ALD database, diagnostic dilemmas and the need for translational metabolism

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Academic Medical Center, Amsterdam
www.amc.nl/ALDgroup

ALD Newborn Screening: Identifying ALD Standards of Care, NY
Adrenoleukodystrophy (ALD)

- Most prevalent peroxisomal disorder
- Birth incidence: ~1:15,000 (♂ & ♀)
  - Europe: ~350 new patients born each year
- X-chromosome (ABCD1)
- Affects the myelin and endocrine organs (adrenal cortex and testis)
Genetics and biochemistry of ALD

endoplasmic reticulum

nucleus

long-chain fatty acids

elongation

very long-chain fatty acids (VLCFA)

ABCD1 gene

ABCD1

peroxisome

degradation

mitochondrion

cell membrane

Kemp and Wanders (2010) Brain Pathology
Genetics and biochemistry of ALD

Mosser et al. (1993) Nature
Genetics and biochemistry of ALD

- endoplasmic reticulum
- nucleus
- long-chain fatty acids
- elongation
- very long-chain fatty acids (VLCFA)
- ABCD1
- peroxisome
- degradation
- mitochondrion
- cell membrane

Mosser et al. (1993) Nature
Genetics and biochemistry of ALD
Genetics and biochemistry of ALD

Moser et al. (1981) Neurology
Genetics and biochemistry of ALD

endoplasmic reticulum

long-chain fatty acids

elongation

very long-chain fatty acids (VLCFA)

storage of VLCFA

ABCD1

peroxisome

degradation

mitochondrion

cell membrane

LD

Gly

ser

m

L-L
The clinical presentation of ALD in men and women

Age of onset, site of initial pathology, rate of progression

adrenal insufficiency

adrenomyeloneuropathy

childhood cerebral ALD

Moser et al. (2007) Nature Clinical Practice
Engelen et al. (2012) Orphanet J Rare Dis
Clinical outcome cannot be predicted

- All ALD patients have a mutation in the *ABCD1* gene

- All ALD males have elevated VLCFA levels
  - 85% of women with ALD have elevated VLCFA levels

- VLCFA levels (plasma, blood cells, fibroblasts) do not correlate with phenotype

- *ABCD1* mutations have no predictive value towards clinical outcome

- Identical mutations are associated with different ALD phenotypes
No genotype-phenotype correlation

- **p.Pro484Arg**

![Genogram showing family members with different ages and phenotypes.](image-url)
No genotype-phenotype correlation

- p.Gln472fsX83

<table>
<thead>
<tr>
<th>X-ALD Kindred</th>
<th>Phenotypes</th>
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<tr>
<td>1</td>
<td>CCER</td>
</tr>
<tr>
<td>2</td>
<td>AMN</td>
</tr>
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<td>3</td>
<td>AMN</td>
</tr>
<tr>
<td>4</td>
<td>CCER</td>
</tr>
<tr>
<td>5</td>
<td>AMN</td>
</tr>
<tr>
<td>6</td>
<td>CCER, AMN, Addison</td>
</tr>
<tr>
<td>7</td>
<td>CCER, AMN, Addison</td>
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<td>AMN</td>
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<td>CCER, AMN, Addison</td>
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<td>14</td>
<td>AMN</td>
</tr>
<tr>
<td>15</td>
<td>CCER</td>
</tr>
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</table>
“Simple” monogenic diseases are also complex/polygenic

Textbook:
• Identical gene mutations should lead to specific phenotypes.

Real life:
• Identical mutations may lead to widely varying phenotypes
• Phenotype is the result of
  1. Primary gene defect
  2. Interplay of (rare) genetic variants (“modifier” genes)
  3. Environmental factors

Schadt (2009) Nature
Argmann et al. (2016) Cell Metab
ALD is a progressive neurodegenerative disorder

Kemp et al. (2016) Nat Rev Endocrinol
The combination of the SNPs in *ELOVL1* and *CYP4F2* have no absolute predictive value (hence, more modifying factors to be identified)

- rs2108622 (p.V433M) affects protein levels and thereby omega-oxidation activity and VLCFA clearance
- rs839765 in the *ELOVL1* promoter affects GATA transcription factor binding in promoter. Functional consequence not yet confirmed.

**Modifier genes: CYP4F2 and ELOVL1**
Rational for newborn screening

Adrenal insufficiency

• Untreated adrenal insufficiency can be life-threatening.
• 39/49 boys identified by extended family screening (without neurological symptoms) had undiagnosed impaired adrenal function (Dubey et al. (2005) J Pediatr).
• AI is often discovered only after several hospitalizations.

Cerebral ALD

• BMT is curative, but only in early stage disease (Aubourg et al (1990) NEJM).
• Of 283 boys diagnosed with cerebral ALD, only 19 were eligible for BMT (Mahmood et al. (2007) Lancet Neurology).
• Patients who are diagnosed on the basis of neurological symptoms are already too advanced for transplantation.
Newborn screening allows timely intervention

Adrenal insufficiency (< age 4)
Cerebral ALD (< age 18)

Personalized follow-up (outcome cannot be predicted)

Adrenomyeloneuropathy (> age 18)
Newborn screening for ALD

- C26:0 lysophosphatidylcholine (C26:0-lysoPC)

\[ \text{pmol per 1/4" DBS} \]

controls \hspace{1cm} ALD

Sandlers et al. (2012) Mol Genet Metab
Newborn screening for ALD

24% of all life births in the US are screened for ALD

http://adrenoleukodystrophy.info/clinical-diagnosis/newborn-screening
Classical diagnosis versus newborn screening

Classical diagnosis
• Patient with clinical complaints visits doctor.
• Symptoms point towards ALD
• Biochemical and genetic tests to confirm diagnosis ALD

Newborn screening
• Baby without clinical complaints with elevated C26:0-lysoPC
• In the absence of clinical clues
• Is it: ALD, ZSD, ACOX1, DBP1, CADDS, ACBD5, …… ?

Newborn screening ≠ Classical diagnosis
Classical diagnosis ≠ newborn screening

ALD?

Elevated C26:0-lysoPC
In the absence of clinical clues
Is it: ALD, ZSD, ACOX1, DBP1, CADDS, ACBD5, …… ?
Diagnostic dilemmas in newborn screening

- Tier 1 & 2: \([C26:0\text{-lysoPC}] \uparrow\uparrow\)
- Tier 3: confirmed pathogenic \(ABCD1\) mutation \(\rightarrow\) ALD
**Diagnostic dilemmas in newborn screening**

- **Tier 1 & 2:** \([C26:0-lysoPC] \uparrow\uparrow\)
- **Tier 3:** no \(ABCD1\) mutation requires further diagnostic testing

ACBD5 deficiency causes a defect in peroxisomal very long-chain fatty acid metabolism

Sacha Ferdinandusse, Kim D Falkenberg, Janet Koster, Petra A Mooyer, Richard Jones, Carlo W T van Roermund, Amy Pizzino, Michael Schrader, Ronald J A Wanders, Adeline Vanderver, Hans R Waterham

**ABSTRACT**

Background Acyl-CoA binding domain containing protein 5 (ACBD5) is a peroxisomal membrane protein with a cytosolic acyl-CoA binding domain. Because of its acyl-CoA binding domain, ACBD5 has been assumed to function as an intracellular carrier of acyl-CoA esters.

Achloroactodysplasia punctata (RCDP) type 1 and 5 and (2) the single peroxisomal enzyme deficiencies. In the first class, multiple peroxisomal metabolic pathways are impaired, resulting in multiple metabolic abnormalities, whereas in the second class, only the metabolic pathways in which the defective

Neonatal detection of Aicardi Goutières Syndrome by increased C26:0 lysophosphatidylcholine and interferon signature on newborn screening blood spots

Diagnostic dilemmas in newborn screening

- Tier 1 & 2: [C26:0-lysoPC] ↑↑
- Tier 3: a new variant in ABCD1
  - This is a variant of unknown significance (VUS)
  - Is this variant pathogenic??
Diagnostic dilemmas in newborn screening

- Many variants have not been tested experimentally.
- Prediction programs like POLYPHEN-2 and SIFT highly insufficient.
- Interpretation of any new exonic sequence variant is often not straightforward. This is even worse for non-exonic variants.

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Wording to include within reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Clearly not pathogenic</td>
<td>Not pathogenic. “Common” polymorphism and therefore not reported.</td>
</tr>
<tr>
<td>2</td>
<td>Unlikely to be pathogenic</td>
<td>Unlikely to be pathogenic. Diagnosis not confirmed molecularly.</td>
</tr>
<tr>
<td>3</td>
<td>Unknown significance (VUS)</td>
<td>Uncertain pathogenicity. Does not confirm or exclude diagnosis.</td>
</tr>
<tr>
<td>4</td>
<td>Likely to be pathogenic</td>
<td>Likely to be pathogenic. Consistent with the diagnosis.</td>
</tr>
<tr>
<td>5</td>
<td>Clearly pathogenic</td>
<td>Predicted to be pathogenic. This result confirms the diagnosis.</td>
</tr>
</tbody>
</table>
Adrenoleukodystrophy Database

X-linked adrenoleukodystrophy (ALD) is the most frequent inherited disorder of the central nervous system white matter with a minimum incidence of 1 in 14,000 newborns. It is a progressive, neurometabolic disease that affects brain, spinal cord, peripheral nerves, adrenal cortex and testis. The disease is caused by mutations in the ABCD1 gene. More than 750 unique mutations have been identified. For a comprehensive summary, please visit the Facts on ALD page.

The ALD database was initiated in July 1999 by Hugo W. Moser, M.D. and Stephan Kemp, Ph.D.

The primary aims of the database are:

1. Catalog mutations and variations in the ABCD1 gene,
2. Facilitate the analysis and interpretation of mutations and variations in the ABCD1 gene,
3. Provide background information on ALD,
4. Provide links to ALD patient organizations, and
5. Help with contacting and finding (local) ALD health care professionals.

New or updated pages:

- Newborn screening, Neugeborenscreening, Dépistage néonatal (Sep 15, 2017) ACBDS and Aicardi Goutières Syndrome
- Mutations and Variants in ABCD1 (Jul 12, 2017) June 2017: complete revision of all mutations and variants.
- Tatsachen zur ALD (Apr 21, 2017) Major update
- Facts on ALD (Apr 17, 2017) Major update

The ALD database is a collaboration between the Peroxisomal Diseases Laboratory at the Kennedy Krieger Institute, Baltimore MD, USA and the Laboratory for Genetic Metabolic Diseases at the Academic Medical Center / Emma Children’s Hospital, Amsterdam, the Netherlands.

We thank the Netherlands ALD Patient Organization for financial support.
ALD database

Primary aims

• Catalogue mutations and variations in the \textit{ABCD1} gene,
• Facilitate the analysis and interpretation of mutations and variations in the \textit{ABCD1} gene,
• Provide background information on ALD,
• Provide links to ALD patient organizations, and
• Help with contacting and finding (local) ALD health care professionals.

• Info in 5 languages:  
  • No funding, no sponsors
### ALD database mutation registry

<table>
<thead>
<tr>
<th>variant</th>
<th>consequence</th>
<th>exon</th>
<th>n</th>
<th>ALDP</th>
<th>references</th>
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<td>c.1820G&gt;A</td>
<td>p.Gly607Asp</td>
<td>exon 8</td>
<td>2</td>
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<td>52, 58</td>
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<tr>
<td>c.1823G&gt;A</td>
<td>p.Gly608Asp</td>
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<td>c.1823G&gt;C</td>
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<tr>
<td>c.1825G&gt;A</td>
<td>p.Glu609Lys</td>
<td>exon 8</td>
<td>26</td>
<td>2.1 ± 1.3%</td>
<td>15, 16, 32, 33, 49, 58, 60, 62, 72, 88, 97, 141</td>
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<tr>
<td>c.1826A&gt;G</td>
<td>p.Glu609Gly</td>
<td>exon 8</td>
<td>1</td>
<td>1.8 ± 0.9%</td>
<td>16, 97</td>
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<td>c.1832A&gt;G</td>
<td>p.Gln611Arg</td>
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<td>c.1833G&gt;C</td>
<td>p.Gln611His</td>
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<td>n.d.</td>
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<td>c.1838T&gt;A</td>
<td>p.Ile613Asn</td>
<td>exon 8</td>
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<td>n.d.</td>
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<td>c.1846G&gt;A</td>
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<td>4.3 ± 1.7%</td>
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<td>c.1849C&gt;A</td>
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<td>4</td>
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<td>15, 33, 64, 86</td>
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<td>c.1849C&gt;T</td>
<td>p.Arg617Cys</td>
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<td>16</td>
<td>absent</td>
<td>6, 15, 16, 32, 33, 49, 58, 59, 60, 86, 88</td>
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<tr>
<td>c.1849del</td>
<td>p.Arg617Alafs*19</td>
<td>exon 8</td>
<td>1</td>
<td>absent</td>
<td>60</td>
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<tr>
<td>c.1850del</td>
<td>p.Arg617Profs*19</td>
<td>exon 8</td>
<td>1</td>
<td>n.d.</td>
<td>33</td>
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<tr>
<td>c.1850G&gt;A</td>
<td>p.Arg617His</td>
<td>exon 8</td>
<td>29</td>
<td>absent</td>
<td>6, 13, 17, 33, 49, 57, 58, 62, 88, 96, 100, 125</td>
</tr>
</tbody>
</table>
Rebuilding the database

- Track all (mutation) publications from 1993 – 2017
- 173 publications
- Data from 13 collaborating diagnostic laboratories / investigators

- Two numbering systems (1993 - ~2004)
  - ATG = 1 or ATG = 386

\[ 706 - 386 = 320 \]  
\[ \text{c.320T>C} \]

<table>
<thead>
<tr>
<th>Missense</th>
<th>Mutation Site</th>
<th>Amino Acid Change</th>
<th>Codon Change</th>
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<tbody>
<tr>
<td>L107P</td>
<td>T→C at 706</td>
<td>Leu→Pro</td>
<td>(L/C)</td>
</tr>
<tr>
<td>Y174D</td>
<td>T→G at 906</td>
<td>Tyr→Asp</td>
<td>(Y/Y)</td>
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<tr>
<td>L211P</td>
<td>T→C at 1018</td>
<td>Leu→Pro</td>
<td>(L/L)</td>
</tr>
<tr>
<td>T254M</td>
<td>C→T at 1147</td>
<td>Tyr→Pro</td>
<td>(T/S)</td>
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<tr>
<td>G277R</td>
<td>G→A at 1215</td>
<td>Gly→Arg</td>
<td>(G/G)</td>
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<tr>
<td>R389G</td>
<td>C→G at 1551</td>
<td>Arg→Gly</td>
<td>(R/R)</td>
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</tbody>
</table>
Rebuilding the database

• Number of cases for each mutation

• Papers containing additional experimental data supporting the pathogenicity of a variant.

• 2642 mutations (cases) representing 799 different mutations.

• Add (expert opinion) remarks for each variant.

• No genotype – phenotype correlation
  • No clinical information on website
# ALD database: 2642 mutations (799 non-recurrent)

<table>
<thead>
<tr>
<th>Chr. position</th>
<th>variant</th>
<th>consequence</th>
<th>exon</th>
<th>remark</th>
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</thead>
<tbody>
<tr>
<td>153008483</td>
<td>c.1823G&gt;A</td>
<td>p.Gly608Asp</td>
<td>exon 8</td>
<td>Reported in ALD male (49), but considering its frequency of 306/36626 control alleles (X:153008483 G/A) this is not a pathogenic variant.</td>
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<tr>
<td>153008485</td>
<td>c.1825G&gt;A</td>
<td>p.Glu609Lys</td>
<td>exon 8</td>
<td>Pathogenic, identified in 29 ALD patients (15, 16, 32, 33, 49, 60, 62, 72, 88, 90, 97, 141, 146). Reduced (2% of control cells) ALDP in patient cells (97) using a quantitative immunoblot technique, no detectable ALDP by immunofluorescence (32, 49, 60, 72, 141, 146).</td>
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<tr>
<td>153008486</td>
<td>c.1826A&gt;G</td>
<td>p.Glu609Gly</td>
<td>exon 8</td>
<td>Pathogenic, identified in 3 ALD patients (15, 97, 141). Reduced (2% of control cells) ALDP in patient cells (97) using a quantitative immunoblot technique, no detectable ALDP by immunofluorescence (32, 49, 60, 72, 141, 146).</td>
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<td>153008492</td>
<td>c.1832G&gt;C</td>
<td>p.Gln611Arg</td>
<td>exon 8</td>
<td>Pathogenic, identified in ALD patient (33).</td>
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<tr>
<td>153008493</td>
<td>c.1833G&gt;C</td>
<td>p.Gln611His</td>
<td>exon 8</td>
<td>Pathogenic, identified in ALD patient (33).</td>
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<td>Synonymous (X:153008499 C/T).</td>
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<td>c.1840G&gt;C</td>
<td>p.Gly614Arg</td>
<td>exon 8</td>
<td>Pathogenic, identified in ALD patient (88).</td>
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<tr>
<td>153008506</td>
<td>c.1846G&gt;A</td>
<td>p.Ala616Thr</td>
<td>exon 8</td>
<td>Pathogenic, identified in 8 ALD patients (32, 97, 141). Reduced (4% of control cells) ALDP in patient cells (97) using a quantitative immunoblot technique, no detectable ALDP by immunofluorescence (32, 141).</td>
</tr>
<tr>
<td>153008507</td>
<td>c.1847C&gt;A</td>
<td>p.Ala616Asp</td>
<td>exon 8</td>
<td>Pathogenic, identified in ALD patient (90).</td>
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<tr>
<td>153008507</td>
<td>c.1847C&gt;T</td>
<td>p.Ala616Val</td>
<td>exon 8</td>
<td>Pathogenic, identified in ALD patient (29).</td>
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<tr>
<td>153008509</td>
<td>c.1849C&gt;A</td>
<td>p.Arg617Ser</td>
<td>exon 8</td>
<td>Pathogenic, identified in ALD patient (92).</td>
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<tr>
<td>153008509</td>
<td>c.1849C&gt;G</td>
<td>p.Arg617Gly</td>
<td>exon 8</td>
<td>Pathogenic, identified in 10 ALD patients (15, 33, 59, 64, 67, 86, 93, 132, 137).</td>
</tr>
<tr>
<td>153008509</td>
<td>c.1849C&gt;T</td>
<td>p.Arg617Cys</td>
<td>exon 8</td>
<td>Pathogenic, identified in 30 ALD patients (6, 15, 16, 32, 33, 49, 58, 59, 60, 64, 86, 88, 93, 132, 137, 145, 178). No detectable ALDP in patient cells (32, 49, 58).</td>
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<tr>
<td>153008509</td>
<td>c.1849del</td>
<td>p.Arg617Alafs*19</td>
<td>exon 8</td>
<td>Pathogenic, identified in ALD patient (60). Deleterious mutation. No detectable ALDP in patient cells (60).</td>
</tr>
<tr>
<td>153008510</td>
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<td>153008510</td>
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<td>exon 8</td>
<td>Pathogenic, identified in 46 ALD patients (6, 13, 17, 22, 32, 33, 49, 57, 58, 60, 62, 74, 87, 88, 96, 100, 125, 145, 147, 154, 178). No detectable ALDP in patient cells (22, 33, 49, 58, 60, 147).</td>
</tr>
</tbody>
</table>
### ALD database: 2642 mutations (799 non-recurrent)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Protein Change</th>
<th>Exon</th>
<th>Pathogenicity Note</th>
</tr>
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<tbody>
<tr>
<td>c.1826A&gt;G</td>
<td>p.Glu609Gly</td>
<td>8</td>
<td>Pathogenic, identified in 3 ALD patients (16, 97, 141). Reduced (2% of control cells) ALDP in patient cells (97) using a quantitative immunoblot technique, no detectable ALDP by immunofluorescence (32, 49, 60, 72, 141, 146).</td>
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<td>c.1839C&gt;T</td>
<td>p.Ile613Ile</td>
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<td>Synonymous (X:153008499 C/T).</td>
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</tr>
<tr>
<td>c.1849C&gt;T</td>
<td>p.Arg617Cys</td>
<td>8</td>
<td>Pathogenic, identified in 30 ALD patients (6, 15, 16, 32, 33, 49, 58, 59, 60, 64, 86, 88, 93, 132, 137, 145, 178). No detectable ALDP in patient cells (32, 49, 58).</td>
</tr>
<tr>
<td>c.1849del</td>
<td>p.Arg617Alafs*19</td>
<td>8</td>
<td>Pathogenic, identified in ALD patient (60). Deleterious mutation. No detectable ALDP in patient cells (60).</td>
</tr>
<tr>
<td>c.1850del</td>
<td>p.Arg617Profs*19</td>
<td>8</td>
<td>Pathogenic, identified in ALD patient (33). Deleterious mutation.</td>
</tr>
<tr>
<td>c.1850G&gt;A</td>
<td>p.Arg617His</td>
<td>8</td>
<td>Pathogenic, identified in 46 ALD patients (6, 13, 17, 22, 32, 33, 49, 57, 58, 60, 62, 74, 87, 88, 96, 100, 125, 145, 147, 154, 178). No detectable ALDP in patient cells (22, 33, 49, 58, 60, 147).</td>
</tr>
</tbody>
</table>

- p.Gly608Asp: Reported in ALD male (48), but considering its frequency of 306/36626 control alleles (X:153008483 G/A) this is not a pathogenic variant.
- p.Glu609Lys: Pathogenic, identified in 29 ALD patients (15, 16, 32, 33, 49, 60, 62, 72, 88, 90, 97, 141, 146). Reduced (2% of control cells) ALDP in patient cells (97) using a quantitative immunoblot technique, no detectable ALDP by immunofluorescence (32, 49, 60, 72, 141, 146).

- Visitors: 507,403

<table>
<thead>
<tr>
<th>Top 10 visited pages</th>
<th>Visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facts on ALD</td>
<td>75.805</td>
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<tr>
<td>Datos sobre la ALD</td>
<td>72.646</td>
</tr>
<tr>
<td>Very long-chain fatty acids</td>
<td>28.722</td>
</tr>
<tr>
<td>Ácidos grasos de cadena muy larga (VLCFA)</td>
<td>28.711</td>
</tr>
<tr>
<td>Mutations in ABCD1</td>
<td>19.688</td>
</tr>
<tr>
<td>Women with ALD</td>
<td>18.102</td>
</tr>
<tr>
<td>Hematopoietic Stem Cell Transplantation</td>
<td>14.681</td>
</tr>
<tr>
<td>Lorenzo’s oil</td>
<td>14.345</td>
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<tr>
<td>Fakten zur ALD</td>
<td>13.539</td>
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<tr>
<td>Diagnosis of ALD</td>
<td>12.619</td>
</tr>
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</table>
After 18 years …

____________________www.x-ald.nl

www.adrenoleukodystrophy.info
ALD database

X-linked adrenoleukodystrophy (ALD) is the most common leukodystrophy with a birth incidence of 1 in 15,000 newborns. It is a progressive, neurometabolic disease that affects brain, spinal cord, peripheral nerves, adrenal cortex and testis. ALD is caused by mutations in the ABCD1 gene. More than 775 unique mutations have been identified. For a comprehensive summary, please visit the Facts on ALD page.

Mutations & Biochemistry
A catalogue of the >750 unique mutations and variations in the ABCD1 gene; info on biochemistry, the ALD gene, etc.

Clinical & Diagnosis
Read posts on different clinical presentations of ALD in males and females, newborn screening and watch educational videos and webinars.

Treatment options
Read about successful and unsuccessful treatment options.
**Mutation statistics**

- A new variant is by definition a variant of unknown significance

### Statistics of ABCD1 mutations and variants

<table>
<thead>
<tr>
<th></th>
<th>All Mutations</th>
<th>Non-recurrent Mutations</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>All ALD mutations in database</td>
<td>2642</td>
<td>N/A</td>
</tr>
<tr>
<td>missense mutations</td>
<td>1607</td>
<td>61 %</td>
</tr>
<tr>
<td>nonsense mutations</td>
<td>258</td>
<td>10 %</td>
</tr>
<tr>
<td>frame shift mutations</td>
<td>450</td>
<td>17 %</td>
</tr>
<tr>
<td>amino acid insertions/deletions</td>
<td>100</td>
<td>4 %</td>
</tr>
<tr>
<td>splice site mutations</td>
<td>106</td>
<td>4 %</td>
</tr>
<tr>
<td>one or more exons deleted</td>
<td>83</td>
<td>3 %</td>
</tr>
<tr>
<td>benign variants</td>
<td>35</td>
<td>1 %</td>
</tr>
<tr>
<td>Variants of unknown significance (VUS)</td>
<td>227</td>
<td></td>
</tr>
<tr>
<td>Synonymous variants</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>
Diagnostic dilemmas in newborn screening

Newborns

- Tier 1 & 2: [C26:0-lysoPC] ↑↑
- Tier 3: a variant of unknown significance (VUS) in ABCD1
  - Is this variant pathogenic??

MS/MS for C26:0LPC → HPLC-MS/MS for C26:0LPC → ABCD1 gene sequencing → Adrenoleukodystrophy

Requires further diagnostic testing
- Zellweger syndrome deficiency
- ACOX1 or HSD17B4 deficiency
- CADDS
- ACBD5
- Aicardi Goutières Syndrome
Translational metabolism (VLCFA homeostasis)

Need for functional testing

• Fibroblasts

• mRNA analysis (splice defects)
• Beta-oxidation
• VLCFA homeostasis
• ALDP expression
• VLCFA and C26:0-lysoPC analysis
Translational metabolism (VLCFA homeostasis)
Translational metabolism (VLCFA homeostasis)

- endoplasmic reticulum
- cell membrane
- mitochondrion
- peroxisome
- elongation
- D₃-C₂₂:₀
- D₃-C₁₆:₀
- ALDP
Translational metabolism (VLCFA homeostasis)

A. Peroxisomal beta-oxidation

B. de novo VLCFA synthesis

\[
\text{D}_3\text{C}_{16:0}/\text{D}_3\text{C}_{22:0} \text{ ratio}
\]

\[
\text{D}_3\text{C}_{26:0} \text{ (nmol/mg protein)}
\]
ALDP expression

| Immunofluorescence: | - | - | - | - | - | - | - | - |
| Western blot (% of control): | 0% | 7% | 1% | 4% | 2% | 5% | 2% | 3% | 2% |

Zhang et al. (2011) Biochem J
**Diagnostic dilemmas**

**Women with ALD**

- **Two unrelated women**
- **Clinical presentation compatible with ALD**
- **Plasma C26:0 and C26:0/C22:0 normal**
- **Both a new variant in **ABCD1**(p.Arg17His, p.Pro358Ser)**
- **No affected relatives**
- **Are these variants pathogenic?**
- **Desire to have a child**

Pathogenicity of novel **ABCD1** variants: The need for biochemical testing in the era of advanced genetics

Martin J.A. Schackmann, Rob Ofman, Björn M. van Geel, Inge M.E. Dijkstra, Klaartje van Engelen, Ronald J.A. Wanders, Marc Engelen, Stephan Kemp

---

Schackmann et al. (2016) Mol Genet Metab
Diagnostic dilemmas

Mosaicism: cells expressing the normal allele or the variant allele

Immortalize cells and clonal expansion (confirmation which allele is active per clone)

Biochemical studies in 3 individual clones per allelic variant (ALDP, VLCFA, beta-oxidation)

Schackmann et al. (2016) Mol Genet Metab
Diagnostic dilemmas

p.Arg17His and p.Pro358Ser are non-pathogenic variants

p.Arg17His and p.Pro358Ser are non-pathogenic variants
Can we improve the diagnostic test in women?

- Can we reach 100% sensitivity?
- Comparison plasma VLCFA and C26:0-lysoPC in dried blood spots from 20 female controls and 46 women with ALD.
Plasma C26:0 versus C26:0-lysoPC in DBS
Biomarker discovery

The Dutch ALD Cohort

- 58 men and 65 women (and growing)
- Prospective cohort (natural history)
- Annual follow-up (including patient care)
- Clinical evaluation (including 6 MWT)
- Brain imaging: 3T and 7T quantitative MRI
- Bio-banking

- Discordant sib-pairs, well-defined sub groups (AMN vs cerebral ALD)
- Genetics, epigenetics, lipidomics, etc.
Biomarker discovery (lipidomics)
Biomarker discovery (lipidomics)

Volcano plot (plasma control versus ALD)

- P-value (-log10)
- Fold change (log2)

- Symbols represent different lipids:
  - LPA
  - LPC
  - PE
  - PC
  - PS

- Significant differences marked by p-values:
  - p < 0.001
  - p < 0.01
  - p < 0.05

- >4-fold decreased in ALD
- >4-fold increased in ALD
Challenges / ongoing research

• Identification and validation of predictive biomarkers for the clinical outcome in individual ALD patients.

• Need for functional testing of ABCD1 variants.

• Sharing of information on mutations is important.

• Need for more sensitive biomarkers in plasma or dried blood spots.
  • Validation of new biomarkers
The AMC-ALD group (www.amc.nl/ALDgroup)

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