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Genetics

Linkage of Niemann–Pick disease type C to human chromosome 18

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ABSTRACT **We analyzed the involvement of chromosome 18 in Niemann–Pick disease type C (NPC), an autosomal recessive cholesterol-processing disorder. Within affected offspring, the chromosome 18 parental contributions were identified by using allele-specific microsatellite markers. Significant linkage of NPC to an 18p genomic marker, *D18S40*, was indicated by a two-point lod score of 3.84. Analysis of meiotic chromosomal breakpoint patterns among the affected individuals indicated that the NPC gene is pericentromerically localized on human chromosome 18.**

Niemann–Pick disease type C (NPC) is an autosomal-recessive neurovisceral lipid storage disease leading to systemic and neurologic abnormalities (1). Clinical features include hepatosplenomegaly, ataxia, dysarthria, dystonia, vertical supranuclear gaze palsy, spasticity, and dementia. Cataplexy and seizures may occur later in the unrelenting course of the illness. NPC is characterized by phenotypic variability, with onset ranging from birth to early adulthood (2). Type C Niemann–Pick disease differs from types A and B in that the latter two are lipidoses which result from a primary lesion in the sphingomyelinase gene, known to be located on chromosome 11 (3). The underlying genetic defect of NPC remains unknown.

The biochemical hallmark of the NPC cell is the abnormal accumulation of unesterified cholesterol in lysosomes (4). This trapping of cholesterol in lysosomes renders NPC cells deficient in their ability to homeostatically regulate both uptake and esterification of low density lipoprotein (LDL) cholesterol (5). This delay in the induction of homeostatic regulation is responsible for the excess accumulation of unesterified cholesterol (6).

Two independently derived mutant mouse colonies have played a pivotal role in delineating the biochemical basis of NPC. Initially, a mutant BALB/c mouse presenting clinical and biochemical features of NPC was characterized and shown to display a lesion resulting in extensive lysosomal cholesterol accumulation and blocked cellular cholesterol esterification (7). This finding lead to the discovery that a similar lesion was a unique and consistent feature in fibroblasts derived from human NPC patients (8, 9). A murine mutation was independently described in a C57BL/Ks mouse. Like the BALB/c mutation, this lesion featured a generalized cellular lipidosis with prominent accumulation of cholesterol and sphingomyelin (10–12). Due to attenuated sphingomyelinase activity and excess sphingomyelin accumulation, the mutant C57BL/Ks mouse was characterized as a sphingomyelinosis and the genetic locus was designated as *spm* (10). Recently, it has been reported that the *spm* locus resides on chromosome 18 (13). Immortalized cells developed from C57BL/Ks-*spm* mice were studied in culture and were reported to feature deficiencies in cellular cholesterol processing similar to those noted in human NPC cells (14). Cross-breeding of heterozygous C57BL/Ks-*spm* and NPC BALB/c mice produced affected offspring with all the clinical and biochemical features of murine NPC (T. Tokoro, Y. Eto, and P.G.P., unpublished work). Reversal of the murine NPC phenotype was reported in a preliminary communication indicating that the transfer of human chromosome 18 to *spm*-derived mouse cells reduced intracellular cholesterol accumulation (16). These experiments clearly suggest that the *spm* and the NPC BALB/c murine mutations involve the same gene and point to a possible role of chromosome 18 in the human disease.

It has been shown that regions of mouse chromosome 18 are extensively syntenic with human chromosomes 5 and 18 (17). Our investigation examines the possible linkage of NPC to human chromosome 5 or 18. Linkage analysis with markers specific to human chromosome 5 provided no evidence for localization of NPC on this chromosome. Genetic linkage analysis with five polymorphic microsatellite markers specific for chromosome 18, however, provided strong evidence for location of the NPC mutation on human chromosome 18.

SUBJECTS AND METHODS

Patients and Families. DNA samples were collected from members of 12 families (Fig. 1), each containing two to four NPC-affected individuals. In general, samples were made available only from the parents and patients of each family. DNA was available from unaffected subject 8-1, yet not from affected subject 7-2.

Although all patients presented with hepatosplenomegaly and neurologic regression, the degree of severity and onset of symptoms were varied. The diagnosis of NPC was accepted only in patients whose cultured fibroblasts demonstrated the unique combination of extensive filipin fluorescent staining of lysosomal cholesterol and greatly diminished cellular cholesterol esterification (2, 9).

DNA Preparation and PCR Analysis. Genomic DNA was isolated by standard methods from peripheral blood samples of living patients and relatives or from the cultured cells of deceased patients (18). PCR and subsequent polyacrylamide gel electrophoresis of the PCR products were performed as previously described (19). The PCR-based polymorphisms were characterized in the individual DNA samples with AC-repetitive microsatellite markers *D18S34*, *D18S40*, *D18S42*, *D18S46*, and *D18S47* (20). Autoradiography was performed at −70°C for periods of 2 hr to overnight.

Linkage Analysis. Linkage analysis was performed by using ILINK from the LINKAGE package of analysis programs (21).

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Abbreviations: NPC, Niemann–Pick disease type C; LDL, low density lipoprotein.

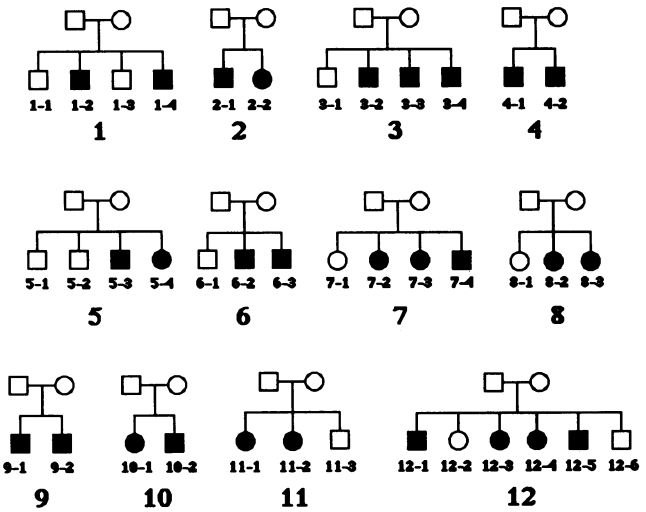

FIG. 1. Pedigrees of informative NPC families. Darkened squares (males) or circles (females) indicate NPC-affected individuals.

RESULTS

On the basis of the murine genetic linkage map that placed the *spm* locus 7.8 centimorgans from the *Grl* locus on mouse chromosome 18 (13), we analyzed comparable genomic regions associated with human *Grl* found on chromosome 5. Employing restriction fragment length polymorphic (RFLP) analysis, we found no evidence for linkage of the NPC gene to human chromosome 5 (data not shown).

To assess the involvement of human chromosome 18 in NPC, two-point linkage analysis was performed using polymorphic markers amplified by PCR. Significant linkage was discovered with marker *D18S40*, which maps to the short arm of chromosome 18 (Table 1). Three recombinants detected among the 28 informative individuals represent a recombination fraction of $\theta = 0.07$ and suggest that *D18S40* recombines in 7% of meiotic crossover events.

Examination of the informative recombination events in NPC-affected individuals is illustrated in Fig. 2. Among others, the recombination events observed in members of families 10 and 11 clearly indicate the unlikelihood that the NPC locus resides within chromosomal intervals C, D, E, or F (delineated by markers *D18S47, D18S34, D18S46*, or *D18S42*). The recombination events observed in families 5, 9, and 10 illustrate the unlikelihood that the NPC locus resides within interval A. Interval B, between polymorphisms *D18S47* and *D18S40*, provides the most likely region to harbor the NPC gene locus. Precise placement of the NPC gene would require more informative markers within interval B and the typing of additional NPC-informative meioses.

Table 1. Maximum two-point lod scores (Z) and maximum recombination fractions (θ) for linkage of NPC to chromosome 18 markers

Polymorphic marker	Regional assignment	Z	θ
D18S40	p11.2–p11.32	3.84	0.07
D18S47	q12.2–q21.2	0.53	0.18
D18S34	q12.2–q21.2	2.38	0.08
D18S46	q12.2–q21.2	0.72	0.20
D18S42	q21.3	0.44	0.79

DISCUSSION

The identification of the NPC gene will allow a more complete understanding of the pathology of this disorder, facilitate the formulation of protocols for potential treatment, and lead to a greater insight into the cellular mechanisms that regulate cholesterol metabolism. Current understanding of the cellular processes that control the uptake and utilization of cholesterol was outlined by Brown and Goldstein in a paper (22) describing their classic studies of the LDL-receptor pathway. We have shown that the NPC mutation is associated with a block in cellular utilization of endocytosed cholesterol. In mutant-derived fibroblast (23), lymphocyte (24), or murine astrocyte cultures (25), receptor-mediated endocytosis of LDL is characterized by abnormal sequestration of cholesterol in lysosomes. This unusual trapping of exogenously derived cholesterol leads to delayed relocation to other cellular organelles (23, 26, 27) and to a deficient utilization of the cholesterol for homeostatic and metabolic processes (6). Just as familial hypercholesterolemia (FH) highlights a plasma membrane receptor that mediates cellular uptake of LDL (22), the NPC mutation points to an additional protein that appears to play a fundamental and critical role in intracellular trafficking and utilization of cholesterol.

Since the physical and biological nature of the NPC protein remains uncertain, we are employing the strategy of reverse genetics, whereby we attempt to locate and characterize the gene as a means of identifying the defective gene product. The initial step in this process has been to genetically link the NPC gene to a particular chromosome. Genomic markers used in this investigation were developed from microsatellite

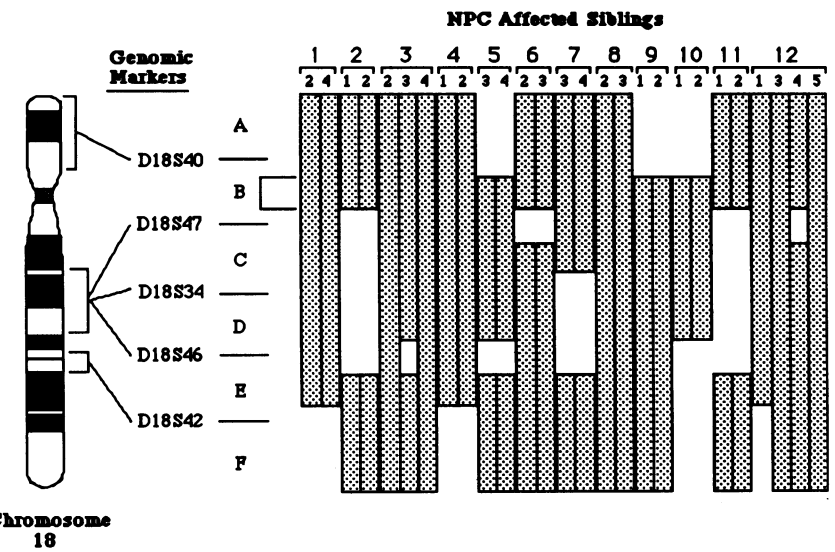

FIG. 2. Meiotic breakpoint map of chromosome 18 as defined by five microsatellite markers and the NPC phenotype. Chromosome intervals are the genomic regions between given markers, depicted as A through F. Gaps in the shaded bars below each affected individual indicate intervals that were involved in meiotic recombination, resulting in divergent segregation of the marker and the NPC phenotype. Unlike intervals A, C, D, E, and F, there is no evidence that interval B has been involved in a meiotic recombination.

AC-repeat sequences that are naturally present and abundant throughout the genome (15). Three important features that make microsatellite markers such powerful tools for PCR-based genetic linkage analysis are (*i*) the sequences that flank the AC-repeat region are unique within the genome and thus confer definitive chromosome identification; (*ii*) their flanking sequences are highly conserved, permitting the development of reliable PCR primers; and (*iii*) the size polymorphism associated within the repeat sequence allows the distinction between the two alleles of a given individual after PCR amplification. The five chromosome 18 microsatellites used in this study each contain a variable stretch of AC nucleotide repeats, usually 15 to 25. In the present study, we show that the NPC gene is significantly linked to marker *D18S40*, a microsatellite found on the small arm of chromosome 18.

Analysis of the polymorphic markers involved in recombinational crossovers permitted formulation of a meiotic breakpoint map to aid in determining the genomic interval most likely to contain the mutated gene. We propose that the NPC locus exists between markers *D18S40* and *D18S47*, a 13.7-centimorgan pericentromeric region of human chromosome 18.

The identification of the NPC-linked marker *D18S40*, which has an NPC recombination frequency of 7%, indicates that this marker segregates with the disease gene in 93% of all meiotic crossover events. This marker may be a useful tool in prenatal diagnosis for families at risk for NPC. The usefulness of this tool should be increased by expanding this study to include additional informative NPC families and concentrate on high-resolution mapping of the genomic interval that spans the centromere of chromosome 18.

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