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


Department of Genetics (P.E.M.T., N.d.V., M.H.B.), Section of Human Genetics, Leiden University, Leiden, Clinical Genetics Center (J.G.P.), Utrecht, Departments of Clinical Genetics (E.J.M.-H., M.H.B.) and Neurology (M.C.B.L.), Erasmus University Rotterdam, Bartimeushage (I.H.), Doorn, Department of Ophthalmology (A.J.L.G.P.), Catholic University Nijmegen, Ophthalmic Research Institute (E.M.B.-W.), Amsterdam, The Netherlands; and Department of Paediatrics (R.M.G.), University College and Middlesex School of Medicine, The Rayne Institute, London, United Kingdom

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-Santaravouri 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, making carrier detection and prenatal diagnosis by haplotype analysis possible. 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...
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-

![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].](image-url)

### Table I. Haplotype Frequencies on Batten Chromosomes in the Dutch Population

<table>
<thead>
<tr>
<th>Markers</th>
<th>Number of haplotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From independent</td>
</tr>
<tr>
<td></td>
<td>Batten patients</td>
</tr>
<tr>
<td>D16S288</td>
<td>7</td>
</tr>
<tr>
<td>D16S299</td>
<td>5</td>
</tr>
<tr>
<td>D16S298</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
</tr>
</tbody>
</table>
ried out on 50 ng genomic DNA in a total volume of 15 μl at a final concentration of 50 mM KCl; 1.5 mM MgCl₂; 200 μM each of dATP, dGTP, dTTP; 0.004 U/μl of SuperTaq (HT Biotechnology Ltd., Cambridge, UK), in the presence of α-32P-dCTP and 6 pmol of each primer. Denaturation was 3 min at 94°C, followed by 27 cycles of amplification with denaturation for 1 min at 94°C, annealing for 2 min at 50°C (D16S297, D16S383, D16S300, D16S261, and D16S304), 55°C (D16S295, D16S296, D16S299, D16S298, and SPN), or 60°C (D16S288), and extension for 1 min at 72°C, with a final extension for 10 min. Twenty microliters of loading buffer was added to the samples before electrophoresis on a 6% denaturing polyacrylamide gel. The gel was autoradiographed for 20 h at −70°C with Kodak XAR5 film.

**Risk Calculation**

Risk calculations were performed as described by Young [1991]. For the sake of simplicity, risk calculations have been performed assuming single consanguineous relationships between the parents.

**RESULTS**

**Allelic Association**

Haplotypes from families with Batten patients were constructed using the markers shown in Figure 1. Allelic association of markers D16S288, D16S299, and D16S298 with the CLN3 locus is also found in the Dutch population (Table I). The D16S288-D16S299-D16S298 haplotype "7 5 6" is found on 23 chromosomes from 25 Batten patients tested. In addition, this haplotype was found on 10 out of 14 chromosomes from parents of deceased Batten patients. The "4 5 6," "8 5 6," and "5 6 5" haplotypes found on 16 chromosomes may be derived from the "7 5 6" haplotype by recombination between D16S288 and D16S299. Recombination between D16S299 and D16S298 may have resulted in the other haplotypes containing D16S298 allele 6 observed on 9 CLN3 chromosomes. Two independent patients are homozygous for D16S298 allele 4. The strong association of the CLN3 mutation with D16S298 allele 6 in almost all Dutch patients suggests that they share a single ancestral mutation. Only 6 out of 64 CLN3 chromosomes carry a different D16S298 allele, suggesting that about 9.4% of the Dutch CLN3 chromosomes contain a different mutation. The frequency of other CLN3 mutations in the Dutch population is estimated to be 0.03% (9.4% of the total CLN3 mutation frequency).

**Carrier Detection**

The linkage disequilibrium between CLN3 and the markers D16S288, D16S299, and D16S298 which is observed in the Dutch population can be used for carrier detection. In a large inbred family from Zeeland, 17 Batten patients are known of whom 7 have been analyzed (NCL 1.2, 1.3, 1.4, 1.5, 1.15, 1.16, 1.17 in Fig. 2).
TABLE II. Risk Calculations

A) Calculation of prior carrier risk for NCL 1.47

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

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

<table>
<thead>
<tr>
<th>Carrier status</th>
<th>Both NCL 1.47 and NCL 1.48 are carriers</th>
<th>NCL 1.47 or NCL 1.48 is a carrier</th>
<th>Neither NCL 1.47 nor NCL 1.48 are carriers</th>
<th>Total risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probability</td>
<td>$\frac{66}{255}^2 = 6.7%$</td>
<td>$\frac{2}{66} \times \frac{66}{255} \times \frac{189}{255} = 38.4%$</td>
<td>$\frac{189}{255}^2 = 54.9%$</td>
<td></td>
</tr>
<tr>
<td>Prior risk for offspring to be</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected</td>
<td>$1/4 \times (\frac{66}{255})^2$</td>
<td>0</td>
<td>0</td>
<td>1.7%</td>
</tr>
<tr>
<td>Carrier</td>
<td>$1/2 \times (\frac{66}{255})^2$</td>
<td>$\frac{66}{255} \times \frac{189}{255}$</td>
<td>0</td>
<td>22.5%</td>
</tr>
<tr>
<td>No carrier</td>
<td>$1/4 \times (\frac{66}{255})^2$</td>
<td>$\frac{66}{255} \times \frac{189}{255}$</td>
<td>$\frac{189}{255}^2$</td>
<td>75.8%</td>
</tr>
</tbody>
</table>

Risk calculations for NCL 1.48 have the same results.

The carrier probability of 66/256 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 Batton disease. The couple NCL 1.47 and NCL 1.48, who are related, have asked for genetic advice concerning Batton disease (see Fig. 2). NCL 1.47 and NCL 1.48 have 4 and 2 cousins suffering from Batton 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 brothers 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 II A). 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 II B).

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 6 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 Batton 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 Batton 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 Batton 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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REFERENCES


