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SHORT REPORT

A benign polymorphism in the aspartoacylase gene may cause misinterpretation of Canavan gene testing

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We found normal individuals whose aspartoacylse gene *Y231X* mutation site consistently gave no signal in a primer extension assay. We determined the nucleotide sequence of the relevant region of the gene in those individuals, and found a new allele with a thymidine residue at the mutation site instead of a cytidine. Since both TAC and TAT code for tyrosine, this sequence polymorphism has no effect on the amino acid sequence of the ASPA protein. We found the relative frequencies of the 693C and the 693T alleles in the tested population to be 0.75 and 0.25 respectively.

Keywords: Canavan disease; aspartoacylase gene; polymorphism; diagnosis; *Y231X* mutation

Introduction

Canavan disease is an autosomal recessive leukodystrophy caused by the deficiency of aspartoacylase (ASPA).[1] The majority of Canavan patients are of Ashkenazi Jewish origin. Mutations in the *ASPA* gene that lead to loss of enzymatic activity have been identified: *E285A* (854A C) and *Y231X* (693C A) account together for over 97% of the mutant chromosomes in Ashkenazi Jewish Canavan patients.[2] In non-Jewish patients of European descent the predominant mutation is 914C A (A305E). It was found in 24 out of 50 chromosomes tested.[3] Ten other mutations were found in 16 chromosomes, whilst the remaining chromosomes had unknown mutations.

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During the development of an assay for mutations in the aspartoacylase gene we discovered a natural polymorphism in the *Y231X* mutation site. Our assay uses a single-nucleotide primer extension reaction to test for point mutations. Two primer extension reactions are carried out for every mutation site tested – one for the normal allele (*wt*) and one for the mutated allele (*mut*). Reaction products are visualised using a simple ELISA procedure. We found several apparently normal individuals whose *wt* and *mut* reactions in the *Y231X* mutation site consistently gave no signal. Suspecting a polymorphism at the tested site, we determined the nucleotide sequence of the relevant region of the gene in those individuals.

Materials and Methods

The sample population of this study consisted of 101 randomly selected individuals who frequented a local clinical laboratory. DNA was extracted from whole blood in EDTA

using a rapid DNA extraction kit (GamidaGen Ltd, Nes Tsiona, Israel). The relevant segment of the *ASPA* gene exon 5 was amplified using PCR and subjected to nucleotide sequence analysis using the GATC 1500 system (GATC GbmH, Konstanz, Germany). For primer extension assays, amplified ASPA exon 5 DNA was prepared as described above and tested using a Pronto™ Canavan kit (GamidaGen Ltd) which was modified to differentiate between the two normal 693 alleles.

Results and Discussion

The published sequence of the normal aspartoacylase gene[4] around the *Y231X* mutation site reads: GAT TAC CCC (the underlined C is the mutation site). We found that individuals with no *wt* signal were homozygous for a different allele with a thymidine residue at the mutation site instead of a cytidine (Figure 1). Since both TAC and TAT code for tyrosine, this sequence polymorphism has no effect on the amino acid sequence of the ASPA protein.

To find out the relative frequency of the 693C/T polymorphism in the Israeli population, we assayed 101 randomly selected individuals who frequented a local clinical laboratory, using a Pronto™ Canavan kit. We modified the kit so that we would be able to distinguish between the two *wt* alleles. Of the 101 random samples tested none had a mutation in the *ASPA* gene; 57 were 693C/C, 37 were C/T and 7 were T/T giving relative frequencies of 0.75 and 0.25 for the 693C and the 693T alleles respectively. This data is consistent with a Hardy-Weinberg equilibrium. To find out whether a linkage disequilibrium exists between the 693C/T polymorphism and the 854A→C mutation, we assayed seven individuals who were previously characterised as carriers and two homozygotes for the 854A→C mutation. We found that three of the carriers were 693C/T, four carriers and the two 854C homozygotes were 693T/T. Although the numbers are small these results may suggest that the 854A→C mutation occurred on the 693T allele.

Those who choose to develop a Canavan diagnostic test, or who might use a 'home-brew' procedure for detection of the *Y231X* mutation in the aspartoacylase gene should be aware of this natural polymorphism, since this benign sequence variation might lead to misdiagnosis of normal individuals.

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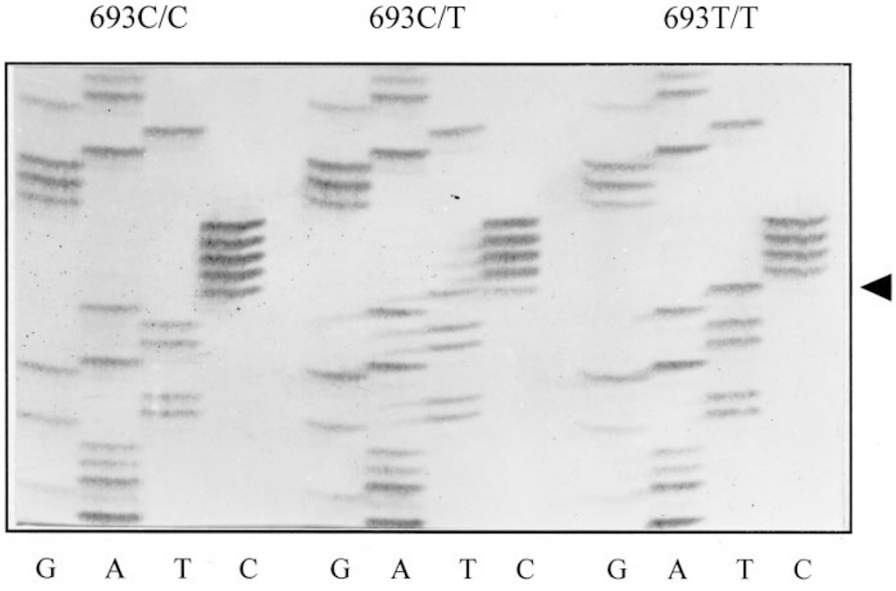

Figure 1 *Sequence determination around the ASPA 693C→A mutation site. The arrow denotes the C/T polymorphism. Crude DNA was extracted from whole blood in EDTA using reagents supplied with the Pronto™ kit and amplified using PCR. Gel purified PCR products were subjected to sequencing using the GATC 1500 system*

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