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The Nova Scotia (Type D) Form of Niemann-Pick Disease Is Caused by a G_{3097}→T Transversion in NPC1

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Summary

Niemann-Pick type D (NPD) disease is a progressive neurodegenerative disorder characterized by the accumulation of tissue cholesterol and sphingomyelin. This disorder is relatively common in southwestern Nova Scotia, because of a founder effect. Our previous studies, using classic linkage analysis of this large extended kindred, defined the critical gene region to a 13-cM chromosome segment between D18S40 and D18S66. A recently isolated gene from this region, NPC1, is mutated in the majority of patients with Niemann-Pick type C disease. We have identified a point mutation within this gene (G_{3097}→T; Gly_{992}→Trp) that shows complete linkage disequilibrium with NPD, confirming that NPD is an allelic variant of NPC1.

Introduction

Niemann-Pick (NP) type II disease is an autosomal recessive lysosomal storage disease characterized by an accumulation of tissue cholesterol and sphingomyelin consequent to abnormal intracellular translocation of LDL-derived cholesterol (Spence and Callahan 1989; Pentchev et al. 1995). Affected children suffer a progressive and severe degeneration of the nervous system and usually die within the first 2 decades of life.

NP type II has been subclassified as type C (NPC) (MIM 257220) and type D (NPD) (MIM 257250). NPD has been reported only in descendants of an Acadian couple who married in ~1700 in what today is Nova Scotia (Winsor and Welch 1978). In contrast, NPC is clinically heterogeneous and has been identified in many ethnic populations. Recent reports have identified two complementation groups within NPC (Steinberg et al. 1994; Vanier et al. 1996). The genetic defect of the major complementation group (representing 27 of 32 patients) has been localized to chromosome 18q11-12 (Carstea et al. 1993), and the gene mutated in these patients, NPC1, has been isolated and has been identified as having a critical role in the regulation of intracellular cholesterol trafficking (Carstea et al. 1997). Linkage analysis of NPD mapped this mutation to the same region of chromosome 18 as that containing NPC1, suggesting that these two disorders are likely to be allelic variants (Greer et al. 1997). We here report mutation analysis of NPC1 in our population, which confirms that this gene is also mutated in NPD disease.

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Subjects and Methods

Subjects

Peripheral blood, fibroblast cell lines, and/or paraffin-embedded tissue was collected, with ethical approval from the IWK Grace Health Centre (Halifax), from >150 members of the extended NPD kindred (Greer et al. 1997). Samples from 7 patients and from 22 obligate carriers were analyzed for mutations in the NPC1 gene. Fifty unrelated, unaffected individuals were used to confirm that mutations detected in NPD patients were not present in the general population.

Mutation Analysis

From the NPC1 cDNA sequence reported by Carstea et. al. (1997), PCR primers were selected to generate three overlapping amplicons that span the entire coding region of the gene; these include a 2.8-kb segment from C_{30} to G_{2835} (F1, 5′-CGG GGT GCT GAA ACA G; and R1, 5′-CCC CTT GGA AGA AGT GTA GT), a 1.1-kb segment from G_{2811} to C_{3909} (F2, 5′-GCA CGA CTA CAC TTC TTC CA; and R2, 5′-GGC ACA ACT TTT GGC TTT A), and a 400-bp segment from G_{3829} to A_{4224} (F3, 5′-GGA GCC ACT CAC GGA TTA; and R3, 5′-TCC TGC TTG CCA AAG AAT). By means of standard reverse-transcriptase–PCR (RT-PCR) techniques (Mauer et al. 1990), amplicons were generated from RNA isolated from two patients and from an unaffected relative

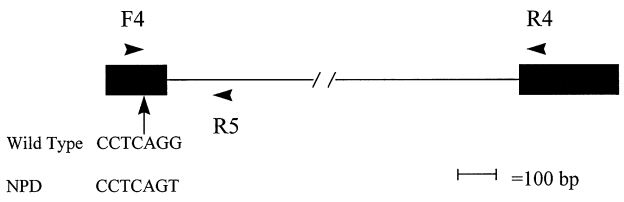

Figure 1 NPD mutation site. The positions of PCR primers F4 (5′-CGG AAG GCA AAC AGA GG), R4 (5′-TTT CTT CAG AGC GTC AAT AAA G), and R5 (5′-CAC CAA AGG ACC AGG ACA) are indicated. Primers F4 and R5 generate a 223-bp amplicon that spans the mutation site (cDNA nucleotide 3097) and the intron/exon boundary following cDNA nucleotide 3164. The seven-base *Bsu*36I restriction-endonuclease recognition sequence present in the wild-type allele and eliminated by the G→T trinucleotide substitution in NPD patients is shown. Blackened boxes denote exons; and intronic sequences are represented by the thin line.

who was not a carrier. Each amplicon was cloned and sequenced by means of the Pharmacia T7 Sequencing Kit (Pharmacia Biotech).

Detection of the NPD Mutation from Genomic DNA

Primers F4 and R4, flanking the NPD mutation site, were used to amplify the intervening genomic DNA. In order to select a reverse primer from within the closest 3′ intron that could be used to synthesize, for mutation analysis, a smaller amplicon directly from genomic DNA, the forward primer F4 was used to sequence into the intron, with the genomic PCR product being used as a template. The intronic sequence was used to design the reverse primer R5. For mutation analysis, primers F4 and R5 were used to generate small genomic amplicons that were subjected to restriction-endonuclease digestion to confirm presence or absence of the NPD mutation (see the Results section).

Results

To test the possibility that the gene recently isolated by Carstea et al. (1997) is also responsible for NPD disease, NPC1 cDNA from NPD patients was cloned and sequenced. A single point mutation, a G_{3097}→T transversion that converts $glycine_{992}$ to tryptophan, was found in eight clones from two patients and was not observed in cDNAs derived from an unaffected individual. This single base change abolishes a *Bsu*36I restriction-enzyme recognition site.

PCR primers F4 and R4 (fig. 1), which flank the NPD mutation and generate a 220-bp amplicon from cDNA, produced a much larger amplicon, ~1.5 kb, from genomic DNA. This indicated the presence of intronic DNA. To generate a small genomic fragment containing the NPD mutation, forward primer F4 was used to sequence through the mutation into the intron. Intronic sequence data were used to generate reverse primer R5 (fig. 1). Primers F4 and R5, which flank the NPD mutation, amplified, from genomic DNA, a 223-bp segment that was used, in conjunction with *Bsu*36I, to screen the remaining 5 patients and 22 obligate carriers (representative data are shown in fig. 2).

With the exception of patient 3114, the affected individuals were homozygous for the mutation. All obligate carriers, with the exception of the mother of patient 3114, were heterozygous for the mutation. None of the 50 unaffected, unrelated individuals had this mutation. The conclusion that patient 3114 is a compound heterozygote is not surprising, because the ancestry of her mother could not be traced into the NPD kindred.

Discussion

NPD disease has been a major health concern for this rural community of Nova Scotia for many years. Despite efforts, both at Dalhousie University (Byers et al. 1989; Sidhu et al. 1993) and elsewhere (Pentchev et al. 1985, 1986, 1995), to study the biochemistry of NP type II disease, the underlying defect remains unknown, and there is little to offer in the way of treatment or cure. Our previous studies, using classic linkage analysis, have defined the NPD critical region to a 13-cM chromosomal segment, between D18S40 and D18S66 (Greer et al. 1997), that contains the NPC1 gene (Carstea et al. 1997). The data reported here show that all of the NPD patients are homozygous for a mutation in this gene and that one compound heterozygote is heterozygous for the NPD mutation. This confirms that NPD and the major complementation group of NPC are allelic variants of the same gene, NPC1. Thus, the genetic symbol for the NPD mutation would be "NPC1, GLY992TRP."

The common ancestry of NPD provides the opportunity to develop a rapid and cost-effective PCR-based

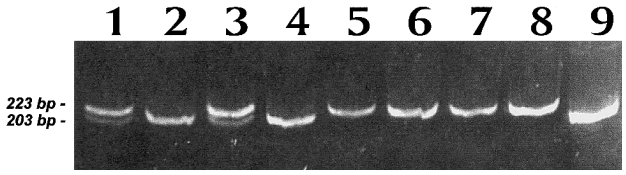

Figure 2 Analysis of the NPD mutation from genomic DNA. PCR amplicons generated by use of primers F4 and R5 were digested with restriction endonuclease *Bsu*36I and were separated by PAGE. NPD mutant alleles produce undigested 223-bp amplicons, and normal alleles are digested to a smaller, 203-bp fragment. Results of analysis of an obligate NPD heterozygote (lane 1), an unaffected relative who is not a carrier (lane 2), patient 3114 (lane 3), the mother of patient 3114 (lane 4), four NPD patients (lanes 5–8), and an NPC patient (lane 9) are shown.

carrier screen for families from Nova Scotia who are at high risk of having affected children. The identification of a compound-heterozygote patient warns that, although this analysis detects the common NPD mutation, it does not identify all NPC1 alleles that may be segregating in the Nova Scotia families.

Although the function of the NPC1 protein is unknown, the predicted amino acid sequence suggests that it is an integral membrane protein containing $\geqslant 13$ transmembrane regions and a putative sterol-sensing domain (Carstea et al. 1997). Carstea et al. (1997) have identified eight distinct NPC1 mutations; these include two frameshift mutations that would produce truncated proteins, a 6-bp deletion (amino acids 200–201), and five missense mutations (located either within or between the transmembrane domains). The NPD mutation reported here (GLY992TRP) is novel, although it is found within the same interval, between transmembrane domains, that contains one of the reported NPC1 missense mutations (GLY928PRO) (Carstea et al. 1997). The NPD mutation produces a nonconservative amino acid change in a glycine residue that is conserved in both the mouse and yeast homologues of the Npc1 protein (Loftus et al. 1997). The less severe biochemical defect observed for NPD cells, relative to most NPC1 mutant cells (Byers et al. 1989; Sidhu et al. 1993), suggests that the protein encoded by the NPD mutant allele may retain partial function, a finding that may have relevance for the development of therapeutic approaches to treat the disease.

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for NPC [MIM 257220] and NPD [MIM 257250])

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