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Chromosome 16 Microdeletion in a Patient with Juvenile Neuronal Ceroid Lipofuscinosis (Batten Disease)

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Summary

The gene that is involved in juvenile neuronal ceroid lipofuscinosis (JNCL), or Batten disease—CLN3—has been localized to 16p12, and the mutation shows a strong association with alleles of microsatellite markers D16S298, D16S299, and D16S288. Recently, haplotype analysis of a Batten patient from a consanguineous relationship indicated homozygosity for a D16S298 null allele. PCR analysis with different primers on DNA from the patient and his family suggests the presence of a cytogenetically undetectable deletion, which was confirmed by Southern blot analysis. The microdeletion is embedded in a region containing chromosome 16–specific repeated sequences. However, putative candidates for CLN3, members of the highly homologous sulfotransferase gene family, which are also present in this region in several copies, were not deleted in the patient. If the microdeletion in this patient is responsible for Batten disease, then we conclude that the sulfotransferase genes are probably not involved in JNCL. By use of markers and probes flanking D16S298, the maximum size of the microdeletion was determined to be ~29 kb. The microdeletion may affect the CLN3 gene, which is expected to be in close proximity to D16S298.

Introduction

Recently, the gene involved in juvenile neuronal ceroid lipofuscinosis (JNCL), or Batten disease—CLN3—has been mapped to chromosome 16p12.1-11.2 (Mitchison et al. 1993). Linkage disequilibrium with polymorphic microsatellite markers D16S288, D16S299, and D16S298 has been observed (Mitchison et al. 1993, 1994; Lerner et al. 1994). The interval of the CLN3 gene is flanked by the markers D16S288 and D16S383 (fig. 1). Further reduction of this interval by linkage analysis requires a large number of families. We set out to collect all patients and their families in The Netherlands.

One of the families was of Moroccan origin. Haplotypes of this family that were constructed with flanking markers were consistent with Mendelian inheritance, with the exception of D16S298. Apparent noninheritance of this marker could be caused by the presence of null alleles. Null alleles for polymorphic microsatellite markers have been observed elsewhere (Phillips et al. 1991; Weber et al. 1991; Callen et al. 1993). In two cases (Phillips et al. 1991; Callen et al. 1993), new primers could amplify the alleles in the family where segregation of a null allele was expected. In the latter case, an 8-bp sequence within the target site had been deleted. Since the presence of a null allele for the marker, which is closest to the CLN3 gene, could indicate the presence of a cytogenetically undetectable chromosomal rearrangement, we decided to analyze this family in more detail.

Patient and Methods

Clinical Evaluation

The patient was the only affected child from Moroccan parents and the second of seven children. He had a normal early youth. At the age of 9 years he was referred because of failing vision (VOD 1/100, VOS 1/150), and a diagnosis of tapetoretinal degeneration was made on the basis of combined ophthalmological and electroretinographic examination. His initial neurological examinations, including electroencephalography and computed-tomography scanning, were normal. He became forgetful shortly after the start of the visual deterioration and had, from the age of 11 years, generalized seizures that were well controlled by valproic acid. More than 1% of the lymphocytes were vacuolated. Electron microscopy showed that part of the vacuoles contained fingerprint material, consistent with JNCL. At the age of 19 years he started to develop a Parkinson syndrome without tremor. At the last examination, at the age of 23 years, he was still able to walk a few steps unsupported, had a friendly mood, and was still able

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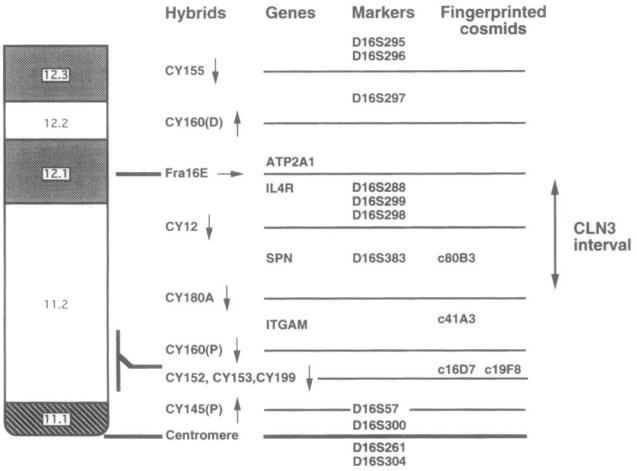

Figure 1 Partial map of the short arm of chromosome 16 (adapted from Hildebrand et al. 1993).

to utter a few meaningful words. There were no other medical complaints.

PCR and Southern Blot Analysis

Genomic DNA of the patient, his parents, and five siblings was isolated from peripheral blood lymphocytes as described elsewhere (Breuning et al. 1990*b*). Fifty nanograms of DNA was used for PCR analysis with sequence-tagged–site (STS) primers 3.12A (5′-GAACCAAAGTCCAGCAGAGC-3′) and 3.12B (5′-AAATAGGTATTGGCCACACCC-3′) and polymorphic microsatellite markers D16S295, D16S296, D16S297, D16S288, D16S299, D16S298, D16S383, SPN, D16S300, D16S261, and D16S304 (Reeders et al. 1991; Shen et al. 1991; Rogaev and Keryanov 1992; Thompson et al. 1992; Hildebrand et al. 1993) (see fig. 1). PCR was carried out in a total volume of 15 μl at a final concentration of 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each of dATP, dGTP, and dTTP, 2.5 μM dCTP, and 0.004 U of SuperTaq (HT Biotechnology)/μl, in the presence of α-^{32}P-dCTP. Primers (synthesized by Isogen Bioscience) were added to a final concentration of 0.33 μM. 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, SPN, and 3.12 STS), or 60°C (D16S288), and extension for 1 min at 72°C. 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. Linkage of CLN3 to the D16S298 locus was analyzed using Linkage (version 5.1) (Lathrop and Lalouel 1984).

Probes were radiolabeled using the Multiprime DNA labeling kit (Amersham) according to the manufacturer's instructions. Southern blots were prepared and hybridized with radiolabeled probes as described by Breuning et al. (1990*a*).

Isolation and Characterization of Clones

PCR screening of superpools, pools, and individual clones from the CEPH YAC library (Albertsen et al. 1990; Cohen et al. 1993) resulted in the isolation of YACs 85D3, 80D12, and 964B10 (D16S298 primers) and YACs 302G12 and 21B11 (D16S299 primers). Filters from a chromosome 16–specific cosmid library (Stallings et al. 1990, 1992) and a human total genomic cosmid library (Blonden et al. 1989) were screened by hybridization to isolate either cosmids containing the 3.12 STS or cosmids extending contig 343 (Stallings et al. 1992), which has been localized within the CLN3 interval. Cosmid DNA was isolated using standard procedures (Sambrook et al. 1989) and digested with *Eco*RI. After separation on agarose gels, fragments were transferred to Hybond N^+ membranes. Membranes with DNA from different cosmids were probed with individual cosmids by competitive hybridization to reveal shared fragments and to construct a contig map. Physical mapping of these clones will be described elsewhere (I. Järvelä, personal communication).

Results

Haplotype Analysis

A patient of Moroccan origin from a consanguineous relationship was diagnosed to have Batten disease. Chromosome 16 markers flanking the CLN3 locus were tested to determine whether the patient had a form of JNCL linked to CLN3 (fig. 2). Results inconsistent with Mendelian inheritance were observed by using marker D16S298. Parent NCL 39.1 seemed to be homozygous for allele 5, while parent NCL 39.2 was homozygous for allele 7. Patient NCL 39.3 did not show any alleles, while his brothers and sisters were also homozygous for either allele 5 or allele 7. The best explanation for these results is that both parents have a null allele for D16S298 (allele 13 in fig. 2) and are hemizygous for either allele 5 or allele 7. The patient has apparently inherited both null alleles, which suggests cosegregation of the null allele with the CLN3 mutation. The odds for linkage of the D16S298 null allele to the CLN3 locus were calculated using a CLN3-mutation frequency of .004 (disease incidence 1/62,500) and a null-allele frequency of .001. The odds for linkage were 63:1 in this family (lod score 1.8), which is suggestive of, but not conclusive for, cosegregation.

Null-Allele Analysis

Null alleles for microsatellite markers can be caused by mutation of (one of) the primer target sites or by deletion of (part of) the amplified region. These two possibilities can be distinguished by designing primers recognizing different target sites. To determine whether

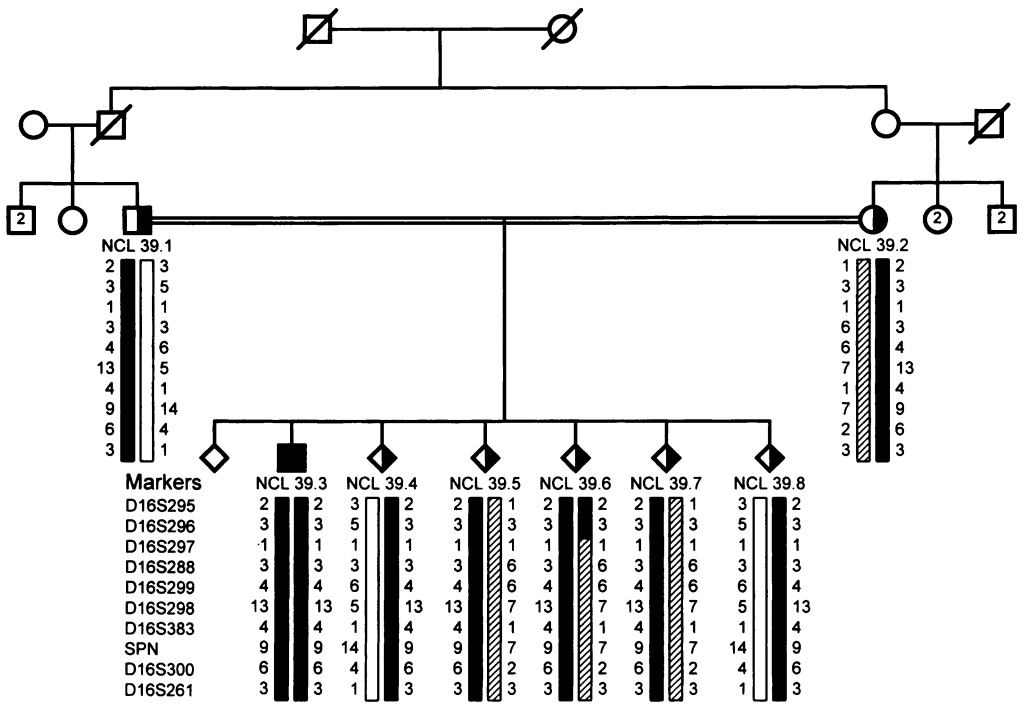

Figure 2 Pedigree of a consanguineous Moroccan family in which Batten disease occurs. Individual haplotypes at polymorphic marker loci flanking the CLN3 locus are shown. D16S298 allele 13 is the null allele.

the null allele is associated with a small mutation, we analyzed the Moroccan family, using the primer combinations shown in figure 3*A*. Close to the D16S298 marker primers, primers for the 3.12 STS were designed. With the 3.12 STS primers, two products were observed in all family members, except the patient, suggesting that two different loci are identified by the 3.12 STS (fig. 3*B*). The patient missed the smaller product, which was the only product amplified on the positive control, the D16S298 containing CEPH Megabase YAC 964BIO. Since the 3.12B primer and the D16S298F primer partly overlap, this could indicate that their target site has been mutated in the patient. By use of the 3.12A primer in combination with the D16S298R primer, a larger product containing the CA repeat is observed in all family members, except the patient, suggesting that at least one of these target sites is also mutated (fig. 3*B*). The possibility that deletion of the primer target sequences causes the absence of PCR products on patient DNA was investigated using a Southern blot with DNA from the patient, his parents, and a healthy control. After competitive hybridization with Cot-1 DNA to prevent intense smearing, the 160-bp 3.12 STS fragment detected faint bands corresponding to *Bam*HI and *Hin*dIII fragments of ~20 kb, which were absent in the patient (fig. 4). The absence of these two fragments and of at least two primer annealing sites is not expected to be a mere coincidence but suggests the presence of a deletion in the patient.

Candidate Gene

Recently, the gene for phenol sulfotransferase (STP) was mapped to 16p12.1-11.2 (Dooley et al. 1993). The STP gene was suggested as a candidate gene for CLN3, on the basis of expression of STP in the brain, the ability of the enzyme to metabolize lipophilic compounds, and the accumulation of lipophilic compounds in JNCL. Recent cDNA cloning of sulfotransferase genes indicates that at least two closely related genes are present in humans (Wilborn et al. 1993; Zhu et al. 1993*a*, 1993*b*). Two members of the highly homologous sulfotransferase gene family, STP and the related catecholamine sulfotransferase gene STM, map to the Batten disease interval on chromosome 16 (T. P. Dooley, personal communication).

To investigate whether the deletion in the Moroccan patient affects the sulfotransferase genes, Southern blots with DNA from the deletion patient were probed with a 525-bp genomic fragment corresponding to the 5′ end of the STP gene, which detects the sulfotransferase gene family (T. P. Dooley and S. E. Mole, unpublished results). No difference could be detected between the patient and his parents (fig. 5). In the unrelated control C, the 525-bp STP fragment detected an extra, smaller *Hin*dIII fragment, while the largest *Hin*dIII fragment is less intense than in the other lanes. This is caused by the presence of a different allele of the STP *Hin*dIII RFLP, which has been observed by others (Henkel et al., in press). Similar results have been obtained with the STP cDNA probe (data not shown). Comparison of the STP-hybridizing restriction patterns of the patient

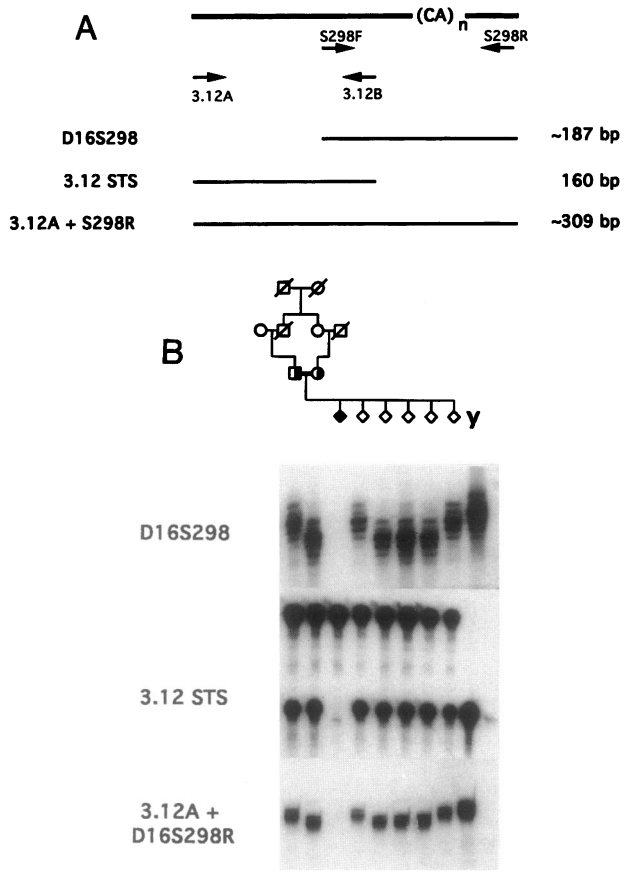

Figure 3 *A*, Schematic representation of primers used to analyze the D16S298 null allele. The size of the PCR products expected is shown. *B*, PCR products obtained on DNA from the Moroccan family by using D16S298 primers, 3.12 STS primers, and a combination of 3.12A and D16S298R primers. YAC 964B10 was used as a positive control.

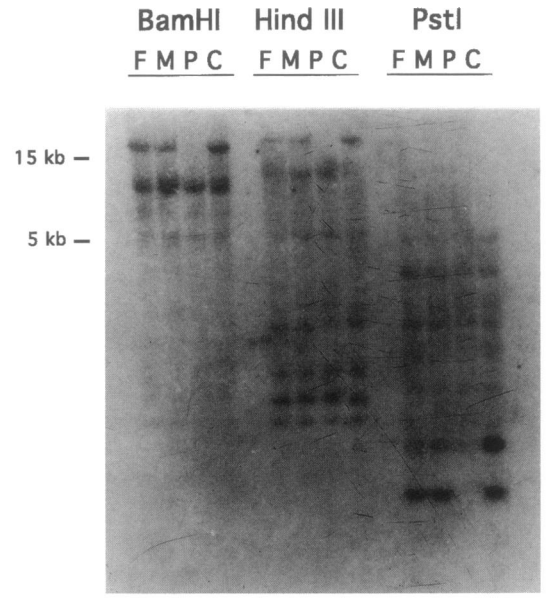

Figure 4 Detection of the deletion in patient DNA by Southern blot analysis with the 160-bp radiolabeled 3.12 STS fragment obtained by PCR on YAC 964B10. DNA from the patient (lanes P), father NCL 39.1 (lanes F), mother NCL 39.2 (lanes M), and healthy control (lanes C) was digested with *Bam*HI, *Hind*III, and *Pst*I. Repeats in the probe were blocked by Cot-1 DNA.

and unaffected individuals indicates that the coding regions of these highly homologous sulfotransferase genes are not involved in the deletion.

Construction of a Cosmid Contig Containing the D16S298 Locus

CEPH YACs 80D12, 85D3, and 964B10, which were positively identified by PCR with D16S298 primers and contain the 3.12 STS, were found to be unstable. Therefore, a gridded subset of a chromosome 16–specific cosmid library and a human total genomic cosmid library were screened with the 3.12 STS product as a probe to obtain clones, which can detect aberrant fragments in the deletion patient. No positive clones were obtained from the chromosome 16–specific cosmid library. One positive clone, NL 11A, obtained from the human total genomic cosmid library, was also positive in the 3.12 STS PCR. Fragments from NL 11A, which were used to screen the cosmid libraries for additional clones, only hybridized to cosmids NL 60D3 and NL 35C1. These clones were already identified during the extension of cosmid contig 343 (Stallings et al. 1992), by cosmid walking using the gridded subset of the chromosome 16–specific cosmid library. Therefore, this contig can be extended with cosmid NL 11A and now contains D16S298 (see fig. 6).

The orientation of the extended cosmid contig 343 has been determined by PCR screening of the cosmids with

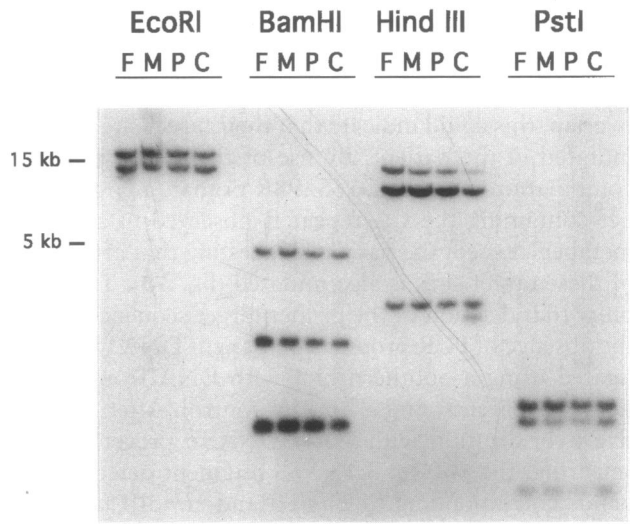

Figure 5 Southern blot analysis of genomic DNA from the patient (lanes P), father NCL 39.1 (lanes F), mother NCL 39.2 (lanes M), and healthy control (lanes C), hybridized with the radiolabeled 5′ 525-bp genomic STP fragment, which detects a highly homologous sulfotransferase gene family of more than two genes.

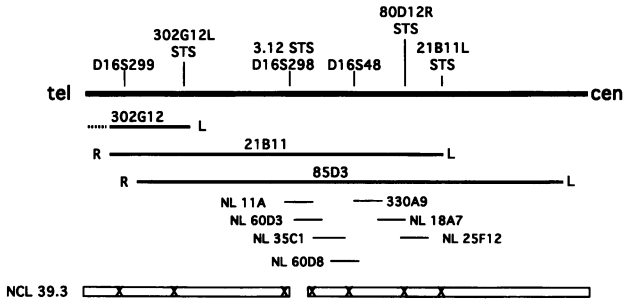

Figure 6 Schematic representation of the maximum size of the deletion in the Moroccan patient NCL 39.3, on the physical map of the distal part of the CLN3 interval. STS and microsatellite markers have been indicated above the CLN3 interval, by the thicker black line; YACs 302G12, 21B11, and 85D3 and the extended cosmid contig 343 have been indicated by thinner black lines; and part of the CLN3 interval detected in the patient with markers (X) has been indicated by the unblackened rectangles.

80D12R and 21B11L STS primers, which were derived from the end clones of the corresponding YACs. The 80D12R end clone has been localized proximal to D16S298 and D16S48. An 80D12R PCR product of the expected size was found with cosmid NL 25F12. Therefore, NL 25F12 represents the proximal side, and NL 11A the distal side, of the extended contig 343.

Extent of the Deletion

The extent of the deletion in the Moroccan patient has been determined by PCR analysis of genomic DNA by using different STS primers and by hybridization with appropriate fragments from contig 343 cosmids (fig. 6). PCR analysis of cosmid NL 11A *Eco*RI fragments indicated that the 3.12 STS is contained within a 29-kb *Eco*RI fragment. The 1.4-kb *Eco*RI fragment and the 2.8-kb *Eco*RI-*Bam*HI fragment of cosmid NL 11A, which flank the 29-kb fragment, detected no aberrant *Eco*RI, *Hin*dIII, *Bam*HI, and *Pst*I fragments in the patient (data not shown). Different parts of the cosmid NL 11A 29-kb *Eco*RI fragment containing the 3.12 STS have been used as probes after competition with Cot-1 DNA to block repeats. Nevertheless, hybridizations with these fragments resulted in smears due to the presence of chromosome 16–specific repeats. Therefore, fragments of aberrant size have not yet been detected in the patient. On the basis of hybridization and PCR results, the maximum size of the deletion is estimated to be ~29 kb, with ~5 kb located distal to, and ~24 kb located proximal to, D16S298.

Discussion

Cytogenetically detectable and submicroscopic deletions along with chromosomal aberrations have played critical roles in the mapping and cloning of increasing numbers of genes. Here, a null allele for D16S298, the closest marker associated with the CLN3 locus, was found in a Batten patient. Subsequent analysis indicated the presence of a microdeletion that could be used for further localization of the CLN3 gene. No clinical manifestations of a contiguous-gene syndrome that suggest the involvement of adjacent genes are apparent. Depending on the density of coding regions in the CLN3 interval, only (part of) the CLN3 gene could be deleted.

Further characterization of this microdeletion is hampered by the presence of chromosome 16–specific repeats. Three blocks of chromosome 16–specific repeats, two on the short arm and one on the long arm, have been identified using FISH (Dauwerse et al. 1992). The CLN3 interval has been localized within the proximal block of repeats on the short arm (N. de Vos, unpublished results). Chromosome 16–specific repeats also interfered with the isolation of the PKD1 gene (The European Polycystic Kidney Disease Consortium 1994).

Present evidence does not exclude the possibility that the microdeletion merely represents a polymorphism within a repetitive region of chromosome 16 in the Moroccan population and leaves the CLN3 gene intact. However, our suggestion that this microdeletion is causing Batten disease could be supported by a Finnish patient found to be heterozygous for the D16S298 null allele (Mitchison et al. 1995 [in this issue]). The probability of the Moroccan patient being homozygous for a microdeletion on chromosome 16, without linkage of the disease to this chromosome, is low.

The presence of deletions in Batten patients is expected to simplify the isolation of the CLN3 gene by reducing the region in which the gene is localized. Additional probes will have to be tested to determine the extent of the deletion. The isolation of cDNA clones, which may be useful single-copy probes, from the deleted region may help to delineate the ends. The characterization of cDNA clones from this region may lead to the identification of both the CLN3 gene and the mutations causing Batten disease.

Acknowledgments

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