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Linkage of Niemann-Pick Disease Type D to the Same Region of Human Chromosome 18 as Niemann-Pick Disease Type C

W. L. Greer,[1] D. C. Riddell,[1,2] D. M. Byers,[2,3] J. P. Welch,[3] G. S. Girouard,[1] S. M. Sparrow,[1] T. L. Gillan,[1] and P. E. Neumann[1,4]

Departments of [1]Pathology, Division of Molecular Pathology and Molecular Genetics, [2]Biochemistry, and [3]Pediatrics, and [4]Department of Anatomy and Neurobiology, Faculty of Medicine, Dalhousie University, Halifax

Summary

Niemann-Pick type II disease is a severe disorder characterized by accumulation of tissue cholesterol and sphingomyelin and by progressive degeneration of the nervous system. This disease has two clinically similar subtypes, type C (NPC) and type D (NPD). NPC is clinically variable and has been identified in many ethnic groups. NPD, on the other hand, has been reported only in descendants of an Acadian couple who lived in Nova Scotia in the early 18th century and has a more homogeneous expression resembling that of less severely affected NPC patients. Despite biochemical differences, it has not been established whether NPC and NPD are allelic variants of the same disease. We report here that NPD is tightly linked (recombination fraction .00; maximum LOD score 4.50) to a microsatellite marker, D18S480, from the centromeric region of chromosome 18q. Carstea et al. have reported that the NPC gene maps to this same site; therefore we suggest that NPC and NPD likely result from mutations in the same gene.

Introduction

Niemann-Pick type II disease is an autosomal recessive disorder usually occurring in childhood. It is characterized clinically by severe, progressive degeneration of the nervous system, coincident with accumulation of tissue cholesterol and sphingomyelin (Spence and Callahan 1989; Pentchev et. al. 1995). The causative gene defect associated with this disorder has not yet been identified.

Yarmouth County of Nova Scotia is reported to have the world's highest incidence of Niemann-Pick type II disease (Vethamany et. al. 1972; Winsor and Welch 1978). An extensive study by Winsor and Welch in 1978 estimated the frequency of affected children in one region of this county to be 1%. The frequency of heterozygous carriers was estimated to be 10%–26%. All of these individuals were shown to have descended from a small set of common ancestors, who emigrated from France to Nova Scotia during the 1600s. These demographics provide an opportunity to localize the defective gene in these patients, through a strategy of linkage analysis.

Niemann-Pick type II disease has been classified into two subtypes, C and D (Crocker and Farber 1958; Crocker 1961). The type C form (NPC) has been documented in many ethnic groups and is biochemically and clinically heterogeneous in nature (Vanier et. al. 1991; Higgins et. al. 1992), whereas the type D form (NPD) is confined to descendants of the Acadian population of southwest Nova Scotia and is more homogeneous. Liscum and Faust (1987) have shown that NPC cells possess the ability to bind, internalize, and degrade LDL normally. However, some cellular responses to LDL—for example, stimulation of cholesterol esterification by acyl-CoA:cholesterol acyltransferase (ACAT) and down-regulation of cholesterol synthesis and LDL-receptor expression—are reduced (Pentchev et. al. 1985, 1986). These observations are consistent with an LDL-specific regulatory defect.

Complementation studies, which assess the possibility of genetic heterogeneity, indicate that NPC comprises at least two complementation groups. Steinberg et al. (1994) have provided evidence of a second complementation group, consisting of 1 of their 12 NPC patients. Vanier et. al. (1996) similarly have described both a major complementation group comprising 27 of 32 unrelated patients and a second group comprising the remaining 5 patients. These five patients include the latter individual described by Steinberg. The full range of clinical and biochemical phenotypes were observed within the major NPC group, and no distinguishing characteristic was associated with the minor group. Carstea et al. (1994) mapped the NPC gene responsible for the major complementation group to 18q11-12, between D18S44 and D18S66. Linkage analysis of one of the larger families in the second group showed that the defective gene did not map to the centromeric region of chromosome 18.

Previous studies by our group have focused on the

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Address for correspondence and reprints: Dr. Wenda Greer, Queen Elizabeth II Health Sciences Centre–VG Site, DNA Laboratory, Hematology, Room 223B, MacKenzie Building, 5788 University Avenue, Halifax, Nova Scotia, B3H 1V8 Canada. E-mail: wgreer@IS.dal.ca

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Table 1

Maximum-Likelihood Estimates of $\hat{\theta}$ between Niemann-Pick Disease Type D and Chromosome 18 Markers, and Associated Two-Point LOD Score (Z)

Polymorphic Marker	Position (cM from D18S40)	$\hat{\theta}$	Z
D18S40	0	.05	1.99
D18S869	5	.00	3.10
D18S44	6	.00	1.93
D18S1101	7	.00	3.33
D18S1108	7	.04	3.38
D18S480	9	.00	4.50
D18S975	11	.00	2.66
D18S66	13	.10	1.90
D18S478	15	.07	1.33
D18S1151	15	.08	1.88

biochemical properties of the Nova Scotia form (NPD) of Niemann-Pick type II disease. Byers et al. (1989) initially showed that NPD fibroblasts exhibit a delayed and partial increase in cholesterol esterification in response to LDL. This is contrasted by the rapid or absent stimulations noted in normal or more severely affected NPC cells, respectively. The distinct mutations in NPC and NPD also result in differences in other regulatory steps in cholesterol metabolism (Sidhu et al. 1993). Down-regulation of LDL receptor, measured by northern blots of LDL receptor mRNA, was substantially delayed only in severely affected NPC cells. Filipin fluorescence staining of stored cholesterol was found to be much less pronounced in NPD than in many NPC fibroblasts. These studies established that NPD, like NPC, is defective in regulation of intracellular cholesterol esterification and storage, although NPD is a homogeneous group with some specificities.

Despite these differences, present evidence suggests that NPD and NPC may be allelic variants. Complementation of cholesterol esterification was not observed either when NPC and NPD fibroblasts were fused with polyethylene glycol (Sidhu et al. 1993) or when activity was measured directly in mixed-cell homogenates (Byers et al. 1989). The linkage analysis presented here examines the hypothesis that the genetic defect segregating in the Nova Scotian NPD population is an allelic variant of NPC, by testing for linkage to the chromosome 18 region that contains NPC.

Subjects and Methods

Subjects

Peripheral blood and/or paraffin-embedded tissue was collected, with ethical approval from the IWK Grace Maternity Health Centre for Children (Halifax), from 130 members of four extended families all of whom can be traced to at least one common ancestor of French origin (Winsor and Welch 1978); these 130 individuals include 7 patients and 20 obligate carriers.

Genotyping by Microsatellite Analysis

The polymorphic microsatellite markers used in this study include D18S40, D18S869, D18S44, D18S1101, D18S1108, D18S480, D18S975, D18S66, D18S478, and D18S1151 from the centromeric region of chromosome 18. Total genomic DNA was extracted from all samples by use of standard high-salt extraction procedures, and the alleles at each locus were amplified by PCR using conditions and primers described in the Genome Data Base (GDB) (http://gdbwww.gdb.org/) and/or the Whitehead Institute Data base (http:/www-genome.wi.MIT.edu).

In brief, ~250 ng of genomic DNA was PCR amplified in a 25-μl reaction mix containing 1.25 U *Taq* polymerase (Gibco BRL), 1.5–2.0 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3 (with the exception of 25 mM Tris-HCl pH 9.3, for amplication of D18S66, D18S40, and D18S1108 allelles), 400 μM each dNTP, 1 μCi ^{32}P-dCTP, and 0.1–1 μM each primer. The reactions were denatured at 95°C for 5 min and then were subjected to 30 cycles of 95°C, 55°C (for D18S869, D18S44, D18S1101, D18S1108, D18S480, D18S478, and D18S1151) or 57°C (for D18S66, D18S975, and

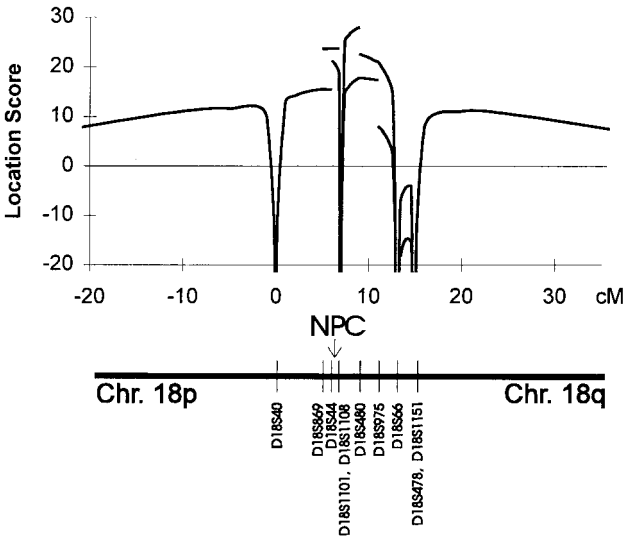

Figure 1 Location scores from multipoint linkage analysis for placement of the NPD gene relative to marker loci. The horizontal axis of this plot represents the genetic-linkage map of marker loci from the centromeric region of chromosome 18, with D18S40 arbitrarily positioned at 0.0 cM. This map was constructed by two-point linkage analysis of the NPD pedigrees. Shown are eight overlapping curves representing the likelihood support for the map position of NPD relative to marker loci, each curve representing a different set of three adjacent marker loci. These data indicate that NPD most likely maps either in the interval between D18S1108 and D18S66 or in the interval between D18S40 and D18S1101. NPC is located between D18S44 and D18S1101.

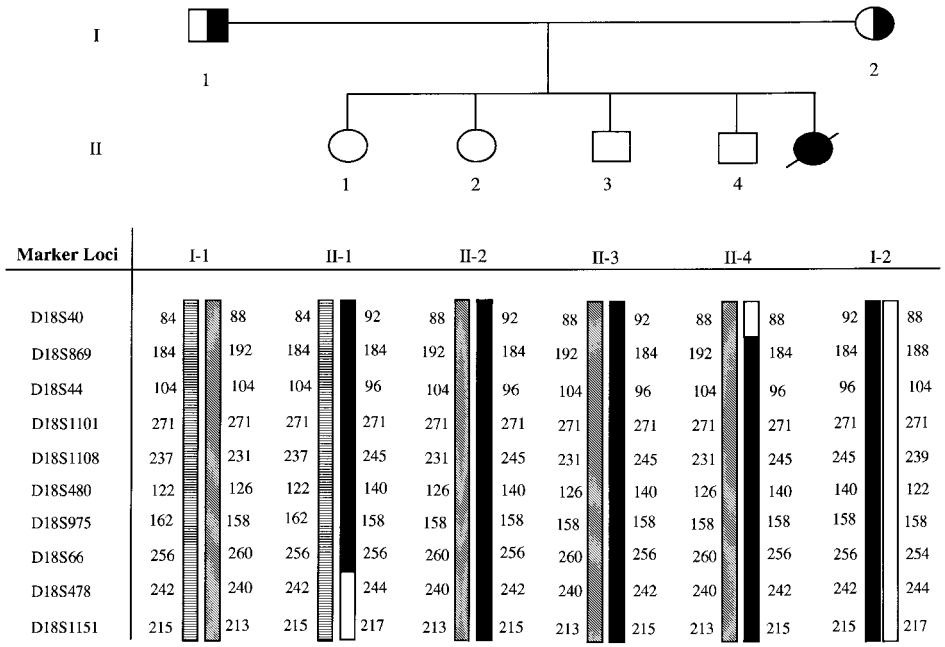

Figure 2 Meiotic recombination, which positions D18S478 distal to D18S66. Directly below each individual in the pedigree, the allele sizes (in bp) at each locus are indicated in the order in which they occur on the chromosome. The phase of these alleles is clearly established by comparison with that in other family members. Haplotypes are further depicted as shaded bars.

D18S40), and 72°C, with a final 7–10-min extension at 72°C. Amplicons were size fractionated by electrophoresis on standard sequencing gels and were sized by comparison with a comigratory M13 sequence ladder generated by use of the Sequenase version 2 kit (Amersham).

Linkage Analysis

Two-point and four-point linkage analyses of NPD and the 10 microsatellite marker loci were performed by use of the FASTLINK versions of the MLINK and LINKMAP programs (Lathrop et al. 1984; Cottingham et al. 1993). In the figure illustrating the results of the multipoint analysis, the location scores for both internal intervals for all four-point analyses were plotted.

Results

The maximum-likelihood estimates for recombination fractions ($\hat{\theta}$) between NPD and each of the 10 marker loci are shown in table 1. These data reveal close linkage ($\hat{\theta} = .00$) of the disease locus to five loci in this region—D18S869, D18S44, D18S1101, D18S480, and D18S975. The highest maximum LOD score (Z_{max}) was 4.50 for NPD–D18S480.

Pairwise linkage analysis between marker loci has generated a genetic linkage map, which is depicted in figure 1. The gene order indicated by these results is generally consistent with other linkage and physical maps of this region. In addition, our data, for the first time, allow positioning of D18S478 and D18S66 relative to each other. Direct analysis of haplotypes reveals two recombinant chromosomes that place D18S478 distal to D18S66 (fig. 2).

To position the NPD locus relative to the linked marker loci, multipoint analyses comparing the relative likelihoods of various NPD locations were performed; the results are represented in figure 1. These data are most consistent with placement of NPD either in the interval between D18S1108 and D18S66 (peak location score 28.0 at locus D18S480) or in the interval between D18S40 and D18S1101 (peak location score 23.8 at D18S44).

Discussion

The results reported here show tight linkage between NPD and polymorphic microsatellite marker loci from the centromeric region of chromosome 18, previously shown by Carstea et al. (1993) to be linked to the NPC locus. Although one cannot exclude the possibility that these disorders result from defects in separate genes located close together, it is reasonable to assume that they are allelic variants. This conclusion is consistent with the apparent lack of complementation observed in previous studies (Byers et al. 1989; Sidhu et al. 1993). The clinical and biochemical phenotypes of NPC patients are variable. NPD likely represents one of the milder variants.

A genetic map of the 10 microsatellite markers studied in this report has been generated from pairwise two-point linkage analyses of our large Nova Scotia NPD kindred. The sizes of the intervals approximate those reported by Carstea et al. (1993, 1994) and others (Ge-

nome Data Base [http://gdbwww.gdb.org]). The data presented here have further characterized this region, ordering the previously inseparable loci D18S478 and D18S66, with the former on the distal side.

We have mapped the NPD gene to proximal chromosome 18q, to one or the other side of D18S1108. NPC is located on the proximal side of these two segments. Carstea et al. (1993) limited NPC to an ~5-cM interval between loci D18S44 and D18S66. We have recently found in an NPC family evidence that greatly reduces the critical region, by establishing D18S1101 as the distal limit (T. L. Gillan, P. E. Neumann, D. M. Byers, D. C. Riddell, and W. L. Greer, unpublished results).

Additional microsatellite markers in this region are presently being generated from YACs, to further delineate the NPC/NPD gene–containing region, with a view to positional cloning and characterization of the gene. Despite the efforts of our group and others to understand the basic biochemical defect responsible for NPD disease, the affected protein is still unidentified.

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