Two novel aspartoacylase gene (ASPA) missense mutations specific to Norwegian and Swedish patients with Canavan disease

T R Olsen, L Tranebjærg, E A Kvittingen, L Hagenfeldt, C Møller, Ø Nilsen


Canavan disease (CD) (OMIM 271900) is an autosomal recessive leukodystrophy characterised by swelling and spongy degeneration of the white matter of the brain. The biochemical marker for the disorder is increased level of N-acetylaspartic acid (NAA) in cerebrospinal fluid and urine owing to aspartoacylase (ASPA) deficiency. The disease is caused by mutations in the gene encoding the enzyme aspartoacylase (EC 3.5.1.15) at 17p13-ter.

Clinical features are macrocephaly, head lag, and hypotonia from the age of 3-6 months followed by rapidly progressive severe mental retardation. Life expectancy is usually into the teens and development of optic atrophy is typical. Only symptomatic treatment is possible. The prevalence is highest among Ashkenazi Jews with a carrier frequency of 1 in 38 and two mutations, Y231X and E285A, constitute 98% of the CD alleles in this population. Canavan disease is rare in non-Jewish populations and one mutation, A305E (914C→T), is found in about 40% to 60% of the disease alleles reported so far. Mutations are scattered all over the six exons of the aspartoacylase gene. In total, 38 different mutations have been reported of which 35 are listed in The Human Gene Mutation Data Base, Cardiff, UK. Prenatal diagnosis by enzyme assay is complicated by technical difficulties. Measurement of N-acetylaspartate concentration in amniotic fluid by stable isotope dilution is considered a reliable approach. However, for carrier detection and prenatal diagnosis, mutation detection should be performed. Our aim has been to study the mutational spectrum of the aspartoacylase gene in Norwegian and Swedish patients of non-Jewish origin. This knowledge is essential for patient management and for the design of rational molecular genetic diagnosis.

MATERIALS, METHODS, AND SUBJECTS

Eight Norwegian patients from six families and one Swedish patient (table 1) were identified by clinical characterisation and enzyme investigations. Clinical information was provided by the paediatricians who had followed the patients from the time of diagnosis. All the patients were boys and all parents and sibs were healthy. The pre- and perinatal periods were unremarkable in all cases except in patient N2 where symptoms started after one week and patient N4 where hypotonia was noted from birth (table 1). Through their mother, patients N2/N3 (family 2) were related to patient N1 (family 1) and through their father they were related to patient N4 (family 3). A fourth family (family 4) could not be linked to the other three families but their ancestors originally came from the same small geographical area.

DNA studies

DNA purification

Genomic DNA was isolated from whole blood by use of an automated DNA extractor (341 Nucleic Acid Purification System DNA extractor, Applied Biosystems), or by salting out methods.

cDNA synthesis

RNA was extracted from cultured skin fibroblast cells by TRI-ZOL LS reagents according to the manufacturer’s specifications (GIBCO, BRL). Total RNA was reverse transcribed using 100 pmol oligo dT/pg RNA and 200 U Super ScriptII reverse transcriptase (GIBCO, BRL) at 50°C for 60 minutes.

PCR

Intronic PCR primers flanking each exon were used for PCR amplification and direct sequencing (table 2). All PCR reactions were carried out on a DNA Thermal Cycler/480 (Perkin-Elmer). PCR was performed in a 50 µl volume containing 400 ng genomic DNA, 10 pmol of each primer, 1 mmol/l dNTPs, 10 × Taq polymerase buffer, 25 mmol/l MgCl, and 5U Taq polymerase.

DNA sequencing

The PCR products were sequenced with the appropriate primer by the use of PCR product Presequencing Kit (Amersham Life Science) and ABI PRISM BigDye Termination Cycle Sequencing Kit (Perkin-Elmer). The sequences were analysed on an ABI 377 automated sequencer unit.

Key points

- Canavan disease (CD) (OMIM 271900) is an autosomal recessive neurodegenerative disorder characterised by spongy degeneration of the white matter of the brain. The disease is caused by deficiency of the enzyme aspartoacylase, encoded by the ASPA gene.
- The mutations E285A and Y231X comprise 98% of all mutations in CD patients in the Ashkenazi Jewish population. In non-Jewish CD patients a total of 38 different mutations have been reported of which one, A305E, is particularly prevalent as it is shared by 40-60% of the patients. The majority of patients investigated originate from southern Europe, United Kingdom, Israel, and Japan. No information exists on mutations in CD patients from Norway and Sweden.
- We report the molecular investigation of eight Norwegian and one Swedish patients deriving from seven families. Mutation analysis of these nine patients, of whom four were related, showed two novel missense mutations, 340G→T (D114Y) and 746A→T (D249V), as well as a previously reported insertion, 245insA. Survival into adulthood was found in four patients, not associated with a particular mutation.
(Perkin-Elmer) and mutations were detected by manual inspection of the sequence electropherograms.

**Mutation detection by PCR and restriction enzyme analyses**

In order to facilitate detection of mutations D114Y (340G→T) and D249V (746A→T), assays were developed based on PCR on genomic DNA followed by restriction enzyme digestion and agarose gel electrophoresis. PCR components were as described above. A DNA fragment embracing D114Y (340G→T) was amplified with primers pCANmF and pCANmR (table 2) at the following cycling conditions: denaturation at 95°C for five minutes followed by 36 cycles with denaturation at 94°C for 30 seconds and annealing/extension at 64°C for 90 seconds. A DNA fragment containing mutation 746A→T (D249V) was amplified with primers Caex6F and CAR3 (table 1) under the following conditions: denaturation at 95°C for five minutes followed by 36 cycles with denaturation at 94°C for one minute, annealing at 55°C for 30 seconds, and extension at 72°C for one minute. The use of restriction enzyme *SnaBI* allowed us to discriminate between the D114 and 114Y alleles, whereas the use of *MboI* allowed discrimination between the D249 and 249V alleles. Restriction enzyme digestion was performed according to the manufacturer’s specifications.

**RESULTS**

**Case reports**

The clinical findings were very similar for all the nine cases and typical for CD: hypotonia developing into spastic tetraplegia, macrocephaly, mental retardation, head lag, and visual impairment. As indicated in table 1, brain biopsy or raised urinary concentrations of NAA led to the diagnosis of Canavan disease. Notably, three of the patients are still alive at the ages of 25, 21, and 15 (N1, N7, and S9, respectively).

Patient N1 showed delayed development at 3 months. Later, he also developed epilepsy, asthma, and diabetes mellitus. Patient N2 showed failure to thrive, irritability, and spasticity at the age of 1 week. The diagnosis was not established at the time of death, at the age of 7 months. His younger brother, born 6 years later (patient N3), died at the age of 8 months, severely mentally retarded, blind, and spastic. A brain biopsy showed spongy degeneration and Canavan disease was diagnosed and deduced also for his brother (N2).

Patient N4 showed hypotonia, failure to thrive, and irritability immediately after birth. At three months, he had hyperreflexia, spastic lower legs, and made no visual contact. Urinary NAA levels were markedly raised.

The parents of patients N5 and N6 (brothers) originate from the same small geographical area as the three previously mentioned Norwegian families (N1-N4). Cerebral CT scan

---

**Table 1** Clinical summary and mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Age of onset</th>
<th>Alive at</th>
<th>Age at death</th>
<th>Diagnosis by</th>
<th>Mutation</th>
<th>aa change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N1</td>
<td>3 months</td>
<td></td>
<td>25 years</td>
<td>Brain biopsy</td>
<td>340G→T</td>
<td>D114Y</td>
</tr>
<tr>
<td>2</td>
<td>N2*</td>
<td>1 week</td>
<td></td>
<td>7 months</td>
<td>Brain biopsy</td>
<td>340G→T</td>
<td>D114Y</td>
</tr>
<tr>
<td>3</td>
<td>N3</td>
<td>4 months</td>
<td></td>
<td>8 months</td>
<td>Brain biopsy</td>
<td>340G→T</td>
<td>D114Y</td>
</tr>
<tr>
<td>4</td>
<td>N4</td>
<td>birth</td>
<td></td>
<td>6.5 months</td>
<td>UNAA</td>
<td>340G→T</td>
<td>D114Y</td>
</tr>
<tr>
<td>5</td>
<td>N5*</td>
<td>3-4 months</td>
<td></td>
<td>3.5 months</td>
<td>Brain biopsy</td>
<td>340G→T</td>
<td>D114Y</td>
</tr>
<tr>
<td>6</td>
<td>N6*</td>
<td>8-10 weeks</td>
<td></td>
<td>6.5 years</td>
<td>Brain biopsy</td>
<td>340G→T</td>
<td>D114Y</td>
</tr>
<tr>
<td>7</td>
<td>N7</td>
<td>3 months</td>
<td></td>
<td>21 years</td>
<td>UNAA</td>
<td>245insA</td>
<td>Truncation</td>
</tr>
<tr>
<td>8</td>
<td>N8</td>
<td>3 months</td>
<td></td>
<td>17.4 years</td>
<td>UNAA</td>
<td>245insA</td>
<td>Truncation</td>
</tr>
<tr>
<td>9</td>
<td>S9</td>
<td>3 months</td>
<td></td>
<td>15 years</td>
<td>UNAA</td>
<td>746A→T</td>
<td>D249V</td>
</tr>
</tbody>
</table>

*Identification of the mutations was based on the genotypes of their parents (N5 and N6) or affected brother (N2).

**NOTE:** urn N-acetylaspartic acid.

---

**Table 2** List of primers

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
<th>Derived from GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cDNA primers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAF1</td>
<td>5′CTGACTTGGGCTTGTGGTA</td>
<td>567156.1</td>
</tr>
<tr>
<td>CAF3</td>
<td>5′CTGATGGACATGCCCTCTCCA</td>
<td>567156.1</td>
</tr>
<tr>
<td>CAR3</td>
<td>5′ACACCGTGAAGATGTAAGCT</td>
<td>567156.1</td>
</tr>
<tr>
<td><strong>Genomic DNA primers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caex1F</td>
<td>5′CTCTGAATTGCAGAAATCAAGA</td>
<td>170466</td>
</tr>
<tr>
<td>Caex1R</td>
<td>5′CACACCTACCTTGCACACAA</td>
<td></td>
</tr>
<tr>
<td>Caex2F</td>
<td>5′CTGAGGCAAGATGTTGTTCA</td>
<td>170467</td>
</tr>
<tr>
<td>Caex2R</td>
<td>5′CAAGGCTCTTGCTGACTTATAA</td>
<td></td>
</tr>
<tr>
<td>Caex3F</td>
<td>5′GGGTTTTTACCAAGAAGACGT</td>
<td>170468</td>
</tr>
<tr>
<td>Caex3R</td>
<td>5′CTCTGAGTTTCAGCTAGGACAT</td>
<td></td>
</tr>
<tr>
<td>Caex4F</td>
<td>5′ATGATCTAGTCCCTCTTCTG</td>
<td>170469</td>
</tr>
<tr>
<td>Caex4R</td>
<td>5′TCTGACCCAGGGTCATGTTGCA</td>
<td></td>
</tr>
<tr>
<td>Caex5F</td>
<td>5′CAGAGATGTTGAGTGGGCT</td>
<td>170470</td>
</tr>
<tr>
<td>Caex5R</td>
<td>5′TGCTCTAGGAGCTATAAAGCT</td>
<td></td>
</tr>
<tr>
<td>pCANmF</td>
<td>5′TATAACACGCAAAAAATGATCGTGATTGCCGATAGAAGT</td>
<td>170471</td>
</tr>
<tr>
<td>pCANmR</td>
<td>5′CCCATGATTGAGGGGTTGGTTGGACG</td>
<td></td>
</tr>
</tbody>
</table>

To allow the discrimination between alleles D114 and 114Y (340G→T) the primers pCANmF and pCANmR were modified in the following way. For pCANmF the nine nucleotides at the 5′ end were replaced with A, C, and T (underlined), respectively. For pCANmR, nucleotides A25 and G27 were replaced with T and C (underlined), respectively. These replacements allowed SnaBI digestion of the mutant 114Y allele while leaving the D114 allele resistant.
Homozygosity for nucleotide substitution 746A→T in exon 2, resulting in the replacement of Asp with Tyr at amino acid position 114 (D114Y) (results not shown). Homozygosity for nucleotide substitution 746A→T was identified in one Norwegian as well as in the only Swedish CD patient included in this study (results not shown). This further shows the importance of residue 114 for the structure/function of the ASPA enzyme. Hence, we conclude that 746A→T causes an amino acid replacement only.

PCR based diagnostic tests were developed for both 340G→T (D114Y) and 746A→T (D249V) (fig 1). Neither D114Y nor D249V were found in a panel of 100 Norwegian control alleles (data not shown).

The Norwegian patient N7 was found to be homozygous for the mutation 245insA. This particular mutation in exon 1 causes a shift in the translational reading frame and, hence, a truncated gene product.

**DISCUSSION**

The majority of non-Jewish Canavan patients investigated originate from southern Europe, United Kingdom, Israel, and Japan. In this work we present brief clinical data and the identification of two novel mutations in Norwegian and Swedish patients with Canavan disease.

The mutation D114Y (340G→T) was found in three related families and in a fourth family originating from the same small geographical region in Norway. It is located in exon 2, resulting in the replacement of the charged, acidic side chain of aspartic acid with the hydrophobic, bulky, aromatic side chain of tyrosine. Hence, the severe consequences of such an alteration can easily be envisaged. Moreover, another amino acid substitution at this particular residue has previously been reported: aspartic acid to glutamic acid (D114E), resulting from 342C→A in a patient of Turkish origin. Such a replacement of Asp with Glu, which conserves the charge and polarity of the side chain but extends the length by only one single methyl group, is apparently sufficient to abolish ASPA activity which was only 0.35% of residual activity. This further shows the importance of residue 114 for the structure/function of the ASPA enzyme.

The mutation D249V (746A→T) was found in one Norwegian and the Swedish patient. The replacement of Asp with Val at amino acid position 249 represents a substitution of a charged, polar side group with an aliphatic, hydrophobic, non-polar side group. The difference in the chemical properties of Asp and Val is consistent with the notion that D249V is abolishing ASPA activity and, hence, is disease causing. Since neither D114Y nor D249V have been reported elsewhere, it is likely that these mutations are specific to Canavan patients from the Scandinavian peninsula.

**Figure 1**  
(A) Detection of the D114Y mutation by SnaBI mediated analyses of a normal control (lane 2), undigested control (lane 3), affected 114Y/114Y homozygote (lane 4), and heterozygote (lane 5). Genomic DNA was amplified and digested with SnaBI. Normal, undigested PCR product is 142 bp whereas normal SnaBI digested PCR product is 120 bp (+22 bp control fragment). Lack of the 120 bp band shows homozygosity for the mutant 114Y allele. The mutant allele is shown by the presence of the 93 bp. Lanes 1 and 7 are 100 bp ladder. (B) Detection of the D249V mutation. Analyses of a normal control (lane 2), undigested control (lane 3), affected 249V/249V homozygote (lane 4), and heterozygote (lane 5). Genomic DNA was amplified and digested with Mbol. Normal, undigested PCR product is 298 bp whereas the normal digested PCR product gives rise to bands of 167 bp, 69 bp, 32 bp, and 30 bp. Lack of the 69 bp and 30 bp bands indicates homozygosity for the mutant 249V allele. The mutant allele is seen as the presence of 167 bp, 99 bp, and 32 bp. Lanes 1 and 7 are 100 bp ladder.
The mutation, 245insA, a frameshift mutation resulting in a truncated, non-functional gene product, was found in a family from western Norway. This mutation was previously identified in an Italian patient. It is located in a run of seven adenines, a motif previously documented to be prone to misincorporation of an extra base by the DNA polymerase as a result of slipped mispairing at the replication fork.

Both D114 and D249 are conserved in the ASPA genes of cow (93% identity), mouse (86% identity), and, strikingly, also in Cyanobacteria (46% identity). In the ASPA gene of rat (86% identity), however, D249 is conserved whereas D114 is replaced with valine. The D114Y, D249V, and 245insA were the only mutations found in these patients. The fact that they were absent in 100 normal control alleles strongly indicates the pathogenicity of these mutations.

Clinical data showed survival into adulthood in four out of six Norwegian/Swedish patients (table 1). However, since both 114Y/114Y and 249V/249V homozygotes show variable survival time, no genotype-phenotype correlation was evident. Lack of genotype-phenotype correlation has also been previously reported for other CD mutations. It remains to be elucidated how environmental and epigenetic factors may influence the clinical course of Canavan disease. Few data exist concerning residual aspartoacylase activity in CD patients. Such information is crucial to clarify a possible link between genotype, enzyme activity, and clinical phenotype.

Our findings add to the spectrum of mutations in Canavan disease and are essential to the genetic diagnosis and counseling of CD families of Norwegian and Swedish origin. The location and nature of the mutations may contribute to further understanding of the function and structure of the ASPA gene product.

ACKNOWLEDGEMENTS

The present work was financially supported by The Norwegian Research Council (NFR project No 114575/320), Samordningsrådet for forskningsfondene (SOR), and The Norwegian Research Foundation for Mental Deficiency-Norwegian Red Cross. We are grateful to the participating families and to Drs Dag Veimo, Nordland Central Hospital, Norway, Sigurd Bersting, Innherred Hospital, Levanger, Norway, and Per Erik Waaler, Haukeland University Hospital, Bergen, Norway, for the referral of patients and valuable clinical contributions. We are also grateful to Helle Klenow and Karina Pedersen, University Hospital of Northern Norway, for their technical assistance. We are also grateful to Helle Klenow and Karina Pedersen, University Hospital of Northern Norway, for the referral of patients and valuable clinical contributions.

Authors’ affiliations

I R Olsen, L Tranebjærg*, Ø Nilsen, Department of Medical Genetics, University Hospital of Northern Norway, N-9038 Tromsø, Norway
Eivindrill@fagmed.uit.no

T Møller, Department of Paediatrics, Haukeland University Hospital, Bergen, Norway

Correspondence to: Dr Ø Nilsen, Department of Medical Genetics, University Hospital of Northern Norway, N-9038 Tromsø, Norway

REFERENCES

7. The Human Gene Mutation Data Base Cardif, UK. http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html
Two novel aspartoacylase gene (ASPA) missense mutations specific to Norwegian and Swedish patients with Canavan disease

T R Olsen, L Tranebjaerg, E A Kvittingen, L Hagenfeldt, C Møller and Ø Nilssen

doi: 10.1136/jmg.39.9.e55

Updated information and services can be found at:
http://jmg.bmj.com/content/39/9/e55

**References**

This article cites 17 articles, 1 of which you can access for free at:
http://jmg.bmj.com/content/39/9/e55#BIBL

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Topic Collections**

Articles on similar topics can be found in the following collections

- Molecular genetics (1254)
- Multiple sclerosis (20)
- Eye Diseases (298)
- Reproductive medicine (519)
- JMG Online mutation reports (168)

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/