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Genetic Heterogeneity in Neuronal Ceroid Lipofuscinosis (NCL): Evidence That the Late-Infantile Subtype (Jansky-Bielschowsky Disease; CLN2) Is Not an Allelic Form of the Juvenile or Infantile Subtypes

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Summary

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigment in neurons and other cell types. Inheritance is autosomal recessive. Three main childhood subtypes are recognized: infantile (Haltia-Santavuori disease; MIM 256743), late infantile (Jansky-Bielschowsky disease; MIM 204500), and juvenile (Spielmeyer-Sjögren-Vogt, or Batten, disease; MIM 204200). The gene loci for the juvenile (CLN3) and infantile (CLN1) types have been mapped to human chromosomes 16p and 1p, respectively, by linkage analysis. Linkage analysis of 25 families segregating for late-infantile NCL has excluded these regions as the site of this disease locus (CLN2). The three childhood subtypes of NCL therefore arise from mutations at distinct loci.

Introduction

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigment in neurons and other cell types. Three main childhood subtypes are recognized according to age at onset, clinical features, and neuropathological findings. These are the infantile (Haltia-Santavuori disease; locus CLN1), late-infantile (Jansky-Bielschowsky disease; locus CLN2), and juvenile (Spielmeyer-Sjögren-Vogt, or Batten, disease; locus CLN3) types. Inheritance is autosomal recessive, and the biochemical defect remains unknown.

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The infantile subtype was reported from Finland in 1974 (Santavuori et al. 1974). It is characterized by onset of symptoms at around 1 year of age and a fulminant clinical course including blindness and severe psychomotor retardation. There is severe neuronal loss and cerebral atrophy, with remaining neurons containing autofluorescent granules with a characteristic granular structure. Inclusions are also found in other cell types. CLN1 has been mapped to human chromosome 1p by linkage analysis (Järvelä 1991; Järvelä et al. 1991). Strong linkage disequilibrium has been identified between CLN1 and alleles at the loci HY-TM1, L-Myc, and D1S62 (Järvelä 1991; Hellsten et al. 1993; Vesa et al. 1993).

The juvenile subtype is characterized by onset at age 6–10 years with visual failure followed by progressive intellectual deterioration and seizures. Fingerprint profiles are the characteristic ultrastructural feature, and lymphocytes show vacuolation on light microscopy. CLN3 has been mapped to human chromosome 16p by linkage analysis (Eiberg et al. 1989; Gardiner et al. 1990;

Callen et al. 1991). Current analysis locates CLN3 at 16p12 in the interval between D16S297 and D16S57 (Mitchison et al. 1993). Linkage disequilibrium has been identified between CLN3 and alleles at three loci within this interval: D16S288, D16S298, and D16S299 (Mitchison et al. 1993).

The classical late-infantile subtype is characterized by onset of seizures and progressive dementia at the age of 2–4 years. Ataxia and myoclonus are constant features. The ultrastructural appearances are those of so-called curvilinear profiles. The children become incapacitated by age 3–6 years, at which time ophthalmological abnormalities can be observed. Macular and retinal degeneration plus optic atrophy occur, causing blindness by age 6 years. CLN2 has not yet been mapped.

Atypical forms of NCL constitute about 10% of all cases. In particular, cases occur which are intermediate between the classical late-infantile form and, as described above, the juvenile variety. Lake and Cavanagh (1978) described a group of patients with onset at age 5–6 years and fingerprint bodies on electron microscopy but with absent vacuolated lymphocytes. These were designated "early juvenile." Santavuori et al. (1982) described a group of patients with similar intermediate age at onset (5–7 years), absence of vacuolated lymphocytes, and cytosomes with both fingerprint and curvilinear profiles visible on electron microscopy. These have been designated a "Finnish variant" of the late-infantile disease. The aim of the present study was to determine by linkage analysis whether the gene (CLN2) causing classical late-infantile NCL is an allelic form of either CLN1 or CLN3.

Subjects and Methods

Families

Late-infantile NCL.—Probands with late-infantile NCL were ascertained with the collaboration of pediatric neurologists in several countries. Twenty-five nuclear two-generation kindreds were studied, including a total of 33 affected individuals. Two siblings were classified as "unknown disease status." Their age was either uncertain or <4 years. There were two affected siblings in eight kindreds (table 1). The families originated from the United Kingdom, The Netherlands, the United States, France, Newfoundland, Germany, Australia, and Costa Rica.

Diagnosis was based on clinical features (Santavuori 1988) together with the results of histological and electrophysiological investigations in the majority of patients. Light microscopy and electron microscopy of a variety of tissues were available, including rectal biopsy, conjunctiva, and fibroblast culture. Characteristic autofluorescent lipopigment or "curvilinear" profiles were reported in 28 (85%) of the cases examined. Electrophysiological investigations were available and were known to be typical of classical late-infantile NCL in eight affected individuals. Characteristic findings included spikes inducible by low photic stimulation, early high visual evoked potentials and loss of electroretinogram, and increased somatosensory-evoked potentials. Atypical patients with features of variant late-infantile NCL have been excluded and are being analyzed separately. Diagnosis and inclusion were based on these criteria, without any prior knowledge concerning the results of linkage analysis with markers on 16p and 1p.

Table 1

Late-Infantile Pedigrees

No. of Families	No. of Affected Children/Family	No. of Healthy Children/Family	No. with Uncertain Disease Status/Family
8	1	1	0
7	1	2	0
5	2	1	0
3	2	0	0
1	1	1	1
1	1	0	1

Juvenile NCL.—Families analyzed with chromosome 16p markers included 70 pedigrees including 113 affected individuals and 77 unaffected siblings (Mitchison et al. 1993).

Infantile NCL.—Forty-four families (40 of Finnish origin) were analyzed with chromosome 1p markers. In total, 53 affected children and 51 unaffected siblings were analyzed. The non-Finnish families were from Sweden, Germany, and the United Kingdom (Hellsten et al. 1993).

Marker Typing

DNA was extracted from white cells or autopsy tissue samples by using standard methods. Individuals were phenotyped at marker loci on chromosomes 1p and 16p. Typing for D1S62, D1S57, and D16S148 was carried out by Southern hybridization as described elsewhere (Gardiner et al. 1990; Järvelä 1991). Typing for D16S296, D16S298, D16S285, and HY-TM1 was carried out by PCR amplification and electrophoresis

Table 2

Pairwise Linkage Results between CLN2 and Chromosome 1 Marker Loci D1S62, HY-TM1, and D1S57

	Lod Score at Recombination Fraction of						
	.00	.01	.05	.1	.2	.3	.4
D1S62	$-\infty$	−2.90	−1.03	−.40	−.02	.03	.01
HY-TM1	$-\infty$	−5.08	−1.55	−.51	−.02	.03	.01
D1S57	$-\infty$	−10.06	−4.30	−2.24	−.74	−.23	−.05

of radiolabeled product on 6% denaturing polyacrylamide gels. These microsatellite polymorphisms all have heterozygosities of >75% (Konradi et al. 1991; Mäkelä et al. 1992; Thompson et al. 1992).

Linkage Analysis

Analysis was carried out by using the LINKAGE package of computer programs, version 5.20, updated by Dr. Jurg Ott (Lathrop et al. 1984). Pairwise linkage analyses were performed by using MLINK option, and multipoint linkage analyses were performed by using LINKMAP option.

Female:male sex ratio 2.46:1 for chromosome 1 markers was used for linkage calculations to allow for the different recombination frequencies found in this chromosomal region (Hellsten et al. 1993). Female: male recombination ratio varies across the region within which CLN3 is known to lie. A multipoint analysis which allowed for variation in both male and female recombination across the region was therefore used. The results were plotted on the male map. M-test was used to test the homogeneity between different clinical forms; the homogeneity between CLN1 and CLN2 was tested by using two-point lod scores with HY-TM1, and the homogeneity between CLN2 and CLN3 was tested by using two-point lod scores with D16S298.

Results

Chromosome 1

Pairwise linkage analysis of CLN2 and D1S62, HY-TM1, and D1S57 revealed negative lod scores (table 2). Multipoint linkage analysis allowed exclusion of a region of more than 25 cM as the site of CLN2, including the region to which CLN1 is assigned (fig. 1). In M-test significant evidence against homogeneity was obtained (χ^2 = 10.04, 1 df, P = .0015).

Chromosome 16

Pairwise linkage analysis of CLN2 and the chromosome 16 markers D16S296, D16S298, D16S148, and D16S285 revealed negative lod scores (table 3). Multipoint analysis allowed a region of about 35 cM to be excluded as the site of CLN2, including the region to which CLN3 has been assigned (fig 2). M-test result confirmed significant evidence against homogeneity (χ^2 = 37.66, 1 df, P < .0001). There was no evidence for linkage equilibrium between alleles at these loci and CLN2 (data not shown).

Discussion

The NCLs represent a group of inherited disorders which exemplify the difficulty of clarifying and categorizing genetic diseases before their pathology is understood at a molecular level. Until the molecular genetic basis for phenotypic variation is understood, attempts at classification are essentially arbitrary.

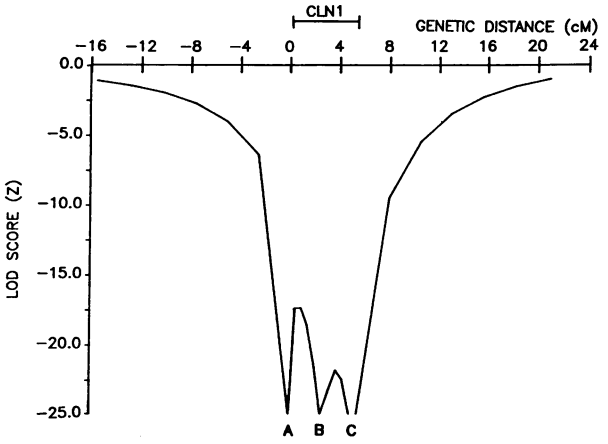

Figure 1 Results of multipoint linkage analysis using the male map of three marker loci on chromosome 1p. A = D1S57; B = HY-TM1; and C = D1S62. Genetic distances are measured from an arbitrary zero at D1S57. Map distance (cM) is calculated by using the Kosambi mapping function. The genetic distances used were as follows: D1S57—2.9 cM—HY-TM1—2.6 cM—D1S62. The current estimate for the position of CLN1 is shown.

Table 3

Pairwise Linkage Results between CLN2 and Chromosome 16 Marker Loci D16S67, D16S296, D16S148, D16S298, and D16S285

	LOD SCORE AT RECOMBINATION FRACTION OF						
	.00	.01	.05	.1	.2	.3	.4
D16S67[a]	−∞	−.04	.47	.54	.41	.21	.06
D16S296	−∞	−5.62	−1.86	−.62	−.08	.14	.05
D16S148	−∞	−2.11	−1.28	−.85	−.40	−.16	−.04
D16S298	−∞	−11.88	−4.93	−2.50	−.78	−.23	−.42
D16S285	−∞	−9.99	−4.66	−2.59	−.93	−.30	−.06

[a] Two-allelic RFLP marker informative only in 40% of families. It is a distant marker and is not in linkage disequilibrium with CLN3 families.

The hallmark of this group of disorders is the accumulation of autofluorescent lipopigment, the ultrastructure of which shows significant variation. Despite intensive investigation their biochemical basis remains unknown. Classification into eponymous subtypes is primarily based on age at onset, although the pattern of neurological progression and ultrastructural findings comprises features which contribute to this classification. It is clear that these entities are not clear cut, and cases occur which are not easy to categorize. Recent observations have provided a further biochemical basis for distinguishing the infantile from late-infantile and juvenile types. Subunit C of mitochondrial ATP synthase accumulates in the latter two varieties, but not in the former (Hall et al. 1991). Both CLN1 and CLN3 have been mapped to well-defined regions by analysis of recombination, and the identification of loci in linkage disequilibrium with each disease locus suggests that these markers are very close.

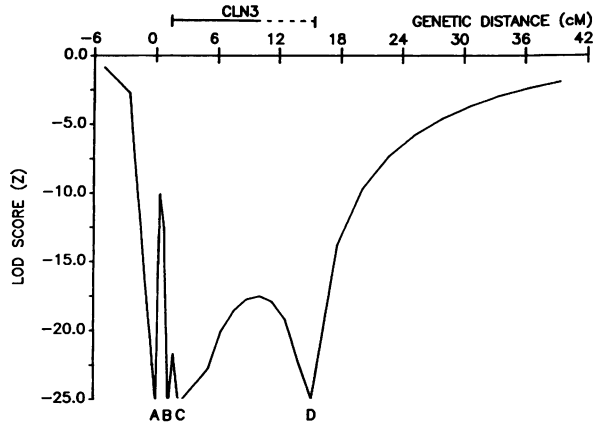

Figure 2 Results of multipoint linkage analysis using the male map of five marker loci on chromosome 16p. A = D16S67; B = D16S296 and D16S148; C = D16S298; and D = D16S285. Genetic distances are measured from an arbitrary zero at D16S67. Map distance (cM) is calculated by using the Kosambi mapping function. The genetic distances used were as follows (M = male; F = female): D16S67—1.2 cM (M), 2.4 cM (F)—D16S296—0.1 cM (M), 2.8 cM (F)—D16S148—1.3 cM (M), 0.057 cM (F)—D16S298—12.5 cM (M), 5.8 cM (F)—D16S285. Current flanking markers for CLN3, as defined by recombination events, are D16S297, which is positioned between D16S296 and D16S148, and D16S57 (Mitchison et al. 1993). The distance between this locus and D16S298 is uncertain, but it is known to lie within the interval D16S298—D16S285. The dashed line gives the maximum possible interval for CLN3, given this map.

The present results clearly demonstrate the existence of nonallelic genetic heterogeneity within this group of disorders. The three childhood subtypes arise from mutant alleles at distinct loci. The nature of the protein products encoded at these loci is of course a matter for speculation, but it can be assumed that they may be subunits of a multimeric enzyme or that they function at separate points of a common metabolic pathway.

Work is in progress to map CLN2 by linkage analysis. Methods now exist which should allow positional cloning of all three disease genes and a complete understanding of the molecular basis of this enigmatic group of disorders.

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