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Prevalence of Canavan Disease Heterozygotes in the New York Metropolitan Ashkenazi Jewish Population

To The Editor:

Canavan disease is a severe neurodegenerative disease that occurs most commonly in the Ashkenazi Jewish population. Previous studies have indicated the carrier

frequency to be between 1/59 (Elpeleg et al. 1994) and 1/45 (Matalon et al. 1994). The disease, now recognized as a deficiency of aspartoacylase (Matalon et al. 1988), is associated with the pathological finding of spongy degeneration of the brain. Patients are usually normal at birth, but by 2–4 mo they lose milestones and develop seizures, macrocephaly, and hypertonia. Death occurs in early childhood (for review, see Beaudet 1995). With the availability of enzymatic testing, an increase in the incidence of newly diagnosed cases has been observed, suggesting that the frequency of the disorder may have been underestimated. In 1993, the cDNA for the aspartoacylase gene was cloned, and an A→C transition at nucleotide 854 was identified in affected individuals (Kaul et al. 1993). This represents a missense mutation from glutamine to alanine at amino acid residue 285 (E285A). This mutation was found in 73/88 Canavan disease–bearing chromosomes from Ashkenazi patients (Kaul et al. 1994*b*). In the same study, a C→A transition at nucleotide 693, which results in the conversion from a tyrosine codon to terminator codon at position 231 (Y231X), was found in 13/88 chromosomes. Thus, these two mutations provide a detection rate >97% in this population. The aspartoacylase gene spans 23 kb of DNA and has been mapped to 17p13-ter by FISH (Kaul et al. 1994*a*). The gene consists of six coding exons. The E285A mutation is located in exon 6 and results in the creation of a new *Eag*I site (Matalon et al. 1993), whereas the Y231X mutation is found in exon 5 and creates a *Mse*I site (Kaul et al. 1994*b*). In this study we have determined the frequency of these two mutations from a panel of unaffected individuals who live in the New York metropolitan area.

Patient samples were collected as part of a carrier-screening program for Gaucher disease, cystic fibrosis, and Tay-Sachs disease. Information concerning the country of family origin was requested from subjects. An additional blood specimen was collected in an EDTA-coated tube at the time of initial testing. Patients provided written consent for testing and were advised that results would not be reported to them. This protocol was approved by the institutional review board at New York University Medical Center. From the period January 1994–May 1995, >500 samples were collected, the majority from individuals of Ashkenazi Jewish origin (see table 1).

Genomic DNA was extracted by standard techniques. Both mutations were analyzed simultaneously by a multiplex PCR to amplify exons 5 and 6 in a microtiter plate format, which enabled 90 samples to be tested at one time. Exon 6 was amplified by primers as described elsewhere (Elpeleg et al. 1994), whereas for exon 5 we used primers CD8 (TTGTCATAGGAAAAGAATTTC) and CD9 (ATTAGGATGGATGATAGCAG). A hot-start procedure was performed with a Hybaid Omnigene thermocycler by use of the following cycle: 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, for 35 cycles, with an additional extension at 72°C for 10 min.

Table 1

Frequency of E285A and Y231X Mutations from Screening Panel

Population Group	No. Tested	Carriers E285A	Carriers Y231X	Canavan Disease Carrier
Ashkenazi	449	10 (1:45)	1	1:41
Non-Ashkenazi	91	0	0	0

For mutational analysis the amplified exon fragments were digested simultaneously with the *Eag*I and *Mse*I restriction enzymes, and the products were separated, by size, on a 4% NuSieve agarose gel. If the mutations were present, the 183-bp exon 6 fragment was cut to 120 and 63 bp, whereas the 117-bp exon 5 product yielded fragments of 72 and 45 bp. Among 449 samples from Ashkenazi Jewish individuals, 10 heterozygotes for the E285A mutation were found. Only one Y231X carrier was identified in this group. The relative frequency of these two mutations obtained in our population was consistent with the estimate based on analysis of Ashkenazi Jewish homozygotes ($\chi^2 = 0.263$, $P < .05$) (Kaul et al. 1994*b*). No carriers for either mutation were found among any of the non-Ashkenazi samples. This resulted in an overall carrier rate of 1/41 among the Ashkenazi Jews in our population, which suggests an incidence of Canavan disease of 1/6,700 Ashkenazi Jewish live births.

Our data revealed a higher frequency of the E285A mutation in the New York metropolitan area, compared with that observed among Ashkenazi Jews in Israel from a previous population study (Elpeleg et al. 1994); but it is consistent with results of another study, on an American Ashkenazi Jewish population (Matalon et al. 1994). Of the 11 carriers identified in our study, 5 indicated eastern Europe and 6 specified Russia as part of their common family origin. The significance of these findings will require larger numbers of subjects and a more detailed specification of family origin.

Tay-Sachs disease carrier testing is the paradigm for genetic screening in the Ashkenazi Jewish population. It has been widely accepted, with nearly 1 million people tested worldwide as of 1992 (Kaback et al. 1993). Canavan disease, like Tay-Sachs disease, is a serious neurodegenerative disorder that is almost universally fatal in early childhood. The calculated incidence of this disorder is approximately half that of Tay-Sachs disease. N-acetylaspartic acid has not been observed in the urine of individuals with other neurodegenerative disorders,

suggesting that mutations in the aspartoacylase gene are likely to be highly specific for Canavan disease. Mutational analysis for Canavan disease meets the previously defined characteristics of Tay-Sachs disease heterozygote-detection programs (Kaback et al. 1977) and could be used for screening in this population. Given the experience with Tay-Sachs disease–carrier programs, it is likely that carrier screening for Canavan disease will be widely accepted in the Ashkenazi community.

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High-Yield Noninvasive Human Genomic DNA Isolation Method for Genetic Studies in Geographically Dispersed Families and Populations

To the Editor:

Human genomic DNA is commonly isolated from peripheral blood samples for genetic studies of families and populations. Blood sampling, however, is expensive and an invasive procedure to which, for ethical reasons, objections may be raised, especially in studies involving older individuals and babies. We have developed a new noninvasive DNA sampling and isolation method involving oral samples taken with cotton swabs. Participants can take mouth swabs themselves, and can send these by mail to the research center, where DNA can be isolated at least up to 3 wk after sampling. DNA isolation from 20 cotton swabs resulted in an average yield of 40 μg of high-molecular-weight DNA per individual, sufficient for complete genome searches with ~800 polymorphic DNA markers when using PCR. Compared with blood sampling, which involves clinically trained personnel, this procedure is fast, less expensive, and suitable especially for DNA collection from geographically scattered subjects.

We have isolated human genomic DNA of family members (ages between 4 and 72 years), young twins (ages between 2 mo and 5 years), and random controls by using mouth swabs. Mouth swabs of the family members and random controls were taken by the participants themselves, and parents took mouth swabs of their twins, following a written protocol. Although twin pairs were sometimes only a few months old, problems were not encountered by parents obtaining samples from their children. At least 10 consecutive samples can be taken from one subject without a significant loss in yield per cotton swab. A second round of 10 cotton swabs can be taken after ~4 h, giving a maximal result of 20 samples in 1 d. The mouth swab sample should be taken in a clean mouth without food remains. After rubbing, the cotton swab, sample-end first, should be placed in a Falcon tube, containing 0.5 ml of STE buffer (100 mM NaCl, 10 mM TrisHCL [pH 8.0] and 10 mM EDTA)