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Carrier Detection of Batten Disease (Juvenile Neuronal Ceroid-Lipofuscinosis)

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Batten disease, or the juvenile form of neuronal ceroid lipofuscinosis, is an autosomal recessive neurodegenerative disorder manifesting with progressive blindness, seizures, and dementia, leading to an early death. The CLN3 locus which is involved in Batten disease had been localized to chromosome 16p11.2. Linkage disequilibrium has been observed between CLN3 and polymorphic microsatellite markers D16S288, D16S299, and D16S298, making carrier detection and prenatal diagnosis by haplotype analysis possible. For the purpose of carrier detection, haplotypes from Dutch Batten patients and their families were constructed. Most patients share the same D16S298 allele, suggesting the presence of a founder effect in the Dutch population. In a large inbred Dutch family, in which Batten disease occurs with high frequency, haplotype analysis has been carried out with high accuracy for carrier detection. © 1995 Wiley-Liss, Inc.

KEY WORDS: Batten disease, carrier detection, haplotype analysis

INTRODUCTION

Batten disease is an autosomal recessive neurodegenerative disorder belonging to the neuronal ceroid lipofuscinoses (NCL), which are characterized by the intracellular accumulation of autofluorescent lipopigments. On the basis of the age at onset, the clinical

manifestations, and the distinct patterns formed by accumulated lipopigments, four major types can be distinguished: infantile NCL or Haltia-Santavuori disease (MIM 256730), late infantile NCL or Jansky-Bielschowsky disease (MIM 204500), juvenile NCL or Batten (Spielmeyer-Vogt) disease (MIM 204200), and adult NCL or Kufs disease (MIM 204300). Batten patients develop progressive blindness, seizures, and dementia, leading to an early death. The clinical diagnosis is confirmed by the presence of fingerprint patterns on ultrastructural examination of different tissues [Brod et al., 1987]. The biochemical defect causing Batten disease still remains unknown.

Linkage of Batten disease locus CLN3 to chromosome 16 was established by Eiberg et al. [1989]. Subsequent linkage analysis placed CLN3 on 16p12 in an 8 cM interval between D16S297 and D16S57 [Mitchison et al., 1993]. CLN3 was observed to be in strong linkage disequilibrium with alleles of the microsatellites D16S288, D16S299, and D16S298. Recently, the map position of CLN3 was refined by recombination to a 2.1 cM interval between D16S288 and D16S383 [Mitchison et al., 1994].

The presence of flanking polymorphic markers closely linked to the CLN3 gene makes carrier detection, presymptomatic diagnosis, and prenatal diagnosis feasible. The frequency of occurrence of Batten disease in the Netherlands is estimated to be 1 in 100,000. In Zeeland (South West Netherlands) clustering of Batten disease occurs in a large inbred family from an isolated Catholic enclave in a predominantly Protestant area. The family is aware of the increased risk for Batten disease. As a consequence, relatives frequently seek genetic counselling. Here, we report the results of carrier detection in this large inbred family.

MATERIAL AND METHODS

Family Material

A total of 25 Dutch families participated in this study, in which 33 Batten patients and 203 relatives

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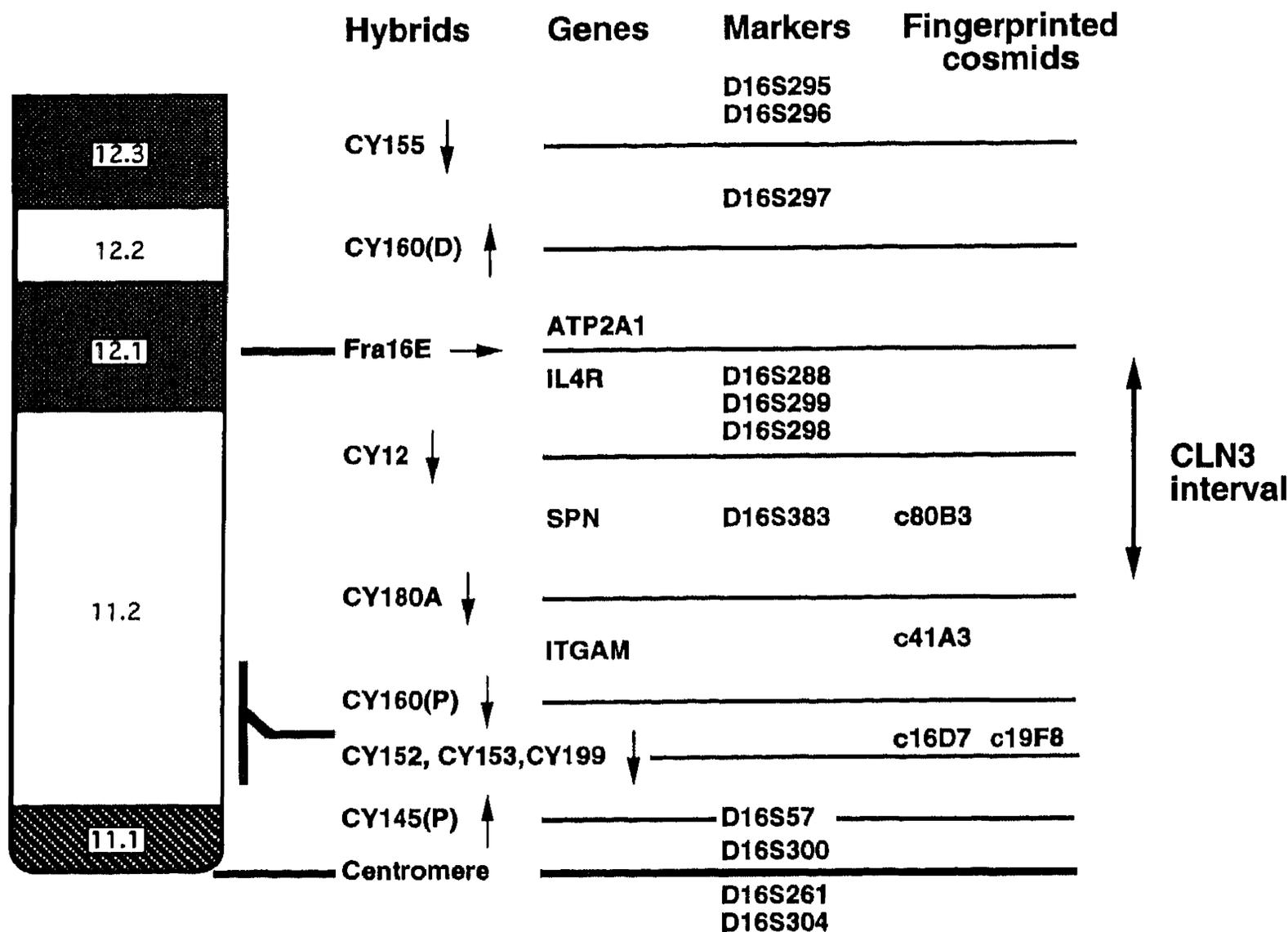


Fig. 1. The CLN3 interval on chromosome 16. The breakpoints of hybrid cell lines containing parts of chromosome 16 are indicated on the left. Arrows point towards the retained part of chromosome 16. Adapted from Hildebrand et al. [1993].

were analyzed. The large inbred family used in the current study has been presented previously [Pinckers and Hoppe, 1978; Pinckers, 1983]. Pedigrees were traced back for at least 5 generations to discover possible relationships. Where possible, grandparents were included for the construction of unambiguous haplotypes.

The diagnosis of Batten disease was established according to the criteria described [Rapola, 1993]. The clinical diagnosis was considered to be confirmed when characteristic fingerprint patterns were observed in lymphocyte inclusion bodies by electron microscopy.

PCR Analysis

Genomic DNA was prepared from peripheral blood samples or from EBV-transformed lymphocyte cell lines by standard procedures [Breuning et al., 1990].

Polymorphic microsatellite markers D16S295, D16S296, D16S297, D16S288, D16S299, D16S298, D16S383, SPN [using new primers described by Mitchison et al. 1994], D16S300, D16S261, and D16S304 were used for haplotype construction [Reeders et al., 1991; Thompson et al., 1992; Hildebrand et al., 1993; Rogaev and Keryanov, 1992; Shen et al., 1991]. PCR was car-

TABLE I. Haplotype Frequencies on Batten Chromosomes in the Dutch Population

Markers			Number of haplotypes	
D16S288	D16S299	D16S298	From independent Batten patients	Deduced from parents of deceased Batten patients
7	5	6	23	10
4	5	6	6	0
8	5	6	6	0
5	5	6	3	1
5	6	6	2	1
3	6	4	4	1
Others			6	1
Total			50	14

TABLE II. Risk Calculations

A) Calculation of prior carrier risk for NCL 1.47^a

	Carrier status				Total risk
	Both NCL 1.49 and NCL 1.50 are carriers	Only NCL 1.49 is a carrier	Only NCL 1.50 is a carrier	Neither NCL 1.49 nor NCL 1.50 are carriers	
Probability	$1/2 \times 1/32 = 1.6\%$	$1/2 \times 31/32 = 48.4\%$	$1/2 \times 1/32 = 1.6\%$	$1/2 \times 31/32 = 48.4\%$	
Prior risk for NCL 1.47 to be—					
Affected	$1/4 \times 1/2 \times 1/32$	0	0	0	0.4%
Carrier	$1/2 \times 1/2 \times 1/32$	$1/2 \times 1/2 \times 31/32$	$1/2 \times 1/2 \times 1/32$	0	25.8%
No carrier	$1/4 \times 1/2 \times 1/32$	$1/2 \times 1/2 \times 31/32$	$1/2 \times 1/2 \times 1/32$	$1/2 \times 31/32$	73.8%

B) Prior risk calculation for offspring from NCL 1.47 and NCL 1.48^b

	Carrier status			Total risk
	Both NCL 1.47 and NCL 1.48 are carriers	NCL 1.47 or NCL 1.48 is a carrier	Neither NCL 1.47 nor NCL 1.48 are carriers	
Probability	$(66/255)^2 = 6.7\%$	$2 \times (66/255) \times (189/255) = 38.4\%$	$(189/255)^2 = 54.9\%$	
Prior risk for offspring to be—				
Affected	$1/4 \times (66/255)^2$	0	0	1.7%
Carrier	$1/2 \times (66/255)^2$	$(66/255) \times (189/255)$	0	22.5%
No carrier	$1/4 \times (66/255)^2$	$(66/255) \times (189/255)$	$(189/255)^2$	75.8%

^aRisk calculations for NCL 1.48 have the same results.

^bThe carrier probability of 66/255 for NCL 1.47 and NCL 1.48 was derived by dividing the total carrier probability (66/256) by the sum of the total probabilities of being carrier and being no carrier (66/256 + 189/256), since the possibility that NCL 1.47 and NCL 1.48 are affected can be excluded.

All carried the D16S288-D16S299-D16S298 haplotype "8 5 6." Haplotype analysis of parents of deceased patients suggests that only two patients carried a different haplotype which was inherited from the parent who was not related to the family (NCL 1.62 in Fig. 2).

Members from this family are aware of the increased risk for Batten disease. The couple NCL 1.47 and NCL 1.48, who are related, have asked for genetic advice concerning Batten disease (see Fig. 2). NCL 1.47 and NCL 1.48 have 4 and 2 cousins suffering from Batten disease, respectively. All parents of NCL 1.47 and NCL 1.48 are related. To simplify the risk calculations, single relationships have been assumed between the parents. Parents NCL 1.49 and NCL 1.53, as siblings of obligate carriers, have a prior carrier probability of at least 50%. Parents NCL 1.50 and NCL 1.54 as second cousins of obligate carriers have a prior carrier probability of at least 3.1% (1/32). These values can be used to calculate the prior carrier risk for their children (Table IIA). The fact that NCL 1.47 and NCL 1.48 are not affected results in a carrier risk of 25.9% (66/255). Therefore, couple NCL 1.47 and NCL 1.48 have a prior risk of 1.7% for an affected child, of 22.5% for a child being a carrier, and of 75.8% for a child who does not inherit the CLN3 mutation (see Table IIB).

Haplotype analysis shows that only father NCL 1.49 of NCL 1.47 is a carrier of the "8 5 6" haplotype. Mother NCL 1.54 of NCL 1.48 carries on one of her chromosomes the "6 6 6" haplotype, which may have arisen by recombination of the "8 5 6" haplotype. NCL 1.47 did not inherit the "8 5 6" haplotype from father NCL 1.49 and NCL 1.48 did not inherit the "6 6 6" haplotype from

mother NCL 1.54. Since the probability of a second unrelated carrier haplotype in this family is estimated to be very low (0.03%), the risk of being a carrier for NCL 1.47 and NCL 1.48 is virtually reduced to the probability of an undetectable double recombination event between the markers flanking CLN3. Since the largest distance between two markers within the CLN3 interval is 0.9 cM, the probability of an undetectable double recombination event between two markers (0.0081%) can be neglected. By contrast, sibling NCL 1.52, who had the same prior carrier risk as NCL 1.47, has inherited the "8 5 6" high risk haplotype from father NCL 1.49. Therefore, the probability of NCL 1.52 not being a carrier is practically reduced to zero.

DISCUSSION

Diagnosis of Batten disease is based on the clinical manifestations and the electron microscopic observation of fingerprint patterns formed by accumulating lipopigments in different tissues. In carriers, no fingerprint patterns can be observed. Therefore, carrier detection for Batten disease has not been possible.

Here, we demonstrate that the recently found allelic association between the CLN3 mutation and polymorphic markers D16S288, D16S299, and D16S298 can be used to offer reliable carrier detection to sibs and to more distant relatives of Batten patients. Carrier detection in more distant relatives is less reliable, due to the increased possibility of recombination. Unfortunately, the allelic association observed between CLN3 and D16S299 or D16S298 is not sufficiently strong to be used for carrier detection among unrelated partners of

known carriers. Although observation of the "7 5 6" high risk haplotype in a partner would strongly suggest that this person is a carrier, the absence of this or of a derived haplotype cannot exclude the carrier status with certainty due to limited information about haplotype frequencies in the Dutch population.

Previously, prenatal diagnosis of Batten disease was performed by electron microscopical screening of chorion villi and fetal tissues for fingerprint patterns [Conradi et al., 1989; Kohlschütter et al., 1989]. Unfortunately, the absence of fingerprints in chorion villi or fetal tissue cannot be considered as conclusive evidence of the absence of Batten disease. DNA-based prenatal diagnosis gives more conclusive evidence: the fetus is affected, carrier or non-carrier depending on its haplotypes. Reliable prenatal diagnosis is possible for new pregnancies of obligate carriers and in consanguineous marriages in the Zeeland cluster of families, when both partners have been identified as carriers by haplotype analysis.

The observed allelic association suggests that the CLN3 gene is in close vicinity of markers D16S299 and D16S298. Isolation of cDNAs from this region is expected to lead to the isolation of the CLN3 gene, which will allow direct mutation analysis and more reliable counselling in the near future.

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