Am. J. Hum. Genet. 57:572–580, 1995

The Molecular Basis of Canavan (Aspartoacylase Deficiency) Disease in European Non-Jewish Patients

A. Shaag,[1] Y. Anikster,[1] E. Christensen,[2] J. Z. Glustein,[1] A. Fois,[3] H. Michelakakis,[4] F. Nigro,[5] E. Pronicka,[6] A. Ribes,[7] M. T. Zabot,[8] and O. N. Elpeleg[1]

[1]Metabolic Unit, Shaare-Zedek Medical Center, Jerusalem; [2]Department of Clinical Genetics, Rigshospitalet, Copenhagen; [3]Clinica Pediatrica, University di Siena, Siena; [4]Division of Enzymology and Cellular Function, Institute of Child Health, Aghia Sophia Children's Hospital, Athens; [5]Clinica Pediatrica, University di Catania, Catania; [6]Department of Metabolic Diseases, Child Health Center, Warsaw; [7]Institut de Bioquimica Clinica, Corporacio Sanitaria, Barcelona; and, [8]Unite Culture Cellulaire, Service de Biochimie, Hopital Debrousse, Lyon

Summary

Canavan disease is an infantile neurodegenerative disease that is due to aspartoacylase deficiency. The disease has been reported mainly in Ashkenazi Jews but also occurs in other ethnic groups. Determination of enzymatic activity for carrier detection and prenatal diagnosis is considered unreliable. In the present study, nine mutations were found in the aspartoacylase gene of 19 non-Jewish patients. These included four point mutations (A305E [39.5% of the mutated alleles], C218X [15.8%], F295S [2.6%], and G274R [5.3%]); four deletion mutations (827delGT [5.3%], 870del4 [2.6%], 566del7 [2.6%], and 527del6 [2.6%]); and one exon skip (527del108 [5.3%]). The A305E mutation is pan-European and probably the most ancient mutation, identified in patients of Greek, Polish, Danish, French, Spanish, Italian, and British origin. In contrast, the G274R and 527del108 mutations were found only in patients of Turkish origin, and the C218X mutation was identified only in patients of Gypsy origin. Homozygosity for the A305E mutation was identified in patients with both the severe and the mild forms of Canavan disease. Mutations were identified in 31 of the 38 alleles, resulting in an overall detection rate of 81.6%. All nine mutations identified in non-Jewish patients reside in exons 4–6 of the aspartoacylase gene. The results would enable accurate genetic counseling in the families of 13 (68.4%) of 19 patients, in whom two mutations were identified in the aspartoacylase cDNA.

Introduction

Canavan disease (CD) is a severe neurodegenerative disorder, primarily affecting the white matter. The disease manifests at several months of age, by poor head control, macrocephaly, marked developmental delay, optic atrophy, seizures, hypertonia, and death in early childhood. A milder course, mainly characterized by moderate developmental delay, has also been reported. CD is transmitted in an autosomal recessive manner and has been reported mainly in Ashkenazi Jewish families. Until recently the diagnosis of CD could be established only by brain biopsy demonstrating spongy degeneration of the white matter, with vacuoles within the myelin sheaths, astrocyte swelling, and deformed mitochondria (Van Bogaert 1970). In 1988, patients with CD were found to excrete excessive amounts of N-acetylaspartic acid in urine, and deficiency of the enzyme aspartoacylase was demonstrated in their cultured skin fibroblasts (Divry et al. 1988; Matalon et al. 1988). The human aspartoacylase gene spans 30 kb, consists of six exons and five introns, and has been localized to chromosome 17p13-ter (Kaul et al. 1994*a*). The mature protein consists of 313 amino acids. Four point mutations have already been reported: three (Y231X, E285A, and 433-2A→G) in the American Ashkenazi Jewish population and one (A305E) in non-Jewish patients (Kaul et al. 1994*b*). In Israel, all diagnosed CD patients were found to be homozygotes for the E285A mutation, and the carrier frequency in the Ashkenazi Jewish population was estimated at 1:59 (Elpeleg et al. 1994*a*). We now report nine mutations in the aspartoacylase gene in 19 non-Jewish CD patients.

Subjects and Methods

Subjects

Fibroblast cell lines of 19 non-Jewish CD patients of different families were available for mutation analysis; these patients included 1 British (B1), 2 Danish (D1 and D2), 2 French (F1 and F2), 3 Greek (Gr1, Gr2, and Gr3), 1 Guadeloupean (Gu1), 3 Italian (I1, I2, and I3), 1 Polish (P1), 3 Spanish (S1, S2, and S3), and 3 Turkish (T1, T2, and T3) individuals. To the best of our knowledge, these were all the diagnosed CD patients in the respective countries whose fibroblasts were available for analysis. The medical records of 18 patients, 12 males

Received October 18, 1994; accepted for publication June 20, 1995.
Address for correspondence and reprints: Dr. Orly N. Elpeleg, Metabolic Unit, Shaare-Zedek Medical Center, Jerusalem 91031, Israel.

0002-9297/95/5703-0006$02.00

and 6 females, were available, and the clinical details of some of these patients have been reported elsewhere (Divry et al. 1988; Nigro et al. 1991; Michelakakis et al. 1991; Bartalini et al. 1992; Toft et al. 1993). In brief, 16 patients presented before age 6 mo (mean ± SD = 2.8 ± 1.7 mo) with an infantile, severe form of CD, and 2 patients (D1 and D2) had a mild form of the disease (table 1). Macrocephaly and increased muscle tone were invariably present before age 1 year in all the severe cases. Funduscopy and neuroimaging studies were not always performed at an early stage of the clinical disease. Nonetheless, optic atrophy and white-matter disease were invariably present by age 4 years in all the severe cases. In the two Danish patients with the mild form of CD, head circumference was not increased, and results of neuroimaging studies were normal at ages 6 and 4½ years. In all cases, excessive excretion of N-acetylaspartic acid in urine and aspartoacylase deficiency in fibroblasts were found.

DNA Analysis

Genomic DNA was extracted from blood samples by standard methods. Total RNA was isolated from fibroblasts by Tri reagent (Molecular Research Center), according to the manufacturer's instructions. First-strand cDNA was generated from total RNA by Moloney murine leukemia virus reverse transcriptase, (Promega) with the antisense oligonucleotide primer CDP6 5′-AGAATGTTGAGCTACCCAAG-3′ (nt 1214–1195) (Sambrook et al. 1989).

A 1,350-bp fragment of cDNA was amplified from the reverse-transcribed product by the oligonucleotide primers CDP6 and CDP5 5′-CTCCACTCAAGG-GAATTCTG-3′ (nt −135 to 116). This amplification reaction was repeated for each patient, to prevent PCR-introduced artifacts, and all the consequent steps were performed in duplicate for each patient. From the 1,350-bp fragment a nested 1,109-bp fragment was amplified by CDP5 and CDP2 5′-ACACCGTGTAAGATG-TAAGC-3′ (nt 974–955). For each of the amplification reactions, a total of 36 cycles of amplification were performed, each consisting of a 1-min denaturation step at 94°C, a 1.5-min period at 60°C for annealing, and a 2-min primer extension at 70°C. The final extension step lasted 10 min (Elpeleg et al. 1994*a*). Sequencing of the PCR products was performed by the dideoxy-chain termination reaction (Sanger et al. 1977) using Sequenase version 2.0 (United States Biochemical, Amersham) and 35S α-dATP. Four more primers—CDP7 5′-TTAC-TAACCCCAGAGCAGTG-3′ (nt 155–174), CDP9 5′-CACTCGTTCCATAGCCAAGT-3′ (nt 498–517), CDP8 5′-GTGGAGCCAGAGAAGTCTTA-3′ (nt 448–429), and CDP12 5′-GGTAATCAACTTTCTCTA-TAA-3′ (nt 694–674)–were used for sequencing of the 1,109-bp fragment. Sequencing reactions were electrophoresed through a 6% denaturing polyacrylamide gel, dried under vacuum, and were exposed to x-ray film for 24–36 h. The entire coding region was sequenced in 16 patients; only exons 4–6 were sequenced in 2 patients, and only exon 6 in 1 patient.

Results

Four point mutations were identified in the 38 non-Jewish CD alleles: an Ala-to-Glu substitution at codon 305 (GCA→GAA), designated "A305E" (15 alleles); a Cys to stop at codon 218 (TGC→TGA), designated "C218X" (6 alleles); a Gly-to-Arg substitution at codon 274 (GGA→AGA), designated "G274R" (2 alleles); and a Phe-to-Ser substitution at codon 295 (TTT→TCT), designated "F295S" (1 allele) (fig. 1). In addition, four microdeletions were identified: 566del7 (fig. 2), 527del6 (fig. 3), and 870del4 (fig. 4), each carried by one allele, and 827delGT (two alleles) (fig. 5). A smaller (~1,000 bp) cDNA fragment was the only amplification product in one patient; this was found to be due to deletion of exon 4 and is designated "527del108" (fig. 6). The genotype of each patient is presented in table 2, and the relative frequencies of the mutations are presented in table 3. It is of note that none of the 38 non-Jewish CD alleles harbored the E285A and the 433-2A→G mutations, previously described in Jewish CD patients (Kaul et al. 1994*b*).

The 566del7 mutation was found in two sisters of Spanish origin (patient S3 and her sister) who were heterozygotes for this mutation. This mutation resulted in a loss of an *Eco*RV recognition site and could therefore be detected in genomic DNA. A 123-bp fragment of genomic DNA was amplified by CDP17 5′-TGTACC-TAGGTATAGAAGT-3′ (nt IVS3-9-536) and CDP18 5′-ACTTACCTTCATTGAAATGA-3′ (nt IVS4+6-621) and incubated with *Eco*RV. When the fragments of the patients and their mother were incubated with *Eco*RV, three bands, of 53 bp, 70 bp, and 116 bp, in addition to two bands representing the heteroduplexes of 123-bp and 116-bp fragments, were produced. Only two bands, of 53 bp and 70 bp, were seen in the father (fig. 7).

A method to detect the C218X mutation in genomic DNA was developed. A 123-bp fragment that contained exon 5 was amplified by the primers CDP13 5′-GTCATAGGAAAAGAATTTCC-3′ (nt IVS4-7-647) and CDP16 5′-TGTTACCTGCAGATTAGGA-3′ (nt IVS5+6-732). The reaction conditions were similar to those used in the above-mentioned reaction. The C218X mutation created a new recognition site for the restriction enzyme *Dde*I. Thus, in patients homozygote for the C218X mutation, the digestion with *Dde*I was associated with the cleavage of the 123-bp fragment into 99-bp and 24-bp fragments. The 99-bp fragment

Table 1

Clinical and Neuroradiological Findings in 18 CD Patients

Patient (sex)	Consanguinity	Age at First Symptom	First Symptom(s)
D1 (M)	−	9 mo	Psychomotor delay
D2 (M)	−	9 mo	Convulsions
F1 (M)	−	4 mo	Axial hypotonia
F2 (M)	−	3 mo	Quadriplegia
Gr1 (M)	+	4 mo	Muscle hypotonia, poor head control
Gr2 (M)	−	5 mo	Poor head control, psychomotor delay
Gr3 (M)	+	5 mo	Poor head control, convulsions
Gu1 (F)	−	6 mo	Psychomotor delay
I1 (F)	−	15 d	Tremor, progressive rigidity
I2 (M)	−	2 mo	Developmental delay, myoclonic jerks
I3 (M)	−	Birth	Macrocephaly, no visual or auditory reaction
P1 (F)	−	2 mo	Tremor, weak reaction to noise
S1 (M)	+	15 d	Convulsions
S2 (F)	−	9 d	Macrocephaly
S3 (F)	+	3 mo	Poor head control, irritability
T1 (M)	+	3 mo	Axial hypotonia
T2 (F)	+	3 mo	Muscle hypertonia, no spontaneous movements
T3 (M)	−	3 mo	Convulsions, poor head control

	Optic Atrophy[a]	White-Matter Disease[a]	Development
D1	3 years −	3 years −	6 years—moderate hypotonia and ataxia, functions at the level of 2 ½ years
D2	4 ½ years −	4 ½ years −	4 ½ years—mild psychomotor retardation
F1	No information	<1 year +	<1 year—profound psychomotor retardation
F2	No information	No information	<1 year—profound psychomotor retardation
Gr1	7 mo +	7 mo +	2 years—profound psychomotor retardation
Gr2	5 mo −	5 mo +	5 mo—profound psychomotor retardation
Gr3	No information	9 mo+	9 mo—profound psychomotor retardation
Gu1	<1 year +	<1 year +	<1 year—profound psychomotor retardation
I1	3 years +	6 mo +	21 mo—profound psychomotor retardation
I2	22 mo +	7 mo +	2 years—profound psychomotor retardation
I3	<1 year +	<1 year +	<1 year—profound psychomotor retardation
P1	9 mo +	9 mo +	15 mo—profound psychomotor retardation
S1	3 years +	4 years +	6 years—profound psychomotor retardation
S2	2 mo +	3 mon +	7 years—profound psychomotor retardation
S3	11 mo +	6 mo +	4 years—profound psychomotor retardation
T1	No information	4 mo +	<1 year—ataxia, dysmetria
T2	7 mo +	3 mo +	1 year—profound psychomotor retardation
T3	27 mo +	8 mo +	8 mo—profound psychomotor retardation

[a] + = Found; and − = not found.

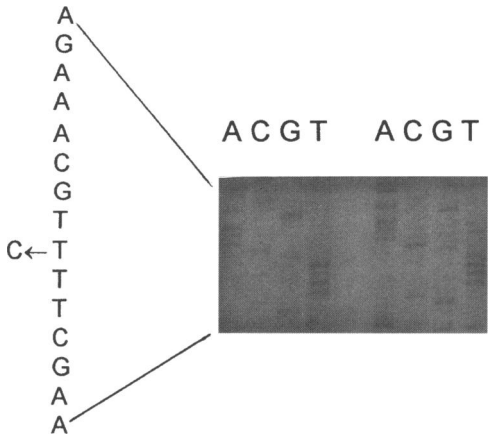

Figure 1 Sequence analysis of part of exon 6 cDNA. A sample from the normal control is shown in the right-hand lanes; and a sample from patient Gr2, who is heterozygous for the F295S mutation, is shown in the left-hand lanes. The T→C mutation at position 884 is shown.

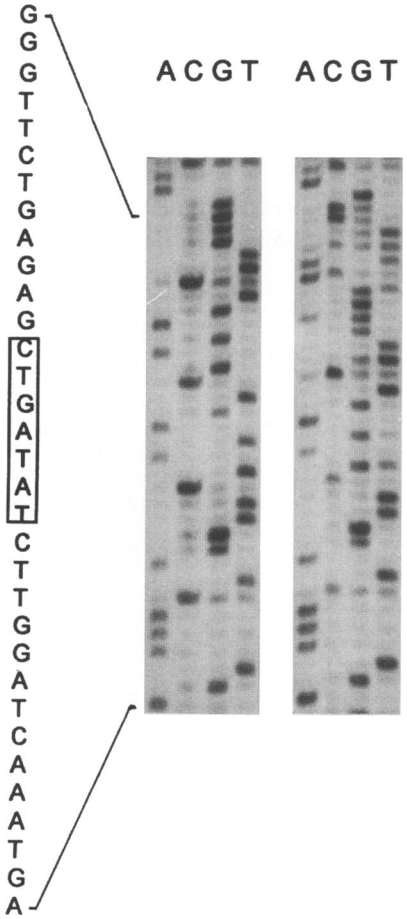

Figure 2 Sequence analysis of part of exon 4 cDNA. A sample from a normal control is shown in the left-hand lanes; and a sample from patient S3, who is heterozygous for the 566del7 mutation, is shown in the right-hand lanes. Deleted nucleotides are inside the box.

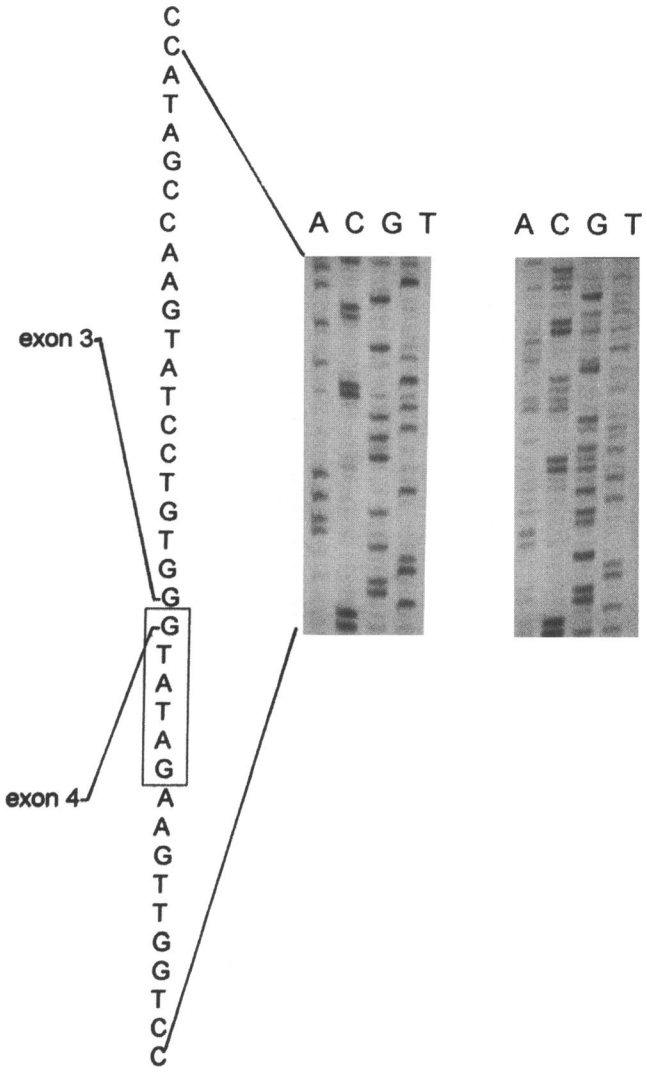

Figure 3 Sequence analysis of a cDNA fragment that includes the exon 3/exon 4 junction. A sample from the normal control is shown in the left-hand lanes; and a sample from patient I2, who is heterozygous for the 527del6 mutation, is shown in the right-hand lanes. Deleted nucleotides are inside the box.

could be distinguished from the 123-bp fragment in a 3% NuSieve agarose gel (fig. 8).

The G274R mutation resulted in the elimination of a recognition site for the restriction enzyme *Bsl*I. The nested 1,109-bp fragment of cDNA, which was amplified by the primers CDP2 and CDP5, was incubated with *Bsl*I. The fragment remained uncleaved in patient T1, who was homozygote for the G274R mutation, and was cleaved into 156-bp and 953-bp fragments in the healthy control (fig. 9). The possibility that patient T1 is a compound heterozygote for the G274R mutation and for a gene that is not expressed—and that all the mRNA analyzed was derived from a single allele—was excluded by repeating the *Bsl*I digestion with a 184-

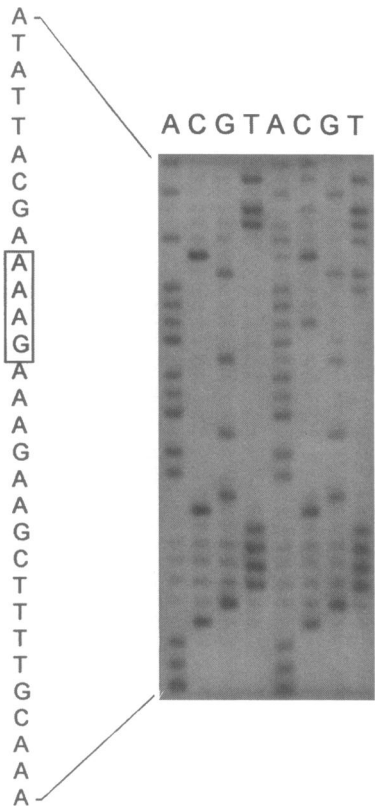

Figure 4 Sequence analysis of part of exon 6 cDNA. A sample from the normal control is shown in the left-hand lanes; and a sample from patient B1, who is heterozygous for the 870del4 mutation, is shown in the right-hand lanes. Deleted nucleotides are inside the box.

bp genomic DNA fragment that was amplified by the primers CDP1 5′-CTCTTGATGGGAAGACGATC-3′ (nt 791–810) and CDP2. The fragment remained uncleaved in patient T1 and was entirely digested, into bands of 157 bp and 27 bp, in healthy controls (data not shown).

Exclusion of a "nonexpressed gene" was also performed for patients D1, D2, Gr1, I1, P1, and S2, who were found by cDNA sequencing to be homozygotes for the A305E mutation. A 145-bp genomic DNA fragment was amplified by primers CDP1 and CDP24 5′-TAAACAGCAGCGAATACTTTaT-3′ (nt 936–915, with a mismatch at nt 916), as recently described elsewhere (Kaul et al. 1994*b*). Incubation with the restriction enzyme *Nsi*I resulted in the cleavage of the genomic DNA fragment of the healthy control into bands of 21 bp and 124 bp, while the fragments of the six patients remained uncleaved (data not shown).

Exclusion of a nonexpressed gene was also performed for patient F1, who was found by cDNA sequencing to be homozygote for the 827delGT mutation. The 184-bp genomic DNA fragment that was amplified by the primers CDP1 and CDP2 was incubated with the restriction enzyme *Rsa*I. Because the mutation eliminated an *Rsa*I recognition sequence, three bands, 139 bp, 38 bp, and 7bp, were found in the healthy control, and only two bands, 139 bp and 43 bp, were found in patient F1, indicating homozygosity for the mutation. The 38-bp and 43-bp bands could be distinguished on 10% acrylamide gel (data not shown).

Discussion

Four point mutations, four microdeletions, and a deletion of exon 4 were identified in 31 of the 38 alleles of non-Jewish CD patients who were available for this study, resulting in an overall detection rate of 81.6%. The structural conformation and functional domains of the aspartoacylase gene are not yet defined. The catalytic region has been tentatively assigned to amino acids 283–289 (Kaul et al. 1993). It is therefore expected that the C218X mutation, resulting in a prematurely truncated protein of 217 amino acids, would manifest by lack of catalytic activity. Mutations 566del7, 827delGT, and 870del4, which change the correct reading frame, starting from residues 187, 276, and 290, respectively, are also expected to manifest by lack of catalytic activity. The A305E mutation has previously been shown to result in the loss of catalytic activity, but the mechanism underlying this phenomenon is unclear (Kaul et al. 1994*b*).

We have not expressed the enzymatic activity of the G274R, F295S, and 527del6 mutations and cannot con-

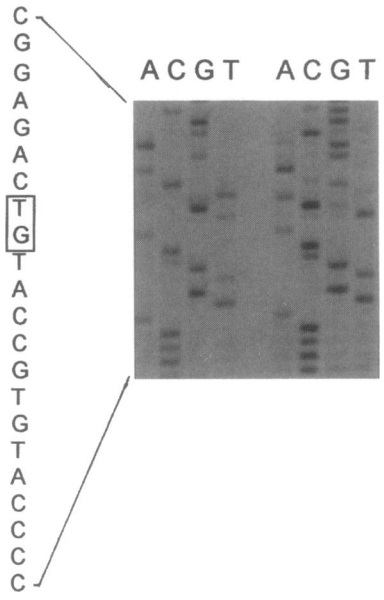

Figure 5 Sequence analysis of part of exon 6 cDNA. A sample from the normal control is shown in the left-hand lanes; and a sample from patient F1, who is homozygous for the 827delGT mutation, is shown in the right-hand lanes. Deleted nucleotides are inside the box.

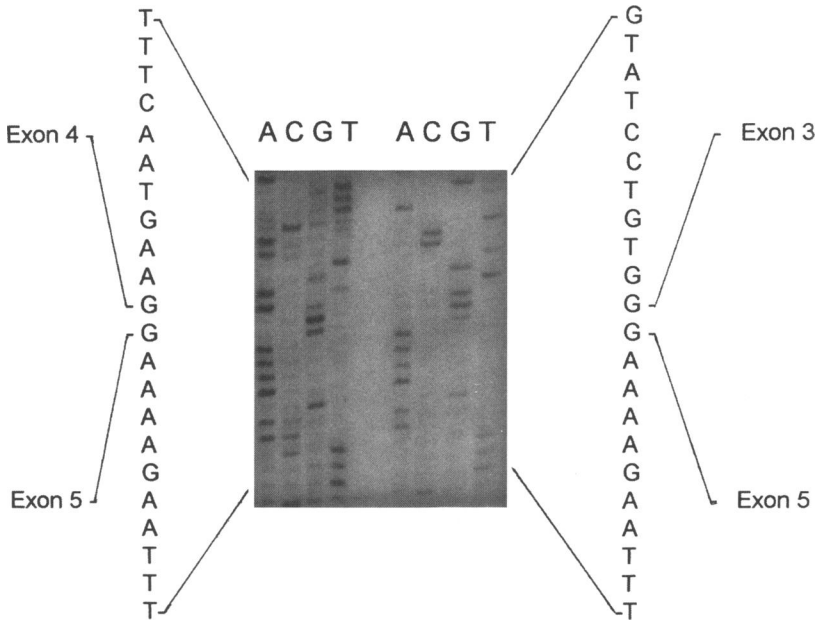

Figure 6 Sequence analysis of cDNA of the 5′ region of exon 5. A normal exon 4/exon 5 junction is shown in the control sample, in the left-hand lanes; and a homozygous state for the 527del108 mutation (exon 4 deletion) is shown in the cDNA of patient T2, in the right-hand lanes.

firm their disease-causing nature. Still, in the case of the G274R mutation, a drastic conformational change is expected because of the substitution of arginine, a positively charged residue with a bulky side chain, for the more flexible, compact, and neutrally charged glycine. Similarly, the F295S mutation substitutes the hydrophobic phenylalanine at codon 295 for serine, which is hydrophilic.

The 527del6 mutation results in the loss of amino acids G176 and I177. Patient I2 was heterozygous for this mutation, which was the only mutation identified in the coding region of his cDNA. The fact that exon 4 splicing remains intact, despite the change of 4 of its first 6 nt, is rather expected. For most exon/intron junctions, the 3′ splice-site consensus sequence is highly redundant. In a comprehensive review of point mutations that lead to missplicing, only 26/101 mutations were located in the 3′ splice site, and all of them reside in the intronic part (Krawczak et al. 1992).

The 527del108 mutation is likely due to a point mutation in the 3′ end of IVS3, leading to missplicing of exon 4. We could not identify this underlying mutation in patient T2, because no data were available for IVS3 sequence. Deletion of exon 4 is likely to result in serious alteration of conformation of the aspartoacylase protein.

A total of 12 mutations are now identified in the cDNA of the aspartoacylase gene; 6 of them reside in exon 6, and the other 6 are located in exons 4 and 5. Sequencing of the entire coding region of the cDNA has not disclosed mutations in the first three exons. The only exception has been an intronic mutation (433-2A→G) with the resultant skipping of exon 3, which has recently been described in a small number of alleles of American

Table 2

Mutations in 19 Non-Jewish CD Patients

Patient	Genotype
B1	A305E/870del4
D1	A305E/A305E
D2	A305E/A305E
F1	827delGT/827delGT
F2[a]	A305E/
Gr1	A305E/A305E
Gr2	F295S/
Gr3	C218X/C218X
Gu1[b]	
I1	A305E/A305E
I2	527del6/
I3[a]	A305E/
P1	A305E/A305E
S1	C218X/C218X
S2	A305E/A305E
S3	566del7/
T1	G274R/G274R
T2	527del108/527del108
T3	C218X/C218X

[a] Only exons 4–6 were sequenced, cDNA is of normal size.
[b] Only exon 6 was sequenced. cDNA is of normal size.

Table 3

Relative Frequency of Mutations among Non-Jewish CD Patients

Position	Mutation	Type	No. (%)	Ethnic Origin
914C→A	A305E	Missense	15 (39.5)	pan-European
654C→A	C218X	Nonsense	6 (15.8)	Gypsy
820G→A	G274R	Missense	2 (5.3)	Turkish
884T→C	F295S	Missense	1 (2.6)	Greek
827delGT		Deletion	2 (5.3)	French
870del4		Deletion	1 (2.6)	British
527del6		Deletion	1 (2.6)	Italian
566del7		Deletion	1 (2.6)	Spanish
527del108		Exon skip	2 (5.3)	Turkish
Total			31 (81.6)	

Jewish CD patients (Kaul et al. 1994*b*). It is possible that mutations in the first three exons of the aspartoacylase gene result in an unstable mRNA and therefore would not be detected by cDNA sequencing.

Our findings indicate that the A305E mutation is pan-European. It is the most prevalent among non-Jewish CD patients and probably the most ancient mutation. While our work was still in progress, this mutation was reported in 24/40 non-Jewish CD alleles, by another group (Kaul et al. 1994*b*). When these studies are taken together, the A305E mutation is found in 39 (50%) of 78 alleles of non-Jewish CD patients.

Patients with a juvenile (childhood or late infantile) form of CD have been described by several investigators (Jellinger and Seitelberger 1969; Goodhue et al. 1979). Most patients studied by us presented in early infancy and were profoundly retarded by age 2 years. The only exceptions were the two Danish patients (D1 and D2), who presented toward the end of the first year of life and had a milder clinical course. Nonetheless, the genotype of these patients (A305E/A305E) was identical to the genotype of patients with the severe form of the disease. Similarly, among Ashkenazi Jewish patients in Israel, the genotype of patients who had the protracted course was identical to the genotype of patients who had the typical course (E285A/E285A) (Elpeleg et al. 1994*a*). We conclude that the clinical severity of CD in patients homozygote for either the A305E mutation or the E285A mutation is not the result of molecular heterogeneity in the aspartoacylase gene.

The discovery of aspartoacylase deficiency in CD in 1988 raised hopes for carrier detection and prenatal diagnosis in this disease. It soon became evident that these goals would not be achieved by determination of aspartoacylase activity. Carrier detection was hampered

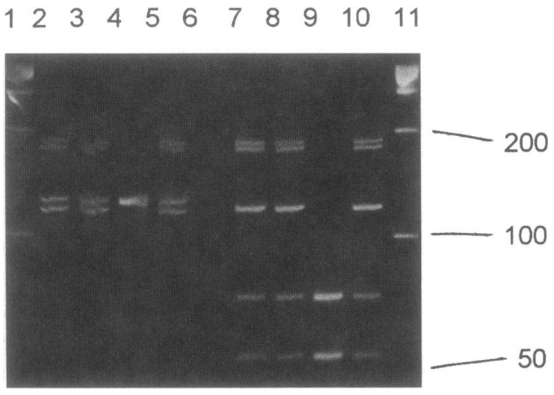

Figure 7 Detection of 566del7 in genomic DNA by digestion with *Eco*RV. Lanes 1 and 11, DNA markers (AmpliSize standard; Bio-Rad Laboratories). Lanes 2–6, PCR products with the oligonucleotide primers CDP17 and CDP18. Lane 2, Patient S3. Lane 3, Affected sister of patient S3. Lane 4, Father of S3. Lane 5, Mother of S3. Lane 6, No DNA. Note both the single band of 123 bp in lane 4 and the heteroduplexes (116 bp and 123 bp) above the deleted (116-bp) band and the normal (123-bp) band, in lanes 2, 3, and 5, indicating a heterozygous state for the deletion. Lanes 7–10, Same fragments after incubation with *Eco*RV. Lane 7, Patient S3. Lane 8, Affected sister. Lane 9, Normal homozygous father of S3. Lane 10, Heterozygous mother of S3. Only the nondeleted (123-bp) fragment is cleaved, into 70-bp and 53-bp fragments.

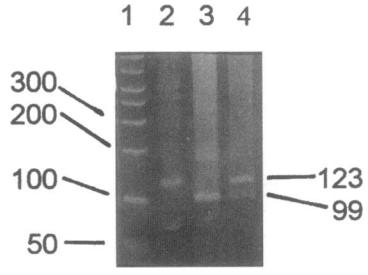

Figure 8 Detection of the C218X mutation by *Dde*I digestion in genomic DNA fragments. The 123-bp fragment, which was the PCR product of the oligonucleotide primers CDP13 and CDP16, was cleaved into 99-bp and 24-bp fragments, in the presence of the mutation. Lane 1, DNA marker (AmpliSize standard; Bio-Rad Laboratories). Lane 2, Normal control. Lane 3, Patient S1. Lane 4, Mother of S1.

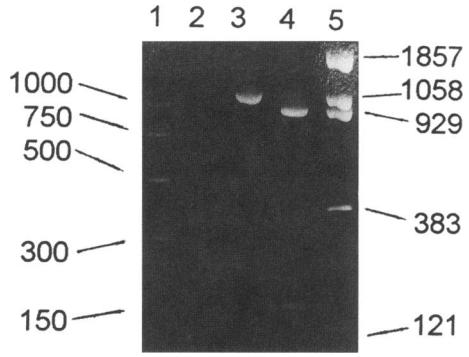

Figure 9 Detection of the G274R mutation by *Bsl*I digestion. A 1,109-bp fragment of cDNA was cleaved into 953-bp and 156-bp fragments in normal controls and remained uncleaved in homozygotes for the mutation. Lane 1, DNA marker (PCR markers; Promega). Lane 2, No DNA. Lane 3, Patient T1. Lane 4, Normal control. Lane 5, DNA marker (pBR322DNA; *Bst*NI digest).

by the overlapping values of enzymatic activity observed in fibroblasts of obligate heterozygotes and normal controls. Prenatal diagnosis was even more unreliable, because of the very low enzymatic activity in skin fibroblasts of normal fetuses obtained before 20 gestational weeks (Rolland et al. 1994). Until recently, accurate prenatal diagnosis could only be established by determination of N-acetylaspartic acid concentration in amniotic fluid by a stable isotope-dilution technique (Bennet et al. 1993). The procedure requires shipment of amniotic fluid on dry ice to the few centers that specialize in this method.

Molecular investigations in CD patients are therefore of major practical importance. The identification of the E285A mutation (Kaul et al. 1993) has enabled us to estimate the carrier frequency among Ashkenazi Jews and to perform prenatal diagnosis in affected families (Elpeleg et al. 1994*a*, 1994*b*). The results of the present study would enable carrier detection and prenatal diagnosis in the families of 13 patients (68.4%) in whom we could identify two mutations in the aspartoacylase cDNA; in an additional 5 patients (26.3%), only one mutation was identified, and no mutations were identified in 1 patient. Six of the 13 patients whose two mutations were found carried the A305E on both alleles. Hence we recommend that new CD patients of European (and, possibly, American) non-Jewish origin be screened for the A305E mutation and that CD patients of Gypsy origin be screened for the C218X mutation. For those whose genotype cannot be fully elucidated by these methods, prenatal diagnosis can still be performed by determination of N-acetylaspartic acid in amniotic fluid.

Acknowledgments

This work was supported in part by Mirski Foundation grant 18002778 and by a grant from the Joint Research Fund of the Hebrew University and Shaare Zedek Medical Center. We are grateful to the following physicians for their helpful cooperation: Dr. Gray (England); Drs. Claverie, Guibaud, M. N. Loiseau-Corvez, Macabeo, Meyer, and Ponsot (France); Drs. C. Dionisi-Vici and M. Dirocco (Italy); and Drs. A. Chabas, J. Campistol, A. Vernet, D. M. Martinez Pardo, and J. M. Garcia Penas (Spain).

References

Bartalini G, Margollicci M, Balestri P, Farnetani MA, Cioni M, Fois A (1992) Biochemical diagnosis of Canavan disease. Child Nerv Syst 8:468–470

Bennet MJ, Gibson KM, Sherwood WG, Divry P, Rolland MO, Elpeleg ON, Rinaldo P, et al (1993) Reliable prenatal diagnosis of Canavan disease (aspartoacylase deficiency): comparison of enzymatic and metabolite analysis. J Inherit Metab Dis 16:831–836

Divry P, Vianey-Liaud C, Gay C, Macabeo V, Rapin F, Echenne B (1988) N-acetylaspartic aciduria: report of three new cases in children with a neurological syndrome associated macrocephaly and leukodystrophy. J Inherit Metab Dis 11:307–308

Elpeleg ON, Anikster Y, Barash V, Branski D, Shaag A (1994*a*) The frequency of the C854 mutation in the aspartoacylase gene in Ashkenazi Jews in Israel. Am J Hum Genet 55:287–288

Elpeleg ON, Shaag A, Anikster Y, Jakobs C (1994*b*) Prenatal detection of Canavan disease by DNA analysis. J Inherit Metab Dis 17:664–666

Goodhue WW, Couch RD, Namiki H (1979) Spongy degeneration of the CNS: an instance of the rare juvenile form. Arch Neurol 36:481–484

Jellinger K, Seitelberger F (1969) Juvenile form of spongy degeneration of the CNS. Acta Neuropathol 13:276–281

Kaul R, Balamurugan K, Gao GP, Matalon R (1994*a*) Canavan disease: genomic organization and localization of human ASPA to 17p13-ter: conservation of the ASPA gene during evolution. Genomics 21:364–370

Kaul R, Gao GP, Aloya M, Balamurugan K, Petrosky A, Michals K, Matalon R (1994*b*) Canavan disease: mutations among Jewish and non-Jewish patients. Am J Hum Genet 55:34–41

Kaul R, Gao GP, Balamurugan K, Matalon R (1993) Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease. Nat Genet 5:118–123

Krawczak M, Reiss J, Cooper DN (1992) The mutational spectrum of single-base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. Hum Genet 90:41–54

Matalon R, Michals K, Sebesta D, Deaching M, Gashkoff P, Casanova J (1988) Aspartoacylase deficiency and N-acetylaspartic aciduria in patients with Canavan disease. Am J Med Genet 29:463–471

Michelakakis H, Giouroukos S, Divry P, Katsarou E, Rolland MO, Skardoutsou A (1991) Canavan disease: finding in four new cases. J Inherit Metab Dis 14:267–268

Nigro F, Ribes A, Fiumara A, Siciliano L, Pavone L (1991) N-acetylaspartic aciduria: report of two new cases with

aspartoacylase assay. The 29th meeting of the Society for the Study of Inborn Errors of Metabolism. Society for the Study of Inborn Errors of Metabolism, Middlesex, England, p P-148

Rolland MO, Mandon G, Bernard A, Zabot MT, Mathieu M (1994) Unreliable verification of prenatal diagnosis of Canavan disease: aspartoacylase activity in deficient and normal fetal skin fibroblasts. J Inherit Metab Dis 17:748

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, 2d ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Toft PB, Geib-Holtorff R, Rolland MO, Pryds O, Muller-Forell W, Christensen E, Lehnert W, et al (1993) Magnetic resonance imaging in juvenile Canavan disease. Eur J Pediatr 152:750–753

Van Bogaert L (1970) Spongy degeneration of the brain. In: Vinken RJ, Bruyn GW (eds) Handbook of clinical neurology. Vol 10: Leucodystrophies and poliodystrophies. North Holland, Amsterdam, pp 203–211