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The Frequency of the C854 Mutation in the Aspartoacylase Gene in Ashkenazi Jews in Israel

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Summary

Canavan disease (CD) is an infantile neurodegenerative disease that is transmitted in an autosomal recessive manner and has mainly been reported in Ashkenazi Jewish families. The primary enzymatic defect is aspartoacylase deficiency, and an A-to-C transition at nucleotide 854 of the cDNA has recently been reported. We screened 18 patients with CD and 879 healthy individuals, all Israeli Ashkenazi Jews, for the mutation. All 18 patients were homozygotes for the mutation, and 15 heterozygotes were found among the healthy individuals. The results disclose a carrier rate of 1:59 and suggest that a screening for the mutation is warranted among Ashkenazi Jewish couples.

Introduction

Canavan disease (CD), a fatal neurodegenerative disorder, primarily involves the white matter. The disease is manifested at several months of age, by macrocephaly, marked developmental delay, optic atrophy, seizures, hypertonia, and death in early childhood (Van Bogaert 1970). It is transmitted in an autosomal recessive manner and has been reported mainly in Ashkenazi Jewish families (Ungar and Goodman 1983). Until recently the diagnosis of CD could only be established 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 abnormal amounts of N-acetylaspartic acid in urine, and deficiency of aspartoacylase was demonstrated in their cultured skin fibroblasts (Divry et al. 1988; Matalon et al. 1988). The cDNA of the aspartoacylase gene was recently cloned, and an A-to-C nucleotide transition at nucleotide 854, which creates restriction-enzyme–recognition sequence for *Eag*I, was identified in 29 of 34 of the mutant alleles (Kaul et al. 1993). The present study was aimed at identifying the frequency of the C854 mutation in mutant alleles of CD patients in Israel and estimating the carrier rate in Ashkenazi Jews.

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Subjects and Methods

Patients and Screened Individuals

Between the years 1989 and 1993, we diagnosed 25 patients with CD. The patients originated from 21 Ashkenazi Jewish families. The diagnosis was suspected on clinical grounds and on the finding of leukodystrophy in brain computed-tomography or magnetic-resonance scan and was verified by the finding of N-acetylaspartic aciduria in the urine. In 18 patients of different families, skin fibroblasts were available, and deficient activity of aspartoacylase was demonstrated (Zelnik et al. 1993).

During a 6-mo period in 1991, 10 ml of cord blood from 600 deliveries of anonymous Ashkenazi Jewish families in Shaare-Zedek Medical Center were collected into EDTA-coated tubes. During a 2-mo period in 1993, 10-ml blood samples of 279 Ashkenazi Jews of the ultra-Orthodox religious community in Jerusalem were collected into EDTA-coated tubes. The samples were obtained as part of the program for carrier detection for Tay-Sachs disease and cystic fibrosis in this community. The results were transmitted to the program organizers.

Genomic DNA Analysis

Genomic DNA was extracted from fibroblasts and blood samples by standard methods. Samples of 0.5–5 ng of DNA were amplified in 100 μl containing 0.25 mM of each of the dXTPs, 78 ng of both oligonucleotide primers—CD1 (CTCTTGATGGGAAGACGATC) and CD2 (ACACCGTGTAAGATGTAAGC)—10 mM Tris HCl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton-X 100, and 0.2 mg of gelatin/ml. After heating of the mixture in boiled water for 5 min and cooling on ice, 2 units of *Taq* DNA polymerase (Appligene) were added. 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 2-min primer extension at 70°C. The final extension step lasted 10 min.

Results

A 183-bp fragment that included the mutation was amplified. After digestion with *Eag*I, affected homozygotes

had two restriction fragments, of 63 and 120 bp; heterozygotes had an undigested 183-bp fragment and two restriction fragments, of 63 and 120 bp; and noncarrier homozygotes had only an undigested 183-bp fragment. All 18 patients were homozygotes for the C854 mutation.

Nine heterozygotes for the C854 mutation were found in the 600 cord blood samples (carrier frequency = 1:67). No homozygotes for the mutation were found. Of 279 individuals of the ultra-Orthodox religious community, 6 heterozygotes for the mutation were found (carrier frequency = 1:47). The carrier frequency for the C854 mutation in the 879 Ashkenazi Jewish individuals is therefore 1:59 ($P = .017 \pm .0087$).

Discussion

Our results confirm that the C854 mutation is indeed a common mutation in CD. Among 88 CD chromosomes of American Ashkenazi Jewish descent, 82.9% harbored the C854 mutation, and another 15.9% harbored two other mutations (Matalon et al., in press). In contrast, all 36 affected alleles of the Israeli Ashkenazi Jewish patients had the C854 mutation. This patients' group included the six CD patients previously reported to have a prolonged survival (Zelnik et al. 1993). Our findings show that this clinical course is not due to mutation heterogeneity but, rather, reflects the improvement in patient care and other unrelated factors. The results also indicate that screening of the Ashkenazi-Jewish community in Jerusalem for the presence of the C854 mutation is likely to identify most CD carriers in this community. Screening of our population for carriers of the two newly identified mutations is pending.

The screening of 879 healthy Ashkenazi-Jewish individuals disclosed a carrier rate of 1:59. The carrier frequency was higher in the ultra-Orthodox community than in the general Ashkenazi population. These results are puzzling because Ashkenazi Jewish CD families were previously reported to originate from eastern Europe (Ungar and Goodman 1983), whereas the ultra-Orthodox community originates mainly from Hungary and Czechoslovakia. It seems that larger numbers should be screened before any conclusion regarding carrier rate in various Ashkenazi Jewish groups can be drawn.

Our findings suggest that the C854 homozygote corresponding to the CD phenotype occurs at a frequency of 1:14,000 live births in the general Ashkenazi Jewish population. This figure may even be higher (1:9,400) if the C854 mutation accounts for only 82.9% of the carriers, as in the American population. Despite small sample size, this estimation is likely to be more accurate than any figure derived from the number of diagnosed patients, because it excludes errors due to undiagnosed patients, aborted patients, and death in utero.

Several genetic disorders are prevalent among Ashkenazi Jews. The carrier frequency of cystic fibrosis is 1:29, and that of Gaucher disease is 1:16 (Abeliovich et al. 1992; Beutler et al. 1993). Unlike CD, mutation heterogeneity is the rule in both disorders, and a carrier state is excluded with a certainty of ~97% only after screening for five different mutations in each disease. Thus, the frequency of the C854 mutation in the general Ashkenazi Jewish population is equal to or higher than those of some of the mutations associated with cystic fibrosis and Gaucher disease in this community. In view of both the grave prognosis of patients with CD and the observed carrier rate, screening is warranted for the C854 mutation and possibly for the two newly discovered mutations in this population.

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References

Abeliovich D, Pashut Lavon I, Lerer I, Cohen T, Springer C, Avital A, Cutting GR (1992) Screening for five mutations detects 97% of cystic fibrosis (CF) chromosomes and predicts a carrier frequency of 1:29 in the Jewish Ashkenazi population. Am J Hum Genet 51:951–956

Beutler E, Nguyen NJ, Henneberger MW, Smolec JM, McPherson RA, West C, Gelbart T (1993) Gaucher disease: gene frequencies in the Ashkenazi Jewish population. Am J Hum Genet 52:85–88

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

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

Matalon R, Kaul R, Michals K. Molecular studies of Canavan disease. Am J Hum Genet (in press)

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

Ungar M, Goodman RM (1983) Spongy degeneration of the brain in Israel: a retrospective study. Clin Genet 23:23–29

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

Zelnik N, Luder A, Elpeleg ON, Gross-Tzur V, Amir N, Hemli JA, Fattal V, et al (1993) A protracted clinical course for patients with Canavan disease. Dev Med Child Neurol 35:346–358