X-Linked Adrenoleukodystrophy
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X-Linked Adrenoleukodystrophy is a disorder involving a defect in a peroxisomal membrane transporter involved in moving substrates including saturated very long chain fatty acids (VLCFA) from the cell cytoplasm into peroxisomes, leading to a characteristic accumulation of VLCHFAs. The main clinical pictures involve inflammatory demyelination, neurodegeneration, and adrenocortical insufficiency (Berger et al, 2006). X-ALD has varied phenotypic manifestations ranging from severe cerebral forms with onset during early childhood to asymptomatic people. The incidence of X-ALD (hemizygotes and heterozygotes – at least half of carrier females develop AMN like symptoms - described below - at mid or later life) is 1:16,800 in all ethnic groups, similar to that of phenylketonuria (Moser et al, 2005), making X-ALD the most frequent monogenetically inherited demyelination disorder and the most frequent peroxisomal disorder.

Basic Genetics

X-linked ALD is an inherited metabolic disorder passed down in the X-linked recessive manner. Males carrying the inherited gene will be affected but the phenotypic expression is highly variable and unpredictable. Female carriers of the gene are usually not seriously affected.

X-ALD is found evenly distributed within all ethnic groups at a frequency of no less than 1:21,000. The frequency of hemizygotes plus heterozygotes in the United States is 1:16,800 (Bezman et al 2001). The disease is found most often in males. As a result of screening at risk family members, many asymptomatic males have been identified that have X-ALD. Heterozygotic females have also been found that exhibit phenotypes similar to adrenomyeloneuropathy, (AMN). There is 100% penetrance in males with regards to the biochemical phenotype of elevated plasma concentration of VLCFA.

The ABCD1 gene is the only gene associated with X-ALD. It is located on the chromosomal locus Xq28. The ABCD1 gene codes for a peroxisomal membrane protein (ALDP), specifically the ATP-binding cassette sub-family D member 1 protein. It is a member of a larger category of proteins described as “ATP binding cassette (ABC) transporters.” The gene ABCD1 contains ten exons and spans 20kb of genomic DNA. Exon 1 is the largest containing 900 bp. There are 2,235 bp of coding sequence in the 3,664 bp transcript. They encode for 745 amino acids. (Kemp et al, 2001). The most common disease causing mutation is an AG deletion at nucleotide 1801-1802 in exon 5. This mutation has been found in 7.9% of families equally in all ethnic groups (Moser et al, 2006). The mutations are clustered around specific regions of the gene. Around 40% of the mutations occur in the transmembrane domain. 30% are clustered in the ATP-binding domain, and 14% are found within exon 5. Exon 5 has been identified as a mutation hot spot on the gene. The remaining 16% are evenly distributed throughout the gene. (Kemp et al, 2001). There have been no reports of promoter mutations or complete gene deletions associated with X-ALD. (Smith et al, 1998).

No correlation has yet been found between the specific mutation and a corresponding phenotype. The same mutation has been found to correspond o each of the
known phenotypes. However, more than 500 different mutations of the ABCD1 gene have been documented with patients exhibiting X-ALD. Large deletions that abolish formation of the gene product have been associated with mild phenotypes. While missense mutations in which abundant immunoreactive protein product is produced cause severe phenotypes.

In order to effectively analyze the unique mutations found in the ABCD1 gene a database, (http://www.x-ald.nl), has been established. At this website established in 1991, information regarding X-ALD mutations cataloging and analysis of different mutations and polymorphisms, with specific focus on the ABCD1 gene and the function of ALDP, as well as phenotypes, biochemistry, genetics, and diagnosis.

**Screening for at-risk family members**

The entire extended family of a known X-ALD patient should be screened for the disease. Early identification and treatment may inhibit progress, and timely therapy and genetic counseling is essential to improve the prognosis. Therapy given to patients already exhibiting symptoms is rarely successful. (Kemp et al, 2001). Also, since no definitive cure is available carrier testing and genetic counseling is crucial. After identifying the DNA mutation in the affected X-ALD patient all other carriers in the family can be identified accurately. Prenatal diagnosis is also recommended by testing the concentration of VLCFA in amniocytes and chorion villus cells. But, confirmation with DNA analysis is also recommended. Prenatal screening could allow early therapy before the nervous system has a chance to be damaged.

It is most likely (93%), that the proband has inherited the disease from one parent. New mutations at most account for only 7% of the individuals with X-ALD. (Moser et al, 2006). Risk to siblings should be assessed depending on the genetic status of the parents. Affected fathers will pass on the gene to all of there daughters and none of their sons. Mothers carrying the gene will have a 50% chance of passing the gene to a child for each pregnancy. Male children will be affected and female children will be carriers. If neither parent is a carrier the risk to siblings is low. Other family members including aunts, uncles, cousins should be tested depending on their family relationship to identified carriers. Extended family screening can lead to identification of males at a time when therapy has the greatest chance of success.

The unpredictable phenotypic variations make it important to test every family member even if the affected proband is only mildly affected. Siblings may exhibit a much more severe form of the same mutation.

**Molecular Biology & Biochemistry**

X-Linked Adrenoleukodystrophy (X-ALD) is a disease characterized by a wide variety of mutations that disrupt the ATP-Binding Cassette transporter subfamily D member 1 (ABCD1) gene. This 19kb gene is located on the q arm of the X-chromosome (Xq28), and its 10 exons typically encode for a 745 amino acid protein product. The product of the ABCD1 gene, designated as the adrenoleukodystrophy protein (ALDP), is thought to be a peroxisomal transmembrane protein with the general structural features of an ATP-binding cassette transporter. ALDP is an integral membrane protein with a hydrophobic transmembrane domain and loops that protrude into the lumen of the
peroxisome as well as a cytoplasmic ATP binding domain. Generally, it is believed that the ALDP protein represents only half of the functional transporter, with homodimer (interaction between two ALDP proteins) or a heterodimer (interaction between ALDP and another peroxisomal transporter) formation required for normal functionality.

Biochemically, a mutation in the ALDP peroxisomal transporter protein leads to the elevation of levels of unbranched and saturated very long-chain fatty acids (VLCFA) in plasma and tissues. The fatty acids commonly found in X-ALD include tetracosanoic (C24:0), hexacosanoic acid (C26:0), and mono-unsaturated hexacosenoic acid (C26:1) and affect cerebral white matter, the spinal cord, peripheral nerves, the adrenal cortex, and the testes. (Moser et al, 2006). A number of studies have shown that the accumulated VLCFAs are of endogenous origin, as changing the lipid content of growth media for cells or X-ALD patient diet has little impact on the course of fatty acid buildup (Tsuji et al, 1985). In normal mammalian tissues, fatty acids are derived from de novo synthesis by fatty acid synthase complex (utilizing acetyl-CoA, malonyl-CoA, and NADPH to elongate fatty acids. Formation of VLCFAs occurs by elongation of shorter fatty acids with enzymes associated with the endoplasmic reticulum. Degradation of fatty acids typically occurs in the mitochondria (or smaller fatty acids) or the peroxisome (for long-chain fatty acids).

Although the precise function of ALDP in the biochemical process of VLCFA accumulation is still unclear, defects in ALDP are postulated to impact peroxisomal catalysis of large fatty acids by β-oxidation. Previous studies linked the absence of ALDP to a lower activity of very long chain acyl-CoA synthetase (VLCS), an enzyme that activates the VCLFAs to CoA derivatives (Lazo et al, 1988), resulting in reduced catabolic activity, and accumulation of the fatty acids in cells. In subsequent experiments, β-oxidation of VCLFAs resumed following ALDP transfection (Braiterman et al, 1998). Furthermore, recent studies suggest that the VLCS enzyme requires a physical interaction with ALDP on the luminal side of the peroxisome membrane to achieve full catabolic functionality. Interestingly, interaction studies indicate that the first 75 residues of ALDP are not required for VLCS activity (Makkar et al, 2006). Similarly, phenotype analysis and mutation mapping also suggest the N-terminal amino acids have a reduced functional importance in the disease state (Berger et al, 2006). Thus, without the presence of the critical ALDP residues in the lumen of the peroxisome, VLCS cannot activate VLCFAs prior to β-oxidation and degradation.

Ultimately, it is believed that VLCFAs and metabolite accumulation incites a neuroinflammatory response with production of cytokines and ICAMS by astrocytes and microglial cells, de-myelination, and a loss of oligodendrocytes. This process therefore begins the degenerative processes associated with the devastating X-ALD phenotypes. (Paintlia et al, 2003).

**Diagnosis and Prognosis**

Diagnosis of X-ALD is based on clinical findings. Although this disorder has an extremely wide range of phenotypic manifestations, Berger et al have classified the clinical picture in six basic categories based on age of onset, organs involved and the rate of progression of neurological symptoms. The symptoms described below are used to make the diagnosis of X-ALD, along with biochemical assays for plasma concentrations
of VCLFAs as well as neuroimaging using MRI. Carrier testing for related females and prenatal testing is clinically available.

**Clinical Pictures** (minor discrepancies exist on the specificities of the classification system but most are generally similar. The following are based on those described by Berger, et al. in X-ALD: Clinical, biochemical and pathogenic aspects, 2006.)

1. **Childhood Cerebral (31-35%)**
   This represents the most severe phenotypic form of the disease with onset between 2 – 10 years of age and rapid progression of symptoms eventually leading to an apparently vegetative state within 2 – 4 years and death following at varied intervals thereafter. Symptoms include adrenal insufficiency and progressive neurological dysfunction. At this age (and into adolescence), X-ALD is often misdiagnosed as ADHD (Moser et al, 2005) based on initial symptoms such as emotional lability, hyperactive behavior, school failure, impaired auditory discrimination and difficulties in vision.

2. **Adolescent and Adult Cerebral (6-12%)**
   For both the adolescent and adult forms, clinical symptoms and progression are similar to those described above, but the age of onset typically occurs somewhat later around ages 11-12 years (adolescent form), and after 21 years for the adult form. Initial misdiagnoses often involve schizophrenia or other psychiatric disorders.

3. **Adrenomyeloneuropathy (40-46%; pure AMN 20-23%; cerebral AMN 20-23%)**
   Symptomatic onset typically occurs between ages 20-50 and involves progressive stiffness and weakness of legs, impaired vibration sense, sphincter disturbances and impotence. AMN mainly involves the spinal cord. Adrenal insufficiency is present in approximately two-thirds of patients; cerebral changes occur in about one-half of patients with the course of illness resembling cerebral ALD as described above. Misdiagnosis of AMN includes multiple sclerosis and familial spastic paraparesis.

4. **Addison-only (10-20%)**
   This form involves primary adrenal insufficiency with no evidence of nervous system involvement. These patients are at high risk of developing AMN later on in life. (At time of publishing of Berger article, oldest described Addison-only patient was 78 years old).

5. **Asymptomatic (decreases with age - very rare after 40 years)**
   Patients with the genetic defect are free of adrenal insufficiency and neurological symptoms but do have elevated plasma levels of saturated VCLFAs. These patients are at high risk for developing adrenal and/or neurological symptoms later in life. According to Berger et al, the oldest described asymptomatic males are in their sixties.

6. **Phenotypes in female carriers (increases with age; ~ 50% after age 40)**
   The presence of neurologic symptoms in females is most likely due to non-random X-inactivation, resulting in a higher number of cells expressing the mutant allele of the gene versus the non-defective allele. Onset of symptoms typically occurs between 40 and 50 years with milder symptoms than those described for males and a slower rate of progression. Cerebral involvement and adrenal insufficiency are rare in female carriers; clinical pictures appear most like AMN and are often misdiagnosed as multiple sclerosis.

   Neuroimaging and biochemical testing are also used to diagnose X-ALD. Neurologically affected males always have an abnormal brain MRI. 85% of affected males show a characteristic pattern of symmetric enhanced T-2 signal in the parieto-occipital region with contract enhancement at the advancing margin (Moser et al, 2006).
Plasma concentrations of VLCFAs are elevated in 99.9% of males with X-ALD at birth and in 85% of carrier females. Approximately 20% of carrier females have normal plasma concentrations. Therefore this assay is extremely useful in distinguishing X-ALD from the other conditions that are often misdiagnosed in its place.

**Treatment Options (Moser et al, 2005)**

There is no definitive cure for X-ALD, however three different treatment options are available that may significantly improve the prognosis of the disease if started early enough.

Adrenal hormone replacement therapy must be provided for patients suffering from adrenal insufficiency. This can prevent an adrenal crisis which was a significant cause of mortality associated with the disease; it can also improve general strength and well-being. This form of steroid replacement therapy does not show any effect on the neurological aspects of the disease.

A 4:1 mixture of glyceryl-trioleate and glyceryl-trierucate commonly called “Lorenzo’s Oil,” has been shown to normalize plasma concentration levels of VLCFAs in affected individuals. Because Lorenzo’s oil has been shown to significantly reduce the risk of developing cerebral disease, but is not useful once the cerebral disease has progressed, therapy is recommended for asymptomatic boys below eight years of age with normal brain MRI results.

Hematopoeitic Stem Cell Transplantation is a more effective form of treatment when inflammatory cerebral symptoms have already progressed, although meeting the criteria to benefit from it are more limited. The best outcomes were achieved in boys who were treated when symptoms were still mild. For further progressed patients, outcome can vary greatly according to the severity of the disease at the time of treatment and treatment is not recommended for patients with more advanced forms of the disease. Stem cell transplantation is also not recommended for patients who do not express neurologic symptoms and who have normal brain MRIs because approximately half of them will never develop the cerebral forms of the disease.

In addition, affected boys benefit from strong familial and social support networks, as well as psychological, educational and vocational counseling and physical therapy to help manage urologic symptoms of the disease (Moser et al, 2006).

**References:**


