Am. J. Hum. Genet. 55:34–41, 1994

Canavan Disease: Mutations among Jewish and Non-Jewish Patients

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Summary

Canavan disease is an autosomal recessive leukodystrophy caused by the deficiency of aspartoacylase (ASPA). Sixty-four probands were analyzed for mutations in the ASPA gene. Three point mutations—693C→A, 854A→C, and 914C→A—were identified in the coding sequence. The 693C→A and 914C→A base changes, resulting in nonsense tyr231→ter and missense ala305→glu mutations, respectively, lead to complete loss of ASPA activity in in vitro expression studies. The 854A→C transversion converted glu to ala in codon 285. The glu285→ala mutant ASPA has 2.5% of the activity expressed by the wild-type enzyme. A fourth mutation, 433 −2(A→G) transition, was identified at the splice-acceptor site in intron 2. The splice-site mutation would lead to skipping of exon 3, accompanied by a frameshift, and thus would produce aberrant ASPA. Of the 128 unrelated Canavan chromosomes analyzed, 88 were from probands of Ashkenazi Jewish descent. The glu285→ala mutation was predominant (82.9%) in this population, followed by the tyr231→ter (14.8%) and 433 −2(A→G) (1.1%) mutations. The three mutations account for 98.8% of the Canavan chromosomes of Ashkenazi Jewish origin. The ala305→glu mutation was found exclusively in non-Jewish probands of European descent and constituted 60% of the 40 mutant chromosomes. Predominant occurrence of certain mutations among Ashkenazi Jewish and non-Jewish patients with Canavan disease would suggest a founding-father effect in propagation of these mutant chromosomes.

Introduction

The deficiency of aspartoacylase (*ASPA*) leads to spongy degeneration of the brain, Canavan disease (CD) (Matalon et al. 1988). The clinical features of this progressive, autosomal recessive leukodystrophy include mental retardation, megalencephaly, and hypotonia. Morphological changes in brain are marked by spongy appearance of the white matter, with swollen astrocytes and elongated mitochondria (Globus and Strauss 1928; Canavan 1931; van Bogaert and Bertrand 1949, 1967; Adachi et al. 1972, 1973; Adornato et al. 1972). CD is prevalent among people of Ashkenazi Jewish extraction, although patients from other diverse ethnic backgrounds have also been diagnosed (Banker and Victor 1979; Matalon et al. 1988, 1989, 1993; Ozand et al. 1990). The incidence of CD may have been underestimated because of the lack, until recently, of a biochemical marker for diagnosis of this disorder (Matalon et al. 1988, 1989, 1993).

Aspartoacylase specifically hydrolyzes its substrate N-acetyl aspartic acid (NAA) to aspartate and acetate (Birnbaum et al. 1952; Birnbaum 1955). Aspartoacylase has been purified and characterized from mammalian sources (Kaul et al. 1991). Human *ASPA* cDNA has recently been cloned and expressed in *Escherichia coli* (Kaul et al. 1993). The coding sequence of human *ASPA* is split into six exons separated by five introns in the genome, and the gene spans 30 kb of DNA. Human *ASPA* has been mapped to the 17p13-ter region (Kaul et al. 1994). Elsewhere we have reported a 854A→C base change in the *ASPA* gene (Kaul et al. 1993). The base change was present in 85% of the 17 probands of Ashkenazi Jewish ancestry (Kaul et al. 1993). We now report identification and characterization of four point mutations in *ASPA* gene from 128 CD chromosomes. Three of the mutations account for 98.8% of the 88 CD chromosomes of Ashkenazi Jewish origin. A fourth mutation, identified exclusively in non-Jewish patients with CD, accounted for 60% of the 40 independent chromosomes.

Patients and Methods

Patients with CD

Sixty-four independent probands with CD were analyzed for mutations in the *ASPA* gene. Forty-four of these probands were of Ashkenazi Jewish extraction, while the other 20 were non-Jewish and of European ancestry. Whenever possible, the mutations observed in probands were confirmed in their parents. The frequencies of mutations among Ashkenazi Jewish and non-Jewish CD chro-

Received January 28, 1994; accepted for publication March 11, 1994.

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0002-9297/94/5501-0006$02.00

mosomes were determined. However, in families with more than one affected child, only one proband was included for such analysis.

Mutation Analysis

Genomic DNA was prepared from cultured skin fibroblast cell lines or from lymphocytes, according to methods described elsewhere (Kaul et al. 1994). In certain instances, "Guthrie" blood spots were used for mutation analysis. Genomic DNA (500 ng) or "Guthrie" blood spots were used for PCR amplification of *ASPA*-specific coding and exon/intron boundary sequences, as described elsewhere (Kaul et al. 1993). The mutations were characterized by (*a*) determination of the nucleotide sequence by using dideoxy chain termination chemistry (Sanger et al. 1977), (*b*) analyses of SSCP (Orita et al. 1989), and (*c*) restriction-endonuclease digestion, as described elsewhere (Kaul et al. 1993). Mutations that did not result in gain or loss of a restriction-endonuclease site were analyzed by PCR-directed site-specific mutagenesis (PDSM). A primer with mismatch at a unique position in its sequence was synthesized. After PCR amplification, the mismatch in the PCR primer created a unique recognition sequence for a restriction endonuclease, either in mutant or wild-type (WT) alleles. The mutant and WT alleles could thus be differentiated by restriction digestion of the PCR-amplified products.

DNA Constructs

The transient expression vector pEUK-C1 (Clontech) was modified to introduce *Sac*I, *Bcl*I, and *Not*I restriction sites and to destroy the *Bam*HI site at the multiple cloning site. The modified vector was called "pGEUK-C1." The WT *ASPA* cDNA was amplified between −108 and +1242 by PCR, using the sense XHASP18 and antisense NHASPC14 strand primers (Kaul et al. 1993). The primers XHASP18 and NHASPC14 had, respectively, *Xba*I and *Not*I linkers at their 5′ termini. The amplified cDNA fragment was digested with *Xba*I and *Not*I restriction endonuclease and was cloned in pGEUK-C1 vector downstream of the SV40 late promoter and VP1 intron. The mutant *ASPA* cDNAs with a 693C→A or 914C→A base change were amplified by reverse transcriptase–PCR of cytoplasmic RNA isolated from patient fibroblast cell lines homozygous for either of these two mutations (Kaul et al. 1993). The amplified mutant cDNAs were cloned as described for WT cDNA. Genomic DNA from a patient homozygous for 854A→C mutation was amplified with G5 and C7 primers as described elsewhere (Kaul et al. 1993). The amplified fragment with the 854A→C mutation was cleaved at unique *Bam*HI and *Hin*dIII restriction sites and was cloned in $pBS^{(+)}$ vector. The *Bam*HI/*Hin*dIII fragment in the WT cDNA clone $pBSHLASP^{(+)}$ (Kaul et al. 1993) was replaced with the corresponding 854A→C mutant cDNA fragment. The 854A→C mutant cDNA insert was amplified using XHASP18 and NHASPC14 primers, was restricted with *Xba*I, and was cloned in pGEUK-C1 vector at *Xba*I/*Sma*I restriction sites.

The WT and mutant *ASPA* expression constructs—$pSV\text{-}ASPA^{(WT)}$, $pSV\text{-}ASPA^{(693A\rightarrow C)}$, $pSV\text{-}ASPA^{(854A\rightarrow C)}$, and $pSV\text{-}ASPA^{(914C\rightarrow A)}$—were isolated and characterized by restriction-endonuclease digestion and by determination of their nucleotide sequence. The plasmid DNAs were isolated and purified on two successive $CSCl_2$ gradients for transfection studies (Sambrook et al. 1989).

Cell Culture, DNA Transfection, and Expression of ASPA

Human *ASPA* cDNA expression constructs were expressed in COS1 cells (a gift from Dr. Jacob Grimberg, ImClone Systems) grown in RPMI-1640 media supplemented with 20% fetal bovine serum and 4% CO_2. Lipofectin-mediated DNA transfections (Loeffler and Behr 1993) were carried out according to the conditions recommended by the manufacturer (Gibco-BRL). After transfection with expression constructs, the COS-cells sonicate were analyzed for aspartoacylase activity, according to a method described elsewhere (Kaul et al. 1991). Either control transfections with pGEUK-C1 vector DNA or mock transfections without any DNA were carried out and analyzed simultaneously. The expression data presented are a mean of at least four independent experiments.

Results

Splice-Acceptor Site 433 −2(A→G) Transition in Intron 2

The exon 3 (Kaul et al. 1994), coding, and splice-junction sequences were amplified using 3A/3B primers. The nucleotide sequence of the 201-bp PCR-amplified product was determined in both strands. The nucleotide sequence data of the sense strand are shown in figure 1. An A→G transition was observed at the splice-acceptor site (433 −2 nucleotide position) in intron 2 of one chromosome. Both A base– and G base–specific signals were observed at this position. The patient is therefore heterozygote for a base change at this position.

The 433 −2(A→G) splice-acceptor-site mutation was also demonstrated by restriction-endonuclease analysis. A sense primer, IVS2SA (5′ GAAAGACGTTTTTGATTT-TTTC*C 3′, with a T→C mismatch marked by the asterisk [*]), was synthesized. The IVS2SA and 3B primers were used for PCR amplification of a 179-bp fragment from the mutant and WT alleles. The results are shown in figure 2. The A→G transition in the mutant allele created a *Hpa*II restriction site by PDSM and is thus cleaved by the *Hpa*II enzyme, into 156- and 23-bp fragments. The amplified product from WT allele is not cleaved by the *Hpa*II enzyme. Restriction analysis confirmed that the patient is heterozygous for the 433 −2(A→G) splice-site mutation.

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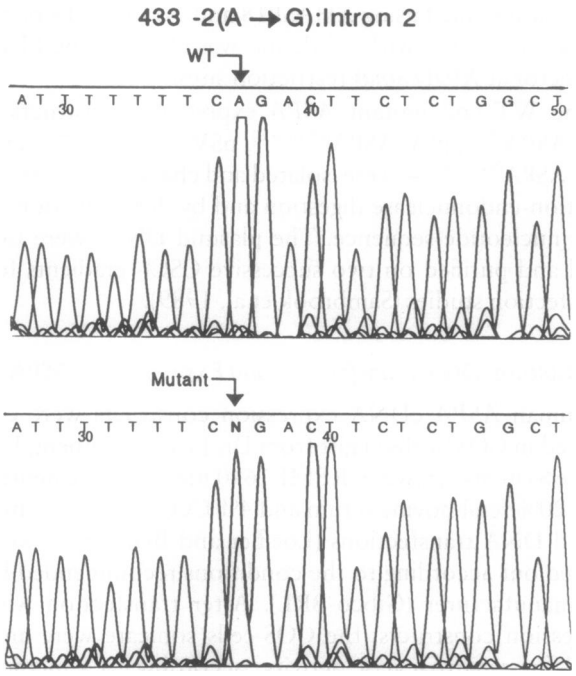

Figure 1 Nucleotide sequence of an exon 3–specific amplified fragment from a control (WT; *upper panel*) and a patient (Mutant; *lower panel*) *ASPA* gene. Exon 3–specific sequences were amplified by PCR with primers 3A and 3B. The nucleotide sequence is shown around the 433 −2(A→G) mutation. The proband was a heterozygote for the A→G transition, and the base is therefore identified as N at this position (*lower panel*).

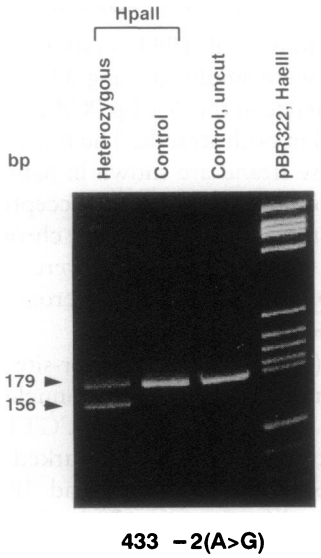

Figure 2 Restriction-endonuclease digestion of PCR-amplified fragments for identification of 433 −2(A→g) mutations. The PCR amplification was carried out with IVS2SA and 3B primers, and a 179-bp amplified fragment was digested with *Hpa*II. The digest was electrophoresed on 6% native polyacrylamide gels and stained with ethidium bromide. The mutant allele results in 156- and 29-bp (not seen in the gel) fragments. The WT allele is not cleaved by *Hpa*II enzyme.

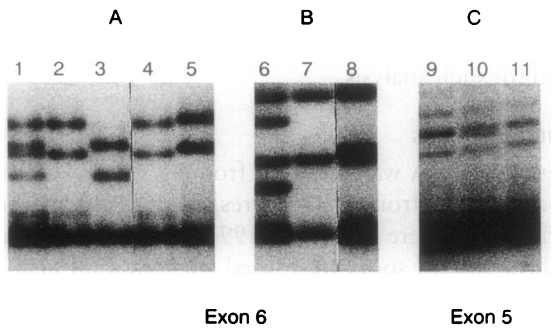

Figure 3 Autoradiography showing SSCP in exon-specific PCR-amplified fragments. Exon 6 sequences (*A* and *B*) were amplified with 6A and RT1 primers as 347-bp fragments and electrophoresed on SSCP gel. Lanes 1 and 6, 854A→C heterozygote. Lanes 2, 4, and 7, Controls. Lane 3, 854A→C homozygote. Lane 5, 914C→A homozygote. Lane 8, 914C→A heterozygote. Exon 5 (*C*) sequences were amplified with 5A/5B primers, and a 235-bp amplified fragment was electrophoresed on SSCP gel. Lane 9, Control. Lane 10, 693C→A heterozygote. Lane 11, 693C→A homozygote.

Identification of a 693C→A Base Change in Exon 5 of the ASPA Gene

Exon 5–specific (Kaul et al. 1994), coding, and boundary sequences were amplified with 5A/5B primers. The 235-bp product was analyzed for SSCP, to indicate base change in the CD alleles. A representative SSCP pattern in exon 5 of *ASPA* gene alleles is shown in figure 3. The PCR-amplified products from mutant and WT alleles had distinct SSCP profiles. Probands homozygous or heterozygous for this base change could be identified by SSCP banding pattern. The nucleotide sequence of the exon 5–specific amplified fragment from such patients was determined. Representative nucleotide sequences of the sense strand are shown in figure 4. A single base change of C→A was observed at position 693 in a patient homozygous for this mutation. The 693C→A base change should result in a nonsense tyr231→ter mutation in the *ASPA* transcript–encoded protein.

The 693C→A base change creates *Mse*I restriction-endonuclease recognition sequence in the mutant alleles. The 693C→A mutation was therefore characterized by digestion of exon 5–specific amplified fragments, with the *Mse*I enzyme. The results of *Mse*I restriction digestion are shown in figure 5. *Mse*I restriction digestion of the mutant alleles resulted in 104-, 73-, and 58-bp bands. The WT alleles yielded 177- and 58-bp fragments. Patients homozygous or heterozygous for the 693C→A mutation could be identified by restriction analysis.

The 693C base position exhibits C→T polymorphism in the general population. This polymorphism is silent in nature, and both the TAT and TAC codons code for tyr231. The 693C→T polymorphism is observed in SSCP gels and is distinct from the 693C→A mutation seen in CD chromosomes.

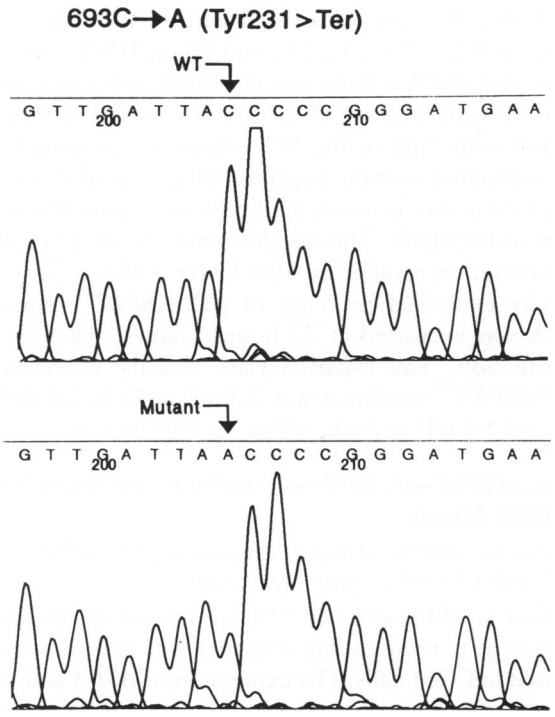

Figure 4 Nucleotide sequence of an exon 5–specific amplified fragment from a control (WT; *upper panel*) and a patient (Mutant; *lower panel*) *ASPA* gene. Exon 5–specific sequences were amplified by PCR with primers 5A and 5B. The nucleotide sequence of the sense strand is shown around the 693C→A mutation. The proband (*lower panel*) was homozygous for the 693C→A base change.

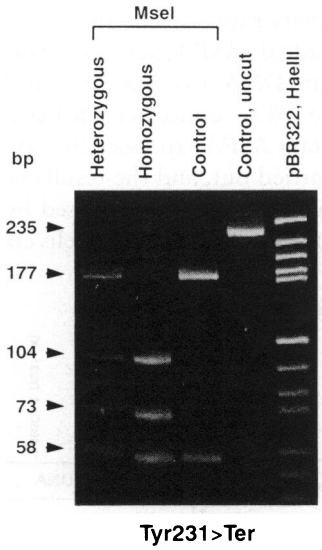

Figure 5 Restriction-endonuclease digestion of PCR-amplified fragments for identification of the 693C→A mutation. Exon 5–specific sequences were amplified using 5A/5B primers. The 235-bp amplified fragment was digested with *Mse*I restriction enzyme and electrophoresed on 6% native polyacrylamide gels and stained with ethidium bromide. The mutant alleles yield 104-, 73-, and 58-bp fragments, and the WT alleles produce 177- and 58-bp fragments.

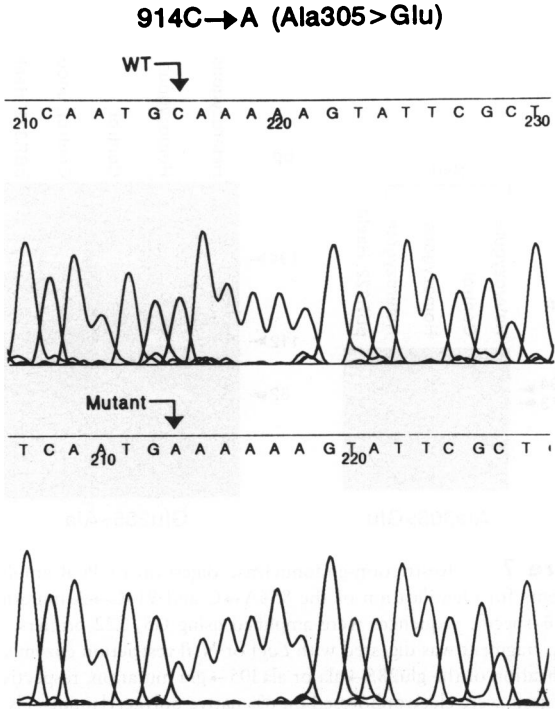

Figure 6 Nucleotide sequence of the sense strand from an exon 6–specific amplified fragment from a control (WT; *upper panel*) and a patient (Mutant; *lower panel*) *ASPA* gene. Exon 6–specific sequences were amplified by PCR with primers 6A and RT1. The nucleotide sequence shown is around the 914C→A base change. The proband (*lower panel*) was homozygous for the 914C→A mutation.

Characterization of the 914C→A Point Mutation in Exon 6 of the ASPA *Gene*

ASPA gene–specific exon 6 sequences and their exon/intron boundaries were amplified using 6A/RT1 primers (Kaul et al. 1993, 1994). The 347-bp amplified products were analyzed for SSCP in CD alleles. A representative SSCP pattern in exon 6 is shown in figure 3. Two distinct SSCP profiles were observed, and probands homozygous or heterozygous for either of the two profiles were identified. One of the SSCP patterns was due to the 854A→C base change described elsewhere (Kaul et al. 1993). The base change responsible for the second SSCP pattern (distinct from that due to 854A→C mutation) was characterized by determination of nucleotide sequence. The nucleotide sequence data for the sense strand are shown in figure 6. A C→A base change at position 914 was observed in a proband homozygous for this mutation. The 914C→A base change would result in a missense ala305→glu mutation.

The 914C→A base change did not result in a gain or loss of restriction site. A primer, C22 (5′ TAAACAGCAGCGAATACTTTA*T 3′, with a T→A mismatch marked by the asterisk [*]), was synthesized and used with the G5 primer (Kaul et al. 1993), for PCR amplification of 194-bp exon 6–specific genomic sequences. The results of such

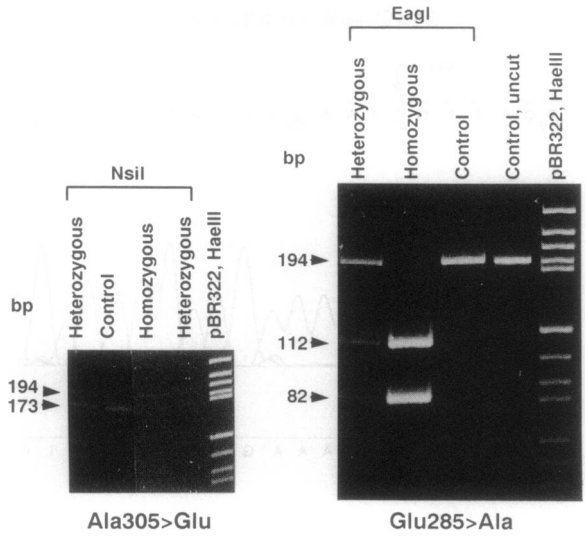

Figure 7 Restriction-endonuclease digestion of PCR-amplified fragments for identification of the 854A→C and 914C→A mutations. Exon 6–specific sequences were amplified using G5/C22 primers. The 194-bp fragment was digested with *Eag*I or *Nsi*I restriction enzyme, for identification of the glu285→ala or ala305→glu mutation, respectively. The digests were electrophoresed on 6% native polyacrylamide gels and stained with ethidium bromide. The mutant allele with the glu285→ala mutation yields 112- and 82-bp fragments on digestion with *Eag*I restriction endonuclease, while the glu285 WT allele is not cleaved. After PCR amplification, the T→A mismatch in the C22 primer creates a *Nsi*I recognition sequence in ala305 WT alleles. Restriction digestion of the amplified WT allele results in 173- and 21-bp (not seen in the gel) bands. The Ala305→glu mutant alleles do not create *Nsi*I recognition sequence after PCR amplification.

analyses are shown in figure 7. The PCR amplification of WT allele created *Nsi*I-specific recognition sequence, so that restriction digestion yielded 173- and 21-bp fragments. The amplified product from 914C→A mutant alleles does not create a *Nsi*I restriction site. Heterozygotes for the 914C→A base change could be differentiated both from homozygous patients and from the control individuals.

The C22/G5 amplification product can also be used for detection of 854A→C mutations. The 194-bp amplified fragment from mutant alleles yields 112- and 82-bp bands, whereas the WT allele is not cleaved by the *Eag*I restriction endonuclease (see fig. 7).

Expression of ASPA *cDNA in COS1 Cells*

The schematics of the human *ASPA* cDNA expression construct pSV-ASPA$^{(WT)}$ are shown in figure 8. The cDNA was cloned at *Xba*I/*Not*I restriction sites in pGEUK-C1 vector downstream of the SV40 late promoter and VP1 intron. The SV40 polyadenylation signal was present downstream of the *ASPA* cDNA insert. The relative locations of three point mutations in the coding sequence are also shown in figure 8.

COS1 cells were transfected with pSV-ASPA$^{(WT)}$ construct at 1.25-, 2.5-, 5-, 10-, and 20-μg DNA concentrations, and *ASPA* activity was measured 36 h posttransfection. Maximal activity of 0.7 mU was observed on transfection with 5 μg of the WT expression construct. This was compared with the background activity of 0–0.02 mU observed in mock-transfected cells or in cells transfected with vector DNA. Transfection with 10- or 20-μg DNA concentration resulted in 40% lower *ASPA* activity. The activity expressed by 5 μg of pSV-ASPA$^{(WT)}$ construct DNA was measured at 72 h and 1 and 2 wk following transfection. The aspartoacylase activity expressed by pSV-ASPA$^{(WT)}$ construct was 2.3 mU at 72 h, 2.4 mU at 1 wk, and 1.8 mU at 2 wk, following transfection.

Effect of 693C→A, 854A→C, and 914C→A Base Change on ASPA *Activity*

Mutant *ASPA* cDNA constructs pSV-ASPA$^{(693C→A)}$, pSV-ASPA$^{(854A→C)}$, and pSV-ASPA$^{(914C→A)}$—with the 693C→A, 854A→C, and 914C→A point mutations, respectively, in their coding sequence (see fig. 8)—were expressed in COS1 cells. The expression of *ASPA* activity by mutant cDNA constructs was compared with the activity expressed simultaneously by the WT construct. The pSV-ASPA$^{(693C→A)}$ and pSV-ASPA$^{(914C→A)}$ mutant cDNA constructs did not express any measurable *ASPA* activity after transfection in COS1 cells. These data establish that the 693C→A or 914C→A point mutations result in complete loss of *ASPA* activity.

The *ASPA* activity expressed by pSV-ASPA$^{(854A→C)}$ construct at 1 wk posttransfection was 0.06 mU, compared with 2.4 mU activity expressed by the pSV-ASPA$^{(WT)}$ construct. The residual *ASPA* activity expressed by the 854A→C mutant cDNA was thus 2.5% of the activity expressed by the WT construct. Kinetic analysis of glu285→ala mutant *ASPA* expressed by pSV-ASPA$^{(854A→C)}$ construct was carried out, and the results were compared with that for the WT enzyme expressed by the pSV-ASPA$^{(WT)}$ construct. The apparent Michaelis constant (K_m) of

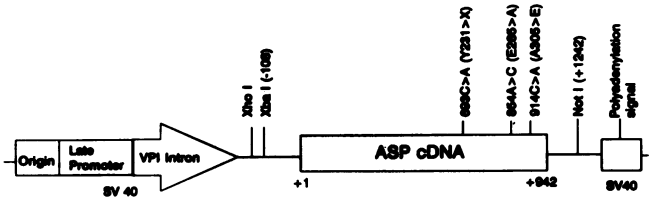

Figure 8 Schematic representation of the pSV-ASPA$^{(WT)}$ expression construct. The WT cDNA insert (base positions −108 to +1248) was cloned in pGEUK-C1 vector downstream of the SV40 late promoter and VP1 intron. The SV40 polyadenylation signal was present 3′ of the cDNA insert. The relative positions of the 693C→A, 854A→C, or 914C→A mutation—present in pSV-ASPA$^{(693C→A)}$, pSV-ASPA$^{(854A→C)}$, or pSV-ASPA$^{(914C→A)}$ expression constructs, respectively—are also indicated. The figure is not drawn to scale.

Table 1

Relative Frequency of Canavan Mutations among Ashkenazi Jewish and Non-Jewish Probands

			No. (%)	
POSITION	MUTATION	TYPE	Ashkenazi Jewish[a]	Non-Jewish[b]
433 − 2	a → g	Intron 2	1 (1.1)	0
693c → a	Y231 → X	Nonsense	13 (14.8)	0
854a → c	E285 → A	Missense	73 (82.9)	1 (2.5)
914c → a	A305 → E	Missense	0	24 (60.0)
Total			87 (98.8)	25 (62.5)

[a] A total of 88 chromosomes from probands were analyzed.
[b] A total of 40 chromosomes from probands were analyzed. Patients studied were Caucasians of European ancestry.

the WT human *ASPA* was determined to be 3.6×10^{-4} M. The glu285→ala mutant enzyme did not reach the apparent maximum velocity (V_{max}) even at substrate concentrations threefold higher than that in a standard assay system. The kinetic parameters thus could not be determined accurately.

Relative Frequency of Four CD Mutations

We have analyzed 128 independent CD chromosomes for four point mutations. Of these, 88 CD chromosomes were from probands of Ashkenazi Jewish extraction. The other 40 chromosomes were from non-Jewish probands of European descent. The frequency of mutations among the two population groups is given in table 1. Only the 433 −2(A→G), tyr231→ter, and glu285→ala mutations were observed among chromosomes of Ashkenazi Jewish ancestry, and together they accounted for 98.8% of the 88 CD chromosomes. In this population, the glu285→ala missense mutation was predominant in 82.9% (73) of the chromosomes, followed by the nonsense tyr231→ter mutation in 14.8% (13) chromosomes and the 433 −2(A→G) splice-site mutation in 1.1% (1) of the chromosomes.

None of the chromosomes from Ashkenazi Jewish probands with CD carried the ala305→glu missense mutation. In the 36 non-Jewish CD chromosomes analyzed, the ala305→glu mutation was identified in 60% (24) of the chromosomes, while the glu285→ala mutation was found in 2.5% (1) of the chromosomes. The tyr231→ter nonsense and 433 −2(A→G) splice-acceptor-site mutations were ruled out in all 40 chromosomes from non-Jewish probands of European descent.

Discussion

CD is a progressive, autosomal recessive leukodystrophy marked by severe neurological impairment. A rapid regression at age 3–8 mo occurs in a majority of these patients. CD is caused by the deficiency of aspartoacylase; and the consequent lack of hydrolysis of its substrate NAA leads to absence or dissociation of myelin by a large number of vacuoles, giving it a spongy appearance (van Bogaert and Bertrand 1949; Matalon et al. 1988, 1989, 1993).

Excretion of NAA in urine of patients with CD is specific and has not been observed in other non-CD white-matter diseases (Matalon et al. 1989). Cultured skin fibroblasts are needed for confirmation of *ASPA* deficiency in patients with CD and for determination of carrier status of individuals at risk. Lack of aspartoacylase activity in blood constituents has made testing of carriers a time-consuming process. CD is prevalent among Ashkenazi Jewish people, and indeed a majority of cases diagnosed at our center have this background (Matalon et al. 1993). However, it has not been possible to carry out epidemiology of CD in the risk population, since cultured cells are required for determination of *ASPA* activity. The isolation and characterization of the human aspartoacylase gene (Kaul et al. 1993, 1994) has made it possible to identify mutations that lead to deficiency of aspartoacylase. Deficiency of ASPA activity due to the nonsense tyr231→ter, the missense glu285→ala, or the ala305→glu mutation establish that the three coding-sequence mutations indeed lead to CD. The 433 −2(A→G) transition in the splice-acceptor site in intron 2 would lead to skipping of exon 3. The skipping of the 94-base exon 3 (Kaul et al. 1994) will also change the reading frame in the final transcript. Such exon

skipping accompanied by frameshift would result in deficiency of aspartoacylase.

The glu285 residue has been predicted to form a triad of ser, his, and glu residues at the catalytic center of *ASPA* (Kaul et al. 1993). Kinetic analysis suggests that the glu285→ala mutation significantly compromises the V_{max} of the mutant *ASPA*. This would be expected of mutations at the catalytic center of the enzyme. The presence of residual activity in glu285→ala mutant *ASPA* would suggest a relatively less severe brain pathology in patients homozygous for this mutation, in contrast to the complete loss of *ASPA* activity observed with the nonsense tyr231→ter and missense ala305→glu mutations. Complete loss of activity because of the tyr231→ter nonsense mutation should be due to premature termination of the *ASPA* polypeptide chain during translation of the mutant mRNA. The reason for complete loss of activity in ala305→glu mutant *ASPA* is not understood at present.

The predominant nature of the glu285→ala mutation (82.9%) among Ashkenazi Jewish patients with CD has now been strengthened by inclusion of a larger sample in the current study. Only one chromosome from a non-Jewish patient carried this mutation. The ala305→glu mutation, on the other hand, is found exclusively among non-Jewish patients of European descent. The ala305→glu mutation was not represented among Ashkenazi Jewish probands. The relative frequency of CD mutations among Ashkenazi Jewish and non-Jewish patients follows a pattern reminiscent of the Tay-Sachs mutations in the two population groups (Paw et al. 1990). Both the CD and the Tay-Sachs mutations in Ashkenazi Jewish people are believed to have originated around the Vilnius region in Lithuania (Banker and Victor 1979; Ungar and Goodman 1983). The predominant nature of certain mutations would suggest a founding-father phenomenon. It would be interesting to explore the genetic background against which the CD and Tay-Sachs mutations might have originally occurred among Ashkenazi Jewish people.

Characterization of CD mutations will make it possible to have a reliable DNA-based method for prenatal diagnosis in informative families (Matalon et al. 1992). The three mutations identified in Jewish probands account for 98.8% of the mutant alleles. This should make it feasible to carry out the epidemiology of CD in the risk population. Furthermore, carrier testing, similar to Tay-Sachs screening (Kaback et al. 1993), can be offered on a routine basis. Genotyping of the risk population for CD mutations should provide a chance at preventing the occurrence of this progressive autosomal recessive leukodystrophy.

Note added in proof.—Since acceptance of the manuscript for this article, we have characterized two Canavan patients of Ashkenazi Jewish background with the 914C→A mutation in one of their alleles. The 914C→A mutation, in both cases, however, was inherited from the parent of non-Jewish origin. It would therefore seem that the 914C→A mutation is indeed of non-Jewish orign.

Acknowledgments

We wish to thank all the physicians who referred patients for diagnosis of CD to our research institute. This study was supported in part by a generous grant from the United Leukodystrophy Foundation.

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