered membrane structure and suppressed the availability of the ACTH receptor. Analogous effects may exist in neural membranes, but this has not been demonstrated experimentally.

**Pathogenesis of adrenal dysfunction**

Adrenal dysfunction in X-ALD is due to primary adrenocortical insufficiency, and elevation of plasma ACTH levels is the initial manifestation (Blevins et al., 1994). Although the extent of clinically evident adrenal insufficiency is variable, as noted above, a substantial proportion of AMN patients, and most heterozygotes, fail to show clinical or biochemical evidence of adrenal insufficiency. Microscopic study reveals some degree of adrenal involvement in nearly all patients. Powers et al. (1987) demonstrated characteristic inclusions in a heterozygous woman who had a normal cortisol response to ACTH stimulation.
Powers et al. (1980) have conducted a correlative morphological and cytochemical study that provides insight into the pathogenesis of adrenal dysfunction. They concluded that the adrenal pathology is due to accumulation of abnormal lipids that contain VLCFA. The earliest change is the appearance of birefringent, cytoplasmic striations with lamellae in cortical cells of the inner zonae fasciculata-reticularis. The lamellae represent the formation or precipitation of lipid-protein aggregates or lipid bilayers (Fig. 10), which contain cholesterol esterified with VLCFA. Cells that contain these inclusions showed a reduction in mitochondrial and microsomal enzymes. Inflammatory cells were not present. As the disease advances, the adrenocortical cells atrophy.

Several factors can contribute to the accumulation of these abnormal cholesterol esters. (i) The impaired capacity to oxidize VLCFA. This leads to an increased proportion of VLCFAs among the fatty acid precursors of cholesterol esters. (ii) Cholesterol esterifying enzyme activity for C26:0 fatty acids is 16–38% that for oleic acid (C18:1), far in excess of the rate of hydrolysis of C26:0-containing cholesterol esters, which is 1/1000 of those that contain C18:1 (Ogino, 1980). This imbalance between the formation and degradation of C26:0-containing esters would be expected to lead to their increasing accumulation, and is the most likely cause for the lipid accumulation and the impaired adrenal function. The VLCFA-containing cholesterol esters would not be available as precursors for steroid hormone synthesis. (iii) The previously cited studies by Whitcomb et al. (1988) indicate that VLCFA excess in the plasma membrane may impair the function of the ACTH receptor.

The nervous system displays two types of pathology

The nervous system pathology in X-ALD displays two apparently disparate types: (i) the distal axonopathy associated with ‘pure’ AMN (Budka et al., 1976; Schaumburg et al., 1977; Powers, 1985), which manifests most commonly in late adolescence or adulthood; and (ii) the inflammatory demyelinating lesion associated with the rapidly progressive cerebral forms of the disease (Schaumburg et al., 1975; Powers et al., 1992), which, in the childhood form, often leads to death before the age at which AMN manifests. Analysis of the long-term course of the asymptomatic and Addison-only phenotypes suggests that all X-ALD patients who survive to adulthood will eventually develop the manifestations of AMN. The pathogenesis of the axonopathy is unknown. We speculate that the alterations in membrane structure and function associated with accumulation of VLCFA may play a pathogenetic role, but at this time there is no direct evidence for this. Studies in cultured Schwann cells (Rutkowski et al., 1995), or oligodendrocytes (Ishii and Volpe, 1992), or other nerve cell culture systems may help to clarify the pathogenesis of the axonopathy.

Pathogenesis of the inflammatory demyelinating lesion

The cerebral lesions in the rapidly progressive inflammatory forms of X-ALD resemble those in multiple sclerosis. In both conditions there is breakdown of myelin with relative sparing of axons, accumulation of cholesterol ester, and a perivascular inflammatory response with breakdown of the blood-brain barrier. Griffin et al. (1985) have typed the cells in the perivascular cuffs. They were able to identify 93% of the cells. Fifty-nine percent were T cells, 24% B cells, and 11% monocytes/macrophages. Fifty-eight percent of the T cells were T4, and 27% T8, while 15% could not be classified. This pattern was similar to that found in the CNS during a cellular immune response (Moench and Griffin, 1984), and this suggested that one component of X-ALD is
Adrenoleukodystrophy

Fig. 10 Adrenocortical cell containing both unilamellate and multilamellate inclusions that are both free in the cytoplasm and attached to various organelles (arrows). Electron micrograph taken from uranyl acetate–lead citrate stained thin sections. Magnification: ×23,625. (With permission, from H. W. Moser et al., 1987.)

immunologically mediated, as is thought to be true for multiple sclerosis.

Role of cytokines
Powers et al. (1992) studied the patterns of expression of cytokine and effector molecules in post-mortem brain tissue of patients with X-ALD and AMN. The most striking finding was the expression of TNF-α in macrophages, and most prominently in astrocytes, at the active edge of the lesion. Expression of TNF-α in astrocytes also occurs in multiple sclerosis (Selmaj et al., 1991a). TNF-α has been shown to be toxic to oligodendrocytes both in vitro (Selmaj and Raine, 1988) and in vivo (Selmaj et al., 1991a); it increases the permeability of brain capillary endothelial cells (Deli et al., 1995), and therapy with an antibody to TNF-α prevents the development of experimental autoimmune encephalitis (Selmaj et al., 1991b). Pro-inflammatory cytokines, including TNF-α, therefore, are thought to be directly responsible for tissue damage in multiple sclerosis (Hauser, 1995). The findings of Powers et al. (1992) provide support for the hypothesis that this is also true for the cerebral forms of X-ALD. Additional support is provided by the demonstration by McGuinness et al. (1993) that the bioactivity of TNF-α is increased in the serum of patients with the inflammatory cerebral form of X-ALD and not in AMN (Fig. 11). Powers
et al. (1992) have formulated the hypothesis that the primary biochemical abnormality in X-ALD, i.e. the abnormal accumulation of VLCFA, causes the liberation of lipids that initiate a cytokine-mediated cascade.

Possible triggers for the inflammatory response

In X-ALD, VLCFA excess is already present in foetal life (Moser et al., 1982) and precedes the development of brain pathology. We postulate that lipids containing an abnormally high proportion of VLCFA can act as triggers to initiate the cascade of inflammatory demyelination that appears to be cytokine-mediated, perhaps by stimulating TNF-α production in a manner analogous to the well-known effect of lipopolysaccharide (Beutler and Cerami, 1988). Whereas a mild to moderate excess of VLCFA is present in all brain lipid species, the greatest excess occurs in the ganglioside, phosphatidylcholine, proteolipid and cholesterol ester fractions. Even though in active demyelinating lesions the cholesterol ester fraction contains the greatest excess of VLCFA, this appears to be a consequence rather than a cause of demyelination, because the fatty acid composition of this fraction was normal in regions of X-ALD brain in which myelin was still intact (Theda et al., 1992). It is thus unlikely that cholesterol esterified with VLCFA acts as a trigger for the inflammatory response. However, each of the three other components could play such a role, with gangliosides a particularly plausible candidate. The gangliosides in X-ALD brain contain 27.8–50% of fatty acids with a chain length >21 (Igarashi et al., 1976b). Such fatty acids are virtually absent in normal brain gangliosides (Igarashi et al., 1976b). Gangliosides have been implicated in a variety of neuroimmunological disorders (Pestronk, 1991). They have been shown to suppress Théier’s murine encephalomyelitis demyelinating disease (Inoue et al., 1996). The immunological properties of gangliosides vary with their fatty acid composition (Kannagi et al., 1982). Gangliosides that contain 22–24 fatty acids are 6–10 times less effective immunosuppressants than are those that contain fatty acids with 16–20 carbons (Ladisch et al., 1994).

Comparison of the inflammatory response in X-ALD with that in multiple sclerosis

Although it is obvious that X-ALD and multiple sclerosis are fundamentally different disorders, the cerebral forms of X-ALD show an inflammatory demyelinating response that has points of resemblance to multiple sclerosis. This suggests that the inflammatory cascades involved in both disorders may have similar mechanisms, and since the primary cause of X-ALD is known, elucidation of the demyelinating process may contribute to an understanding of multiple sclerosis. The points of resemblance between X-ALD and multiple sclerosis have already been cited; however, several differences have been noted in the past, and others have emerged recently. (i) In X-ALD, the lesions are large and confluent, rather than scattered throughout the nervous system. (ii) In multiple sclerosis, the inflammatory infiltrates are located at the active demyelinative edge, while in X-ALD they tend to be behind the active edge (Schaumburg et al., 1975; Powers et al., 1992). (iii) Oligoclonal bands in the cerebrospinal fluid are present in only a small proportion of X-ALD patients (H. W. Moser, unpublished observation), but they are present in >90% of multiple sclerosis patients. (iv) The association between multiple sclerosis and HLA-DR2 haplotypes that has been documented repeatedly (Hillert and Orelup, 1993) does not exist for the cerebral forms of X-ALD, which show a weak association with the HLA B-44 haplotype.
Adrenoleukodystrophy

(v) McGuinness et al., (1997) have recently compared inflammatory cytokine expression in X-ALD and multiple sclerosis post-mortem brain tissue. The expression in multiple sclerosis was more intense than in X-ALD, with Th2 cytokines predominant in multiple sclerosis lesions, and Th1 cytokines in X-ALD.

Taken together these results indicate that the mechanism of the inflammatory response in X-ALD differs from that in multiple sclerosis.

Therapy

Adrenal insufficiency

The adrenal insufficiency responds readily to steroid replacement therapy, but unfortunately may fail to be recognized. This is a serious error since adrenal insufficiency has been the cause of death in several patients who had little or no neurological involvement. Restoration of adrenal function increases general strength and has improved school performance in patients whose brain MRI was normal. Except for one report, in a patient with AMN (Peckham et al., 1982), steroid replacement therapy has not altered neurological disability.

Neurological disability

Three therapeutic approaches are under current investigation: diet, bone-marrow transplantation and immunosuppression. Although none is as yet satisfactory, bone-marrow transplantation shows the greatest promise. Evaluation is hampered by the variability of X-ALD, and still insufficient knowledge about its natural history.

Dietary therapy

The previously cited finding by Kishimoto et al. (1980) that the VLCFAs in the brain of an X-ALD patient were of dietary origin, at last in part, led to the design of a diet that restricts the intake of saturated VLCFA acids. Since standard nutritional texts did not list C26: 0 content, we tested 135 common foods and devised a diet that restricted the intake of C26: 0 to <3 mg per day, compared with the 12–40 mg contained in the customary American diet (Brown et al., 1982; Van Duyn et al., 1984). Achievement of this low intake required restriction of fatty foods and the outer coverings of vegetables and fruits. It had been our hope that, analogous to what had been found when phytanic acid intake was restricted in patients with Refsum disease (Steinberg, 1995), the dietary restriction of the fatty acids that the patients were unable to metabolize would lead to a reduction of plasma VLCFA levels and clinical improvement. Five patients with CCER and one man with AMN received the diet for periods ranging from 3 months to 2 years. Unfortunately there was no effect on either the plasma C26: 0 levels or clinical progression (Fig. 12). The difference from the results in Refsum disease can be attributed to the demonstration that phytanic acid is of dietary origin only (Steinberg, 1995), while, as already noted, VLCFA are derived both from endogenous synthesis as well as the diet.

The previously cited studies of Rizzo et al. (1986), that addition of the mono-unsaturated oleic acid reduces the levels of C26: 0 in cultured skin fibroblasts of X-ALD patients, led to the trial of a regimen in which dietary restriction of VLCFA was combined with the administration of glyceryl trioleate (GTO) oil (A. B. Moser et al., 1987; Rizzo et al., 1987). This regimen led to a 50% reduction of plasma VLCFA levels in ~4 months (Fig. 13) and to a statistically significant improvement in one aspect of peripheral nerve function, peroneal amplitude (H. W. Moser et al., 1991b), although the statistical significance is not sustained when Bonferroni’s inequality is applied (Ingellinger et al., 1987). As a result of the efforts of Mr and Mrs Odone, the diet was altered to a 4:1 mixture of GTO and glyceryl trierucate (GTE) oil, popularly referred to as Lorenzo’s oil, which will subsequently be referred to as GTE. Erucic acid is an omega 9 mono-unsaturated 22-carbon fatty acid, which, as noted previously, acts by competing with saturated fatty acids for the microsomal fatty acid elongating enzyme system (Bourre...
before initiation of the GTE–GTO diet. The rapid drop in C26: 0 for the other groups because some of them had received GTO text. The baseline value for the GTE–GTO group is lower than

lengths of time (Poulos et al., 1993, 1995; Korenke et al., 1976). Erucic acid has a powerful effect on plasma C26: 0 levels in X-ALD patients (Fig. 13). In most patients, the level is normalized within 4 weeks. Since erucic acid produces a cardiac lipidosis in rodents (Corner, 1983), there had been initial concern that it could cause heart damage. However, cardiac side effects have not been documented clinically or by ECG or echocardiography in more than 100 patients (H. W. Moser, unpublished observation). Moderate reduction in platelet count is observed frequently, but has not been associated with clinically significant bleeding, and can be managed by reduction and monitoring of GTE intake (Kickler et al., 1996).

The dramatic effect of GTE oil on the plasma levels of VLCFA led to a series of therapeutic trials that have involved more than 300 patients. For ethical reasons these were conducted as open, nonrandomized trials. Although analysis of data is not yet fully complete, the consensus is that when the diet is administered to patients who are already neurologically involved, it has little or no effect on the neurological progression (Rizzo et al., 1990; Rizzo, 1993; Uziel et al., 1990; Aubourg et al., 1993; Kaplan et al., 1993; Moser, 1993, 1995; Korenke et al., 1995, 1996). A likely explanation for these disappointing results is provided by the results of biochemical assays of the post-mortem tissues of five X-ALD patients who had received the oil for various lengths of time (Poulos et al., 1994; Rasmussen et al., 1994). Whereas substantial amounts of erucic acid were present in adipose tissues and liver even months after the diet had been discontinued, the erucic acid level in brain was minimal and no higher than that in patients who had never received the oil (Table 5). It appears unlikely, therefore, that the GTE diet affected the synthesis of C26: 0 and other saturated VLCFA in brain. Consistent with this conclusion is the finding that in three of the four treated patients the levels of C26: 0 and saturated VLCFA in various brain lipids were similar to those in untreated X-ALD patients (Table 6). Of interest, however, is that in the one patient who had been treated with GTE for the longest time (36 months) the C26: 0 levels in the total lipid, glycolipid and phospholipid fractions were normal (Table 6). Subject to the caution that this is only a single case, the data raise the possibility that prolonged normalization of plasma C26: 0 levels can bring about a reduction of the brain C26: 0 levels. This result adds to the rationale for the prevention trial described below. The C26: 0 level in the brain cholesterol fraction continued to be elevated, but, as was noted previously, this fraction may reflect the lipid composition of myelin lipids that had broken down at an earlier time. Furthermore, cholesterol esterified with VLCFA is hydrolysed exceedingly slowly (Ogino and Suzuki, 1981).

**Possible preventive effect in asymptomatic patients**

Experience with disorders such as phenylketonuria and hypothyroidism indicate that the timing of the correction of the metabolic defect is crucially important. Therapy initiated in early life prevents mental retardation, but is not effective once the neurological defect has become established. A trial of preventive therapy is particularly relevant for X-ALD, since the disorder can be diagnosed at birth, and neurological involvement almost never begins before 4 years of age, and usually later (Fig. 1), thus providing a wide window of opportunity for preventive therapy. We have identified 123 neurologically asymptomatic X-ALD patients by testing plasma VLCFA levels in at-risk relatives of symptomatic patients, as well as children with Addison’s disease. Patients were assigned to the neurologically asymptomatic category if neither the patient, family, physician nor the teacher had reported neurological symptoms, and the neurological examination is normal. All of these patients were invited to participate in the dietary therapy protocol. The GTO-GTE mixture provides ~20% of total caloric intake, which is accomplished with a daily dosage of 2–3 ml/kg. Other sources of fat provide 10–15% of total calories. Supplements of essential fatty acids, vitamins and minerals were provided, and platelet count, liver function and electrocardiogram were monitored. More than 80% of the patients who maintained the diet have had significant reduction or normalization of plasma VLCFA levels. Details of the treatment protocol have been presented (A. B. Moser et al., 1987; H. W. Moser et al., 1991b).

Evaluation of clinical efficacy presents a difficult challenge. For ethical reasons, randomized placebo-controlled study design proved impossible. The hope engendered by the
Table 5  Erucic acid (C22: 1) and nervonic acid (C24: 1) levels in ALD patients treated with GTE

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age at death (years)</th>
<th>Time on therapy (months)</th>
<th>Interval* (months)</th>
<th>Total lipid (% of total fatty acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adipose</td>
</tr>
<tr>
<td>Untreated (n = 7)</td>
<td>11.3–51</td>
<td></td>
<td></td>
<td>0.02 ± 0.13</td>
</tr>
<tr>
<td>Treated Patient 1</td>
<td>52.7</td>
<td>5</td>
<td>6</td>
<td>0.26</td>
</tr>
<tr>
<td>Treated Patient 2</td>
<td>7.3</td>
<td>5</td>
<td>9</td>
<td>1.29</td>
</tr>
<tr>
<td>Treated Patient 3</td>
<td>35.7</td>
<td>16</td>
<td>12</td>
<td>1.83</td>
</tr>
<tr>
<td>Treated Patient 4</td>
<td>10.2</td>
<td>35</td>
<td>3</td>
<td>3.00</td>
</tr>
</tbody>
</table>

*Interval between cessation of therapy and death. Note that the adipose tissue and liver of GTE-treated patients contained a 10- to 50-fold excess of erucic acid (C22: 1) or of its elongation product (C24: 1) even 12 months after the dietary therapy had been discontinued. In contrast, their content in brain was equal to that in untreated patients. Poulos et al. (1994) reported comparable results in an X-ALD patient who had continued GTE therapy until the day he died.

Table 6  Brain C26: 0 levels as percentages of total fatty acids in ALD patients treated with Lorenzo oil

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total lipid</th>
<th>Cholesterol ester</th>
<th>Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects (n = 19)</td>
<td>0.48 ± 0.09</td>
<td>1.53 ± 1.08</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Untreated ALD Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early (n = 6)</td>
<td>0.70 ± 0.40</td>
<td>12.05 ± 1.82</td>
<td>0.29 ± 0.13</td>
</tr>
<tr>
<td>Late (n = 10)</td>
<td>3.51 ± 2.61</td>
<td>11.47 ± 9.37</td>
<td>0.41 ± 0.24</td>
</tr>
<tr>
<td>Adults (n = 12)</td>
<td>1.09 ± 0.50</td>
<td>5.05 ± 1.36</td>
<td>0.35 ± 0.09</td>
</tr>
<tr>
<td>Treated #1</td>
<td>1.01 ± 0.34</td>
<td>2.66</td>
<td>0.65</td>
</tr>
<tr>
<td>Treated #2</td>
<td>2.69 ± 0.55</td>
<td>7.15</td>
<td>1.22 ± 0.33</td>
</tr>
<tr>
<td>Treated #3</td>
<td>0.68 ± 0.11</td>
<td>2.89</td>
<td>0.35</td>
</tr>
<tr>
<td>Treated #4</td>
<td>0.20 ± 0.06</td>
<td>6.82</td>
<td>0.14</td>
</tr>
</tbody>
</table>

See text for discussion.

striking biochemical effect, i.e. normalization of plasma VLCFA levels, combined with the devastating course of the CCER phenotype prevented us from proposing a randomized study design, and this position was sanctioned by our Institutional Review Board and by the National Institutes of Health who supported the study. The difficulty of evaluation is compounded by the complete lack of a historical control group for the asymptomatic phenotype since the capacity to identify asymptomatic patients by plasma VLCFA assay was not achieved until 1981, only a few years before the development of dietary therapy.

Since the usual procedures for therapeutic evaluation are foreclosed, the appraisal will be based upon a comparison of the incidence of the CCER phenotype in the treated X-ALD patients with that in a representative sample of the untreated X-ALD population. As discussed previously, and shown in Tables 2 and 3, we estimate that 33–39% of untreated X-ALD patients will develop the CCER phenotype, and that approximately two-thirds of untreated X-ALD patients will escape it. Therefore, dietary therapy can be judged to be an effective preventive only if significantly more than two-thirds of the treated patients escape the CCER phenotype. For the comparison to be meaningful, analysis of the treated patients should focus on those asymptomatic patients who began therapy before the usual age of onset of the CCER, and patients must be followed until they have passed the age of greatest risk of developing the severe cerebral disease (12–14 years, as shown in Fig. 1).

Figure 14 shows the neurological status of the current cohort. Neurological status has remained normal in 96 of
115 patients (83%). The CCER phenotype, leading to death in four, occurred in seven (6%). Mild abnormalities, which would not be classified as CCER, occurred in 12%. Although this overall result appears encouraging, it should be considered with caution. Five of the patients with severe outcome had started the diet before the age of 5 years, and two had normalized their plasma levels completely. The therapy therefore is not an absolute preventive. At this time, only 10 of the patients meet the criteria for definitive evaluation, namely initiation of therapy before the age of 6 years and follow-up until the age of 12 years or later. These are the patients clustered in the upper left-hand corner of the figure. Three of these 10 patients died or developed CCER. However, the number is far too small to draw conclusions.

We plan to continue this prevention trial for a further 3–5 years, and more patients are being enrolled. In addition to the neurological appraisals, the evaluations include MRI studies at yearly intervals, with quantitative scores assigned utilizing the system designed for this purpose by Loes et al. (1994) and detailed neuropsychological appraisals designed for the study of leukodystrophy patients by Shapiro and colleagues (Shapiro and Klein, 1994; Shapiro et al., 1995). We have also established a collaborative agreement with a European study group coordinated by Drs Frank Roels and Wolfgang Köhler, which will permit at least a doubling of the study population. We have reached agreement on a common study protocol and data will be pooled. Biostatistical analysis will utilize newly developed techniques of survival analysis (Wang, 1991) and longitudinal analysis (Liang and Zeger, 1986). We will also correlate clinical outcome with the degree of normalization of plasma VLCFA levels. These combined approaches should permit a definitive answer to the important question of whether early dietary therapy can reduce the frequency and severity of subsequent neurological disability. Even if dietary therapy proves to be of limited value, the data will provide key baseline information for the evaluation of other modes of therapy, such as bone-marrow transplantation and gene therapy.

**Bone marrow transplantation**

The first bone-marrow transplantation for an X-ALD patient was reported in 1984 (Moser et al., 1984). The rationale was based upon the demonstration that normal bone marrow-derived cells can degrade VLCFA and that this capacity is deficient in X-ALD patients (Singh et al., 1984a). Furthermore, the intense perivascular lymphocyte and macrophage infiltrates in X-ALD brain lesions make it likely that substantial numbers of bone marrow-derived cells would enter the brain. At that time, the defective gene product had not yet been identified, and it was hoped that, as had been shown to be the case in lysosomal disorders (Walkley et al., 1994), the gene product that is deficient in X-ALD might be transferred from bone marrow-derived cells to nervous tissue cells. The patient was in the rapidly advancing stage of the adolescent cerebral form of X-ALD. The study did show encouraging biochemical effects (Fig. 15). Circulating white blood cells acquired the capacity to degrade VLCFA, and the plasma VLCFA level diminished 2 months after the transplant, suggesting that the correction of the enzyme defect in bone marrow-derived cells was sufficient to alter the overall metabolism of VLCFA. The rise in C26:0 levels during and immediately following the transplant is probably due to its mobilization from adipose tissues. Unfortunately, the clinical results were discouraging. The patient lost his vision on the seventeenth day after bone-marrow transplantation, his neurological disability continued to advance, and he died of an adenovirus infection 141 days after the transplant; thus, no additional transplants were performed during the next 4 years. Experience with 70 X-ALD patients who have received bone-marrow transplantation since then indicates that when transplants are performed in patients who are already in the rapidly advancing stage of the disease, the neurological disability continues to advance in the immediate post-transplant period, and the rate of progression may even accelerate. On the basis of this experience, this first patient would now not be recommended for transplantation.

The attitude toward bone-marrow transplantation was revolutionized by the report of Aubourg et al., (1990), who performed a transplant in a 7.5-year-old patient with mild cognitive, neurological and MRI abnormalities; he demon-
strated not only stabilization of the course, but reversal of the deficit 24 months later. Subsequent follow-up 8 years after the transplant indicates that neurological examination and MRI are normal, and that neuropsychological function is equal to that of his unaffected non-identical twin who had served as the donor (P. Aubourg, personal communication). Partial reversal of neurological disability, over and above the variation of the natural history of the disease, has been observed in seven additional patients (W. Krivit and P. Aubourg, unpublished observation). The improvement appears to occur after the first 6 months and may continue for several years. Although additional follow-up and more rigorous comparison with untreated patients matched for age and disease severity are required, these preliminary data do suggest that bone-marrow transplantation can lead to significant neurological benefit. The mechanism by which this benefit is achieved is unknown. The demonstration that the defective gene product in X-ALD is a peroxisomal membrane protein (Mosser et al., 1993) appears to rule out the possibility that this product could be transferred from bone marrow-derived cells to neuronal elements. It has been suggested that the improvement is a result of the intense immunosuppression that precedes the transplant procedure. Against this possibility is the observation that, in the patients who have had favourable results, improvement has continued long after cessation of immunosuppression therapy. In addition, in one patient who had received intense immunosuppression, but in whom engraftment did not occur, neurological disability continued to advance rapidly. In our view, the most likely explanation for the favourable effect is that normally functioning bone marrow-derived cells that include microglia (Hickey and Kimura, 1988) metabolize VLCFA and thus reduce their levels in brain, and interrupt the cascade of demyelination. The observation that improvement appears to commence only 6 months after bone-marrow transplantation would be compatible with the slow turnover of microglia (Lawson et al., 1992), which may thus be replaced by enzymatically competent cells at only a slow rate. In accordance with this hypothesis the key factor in improvement is the lowering of VLCFA levels in brain. At present this must remain a hypothesis since, for ethical reasons, direct measurement in patients is not feasible. The plasma VLCFA levels appear to be an unreliable indicator of the level of VLCFA in brain. GTE therapy can normalize plasma VLCFA levels (Fig. 13) without altering those in brain (Table 6). Although bone-marrow transplantation has lowered plasma VLCFA levels in some X-ALD patients (Aubourg et al., 1990; Moser et al., 1984), it has not done so in others, and the post-transplant alteration in plasma VLCFA levels does not correlate with the degree of clinical benefit (Krivit et al., 1995).

Although bone-marrow transplantation appears to be the most effective therapy for X-ALD, its application is in a state of flux and requires considerable care and judgement. It is not recommended for patients with rapidly advancing or severe cerebral involvement (Krivit et al., 1995) because these patients cannot tolerate the temporary worsening that is associated with the stress of the procedure. It is also not recommended for asymptomatic patients with normal MRI, because these patients have a >50% chance of not developing severe forms of the disease, and therefore should not be subjected to the risk of the procedure, considering that the overall mortality in the 70 patients who have been transplanted is 30%. The prime indication is in patients who show early evidence of cerebral involvement on the basis of MRI and neuropsychological testing (Krivit et al., 1995). Detection of such abnormalities at an early stage, when therapy appears to be most effective, requires monitoring of neurologically asymptomatic patients at 6-month to 1-year intervals. Techniques for the prevention of graft versus host disease, and the design of immunosuppression regimens are under review, and promise to lower the risk of the procedure (Krivit et al., 1995). In view of the rise of C26: 0 levels that may occur in connection with the transplant (Fig. 15), Dr Krivit recommends that plasma VLCFA levels be lowered with the aid of the GTE regimen, before, during, and after the transplant. If the apparently favourable effects of bone marrow transplantation are confirmed, and the risk of the procedure diminishes, the threshold for recommending it will be lowered, and it may then be considered for asymptomatic patients with abnormal MRI and for patients with AMN who, at this time, are generally not considered to be suitable candidates.

**Immunosuppression**

Immunosuppression is administered in order to abolish or reduce the brain inflammatory response that is associated with the rapidly progressive cerebral forms of the disease. The aim is to convert these severe disorders into the somewhat milder AMN. These approaches have not been successful so far.

Immunosuppression with cytoxan has not been effective (Stumpf et al., 1981; Naidu et al., 1988). A double-blinded therapeutic trial of thalidomide and of β-interferon therapy is in progress in our clinic. Eighteen patients with cerebral inflammatory disease in whom the disease was considered too advanced for bone marrow transplantation are participating at this time, and a total enrollment of 60 is planned. Thalidomide was selected because it reduces the activity of TNF-α, which, as has been discussed, may have a pathogenetic role in X-ALD (Powers et al., 1992), and it has been of benefit in lepromatous leprosy (Sampaio et al., 1993). Interferon β-1b was selected because of the points of resemblance between the inflammatory forms of X-ALD and multiple sclerosis, and the demonstrated benefit of β interferon for certain forms of multiple sclerosis (Paty et al., 1993). Since the code in this double-blinded study has not yet been broken, we cannot comment on the therapeutic effectiveness. There are anecdotal reports that immunoglobulins have been of benefit (Oka et al., 1996).
**Gene therapy**

X-ALD is a promising candidate for gene therapy, since the condition can be diagnosed years before neurological damage occurs. The gene has been isolated and shown to correct the defect in VLCFA metabolism in X-ALD cells (Cartier et al., 1995). Further experience with bone-marrow transplantation and studies in animal models of the disease will be required to determine whether transfection of haematopoietic cells will suffice, or if additional approaches need to be considered.

**Concluding remarks**

Adrenoleukodystrophy has become the topic of intensive study in several laboratories and clinics in the United States, Europe and Japan. Analysis of the advances that have been achieved, and of the problems that remain, points to several issues that may apply to other neurogenetic disorders. The application of precise and non-invasive diagnostic techniques, in this instance the plasma assay of VLCFA, has demonstrated that the disorder is more common, and that the range of phenotypic expression is much wider, than had been recognized in the past. Identification of the gene defect was accomplished by positional cloning, and the gene product has unexpected properties that do not readily account for the biochemical abnormalities or the pathogenesis of the disease. While the improved capacity for precise prenatal and postnatal diagnosis has facilitated genetic counselling, many new patients continue to be identified in families that had not been known to be at risk. Effective therapy still escapes us. Several urgent research needs remain. These include more detailed knowledge about the natural history of each of varied phenotypes that may be associated with the same primary gene defect. Recently developed transgenic mouse models of X-ALD provide new opportunities to elucidate pathogenesis, and to design and evaluate new therapies. One of these models has been developed in Japan (Kobayashi et al., 1997), the other at the Kennedy Krieger Institute in Baltimore (Lu et al., in press). Both models are deficient in ALD protein, also oxidation of VLCFA is defective and VLCFA levels in brain and adrenal glands are increased. The model produced in Baltimore shows lamellar inclusions in the adrenal gland. The animals do not show neurological changes at 6 months (Baltimore) or at 12 months of age (Kobayashi et al., 1997). Strategies to enhance the phenotype include dietary loading with VLCFA, the additional knockout of other ABC transporters such as 70-kDa peroxisomal membrane protein (PMP70) and ALD related protein, which have been referred to previously, and cross-breeding with strains that are highly susceptible to experimental allergic encephalitis. If and when effective therapy becomes available, the development and implementation of mass screening techniques in the newborn, which should permit identification of affected individuals before they develop symptoms since VLCFA in plasma are already increased at birth (H. W. Moser, unpublished observation), could lead to a truly effective approach to this devastating disorder.

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