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Regional Mapping of the Batten Disease Locus (CLN3) to Human Chromosome 16p12

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Summary

The gene for Batten disease (CLN3) has been mapped to human chromosome 16 by demonstration of linkage to the haptoglobin locus, and its localization has been further refined using a panel of DNA markers. The aim of this work was to refine the genetic and physical mapping of this disease locus. Genetic linkage analysis was carried out in a larger group of families by using markers for five linked loci. Multipoint analysis indicated a most likely location for CLN3 in the interval between D16S67 and D16S148 ($\hat{Z}$ = 12.5). Physical mapping of linked markers was carried out using somatic cell hybrid analysis and in situ hybridization. A mouse/human hybrid cell panel containing various segments of chromosome 16 has been constructed. The relative order and physical location of breakpoints in the proximal portion of 16p were determined. Physical mapping in this panel of the markers for the loci flanking CLN3 positioned them to the bands 16p12.1→16p12.3. Fluorescent in situ hybridization of metaphase chromosomes by using these markers positioned them to the region 16p11.2-16p12.1. These results localize CLN3 to an interval of about 2 cM in the region 16p12.

Introduction

Batten disease, the juvenile-onset subtype of neuronal ceroid lipofuscinosis, is an autosomal recessive neurodegenerative disease characterized by the accumulation of autofluorescent lipopigment in neurones and other tissues. Genetic linkage studies using "classical" protein markers identified linkage to the haptoglobin locus, allowing assignment of this locus (CLN3) to human chromosome 16 (Eiberg et al. 1989). Further studies, using DNA markers in 42 families, indicated that the most likely map location for CLN3 was in the interval between D16S148 and D16S150 (Gardiner et al. 1990).

The aim of the present work was to further refine the genetic and physical localization of CLN3. Linkage analysis was carried out in a total of 58 families by using an additional DNA marker (CRI-0391, identifying D16S67).

Physical mapping of linked markers was carried out using a mouse-human hybrid cell panel containing various segments of human chromosome 16. This has been constructed from constitutional chromosome 16 rearrangements ascertained in patients by cytogenetic studies (Callen 1986; Callen et al., in press). The relative order and physical location of these breakpoints have been determined by cytogenetic analysis and by mapping genes or anonymous DNA probes by Southern analysis and PCR (Callen et al. 1988, 1989, and in press; Richards et al., in press). In the present study, breakpoints in the proximal portion of the short arm of chromosome 16 were utilized to physically localize the markers which genetically flank CLN3. Fluorescent in situ hybridization provided evidence for local-

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ization to an overlapping region extending more proximally. This combination of genetic and physical mapping has allowed the CLN3 locus to be mapped to chromosome 16p12.

Subjects and Methods

Families

The families used in the present study were (*a*) the two-generation kindreds analyzed elsewhere (Gardiner et al. 1990) and (*b*) an additional 16 families. In total, 97 affected individuals and 70 unaffected siblings were studied. There were three affected siblings in each of five pedigrees, two affected siblings in each of 30 pedigrees, and one affected sibling with one to five unaffected sibling(s) in each of 23 pedigrees. Diagnostic criteria were as described elsewhere (Gardiner et al. 1990).

Genetic Mapping

DNA marker phenotyping was carried out by Southern hybridization using standard methods described elsewhere (Gardiner et al. 1990). Members of these kindreds were typed with four of the markers used in two previous studies: Julier et al. (1990) detected loci D16S159 (by pCJ52-94T1), D16S148 (by pCJ52-95M1), D16S150 (by CJ52-161), and D16S151 (by pCJ52-209M1), and Keith et al. (1990) detected D16S67 (by CRI-0391). The multipoint method was used for linkage analysis (Lathrop et al. 1985). The lod scores were calculated by dividing the location score by 4.6. The map of five marker loci was assumed to be fixed, and the location of CLN3 was allowed to vary over a range large enough to encompass free recombination on either side of the marker cluster. The sex-averaged genetic distance between marker loci was calculated from previously published data and information from the CEPH data base.

Physical Mapping

Construction and characterization of the hybrid cell lines have been described elsewhere (Callen 1986; Callen et al., in press). For the hybrid cell lines CY12, CY165, CY15, and CY13 the portion of chromosome 16 retained in each hybrid consisted of the region from the breakpoint on the short arm to the end of the long arm. The cell line CY160 contained an interstitial deletion of the region 16p11.2→p12.1.

The marker probes used for linkage analysis were examined, in addition to the following: the gene ATP2A, detected by a cDNA probe encoding the human fast-twitch muscle Ca-ATPase (MacLennan et al. 1987), and the gene IL4R, detected by a 670-bp *Pst*I-*Eco*RI fragment of the mouse IL4R clone B4 cDNA. DNA samples isolated from cell hybrids, human leukocytes, and the mouse cell line A9 were analyzed by Southern hybridization according to a method described elsewhere (Hyland et al. 1988). Some probes contained repetitive sequences, and preassociation with an excess of sonicated human DNA was necessary (Sealey et al. 1985). The CR3A gene was mapped by PCR using DNA primer sequences constructed from the known gene sequences (Richards et al., in press). The mouse line A9, the hybrid mouse parent, and CY18, a mouse human hybrid which contains a chromosome 16 as the only human chromosome, provided appropriate controls.

The fragile site FRA16E was expressed in lymphoblastoid cell lines by addition of Hoechst 33258 24 h before harvest (Hori et al. 1988). Location of gene or anonymous DNA probes with respect to this fragile site was carried out by in situ hybridization using tritium-labeled probes according to a method described elsewhere (Pritchard et al., in press).

Competitive in situ hybridization experiments were performed according to the method of Cherif et al. (1990). Metaphase chromosomes from phytohemagglutinin-stimulated blood cells of healthy males were cultured according to the methotrexate-5-bromeodeoxyuridine (BrdU) method. Cosmid probes were labeled by nick translation with bio-11 dUTP and were hybridized. The hybridization signal was revealed with avidin FITC and was amplified according to a method described by Pinkel et al. (1986). The hybridized probes appeared as yellow-green spots on red R-banded chromosomes.

Results

Linkage Analysis

A map summarizing the lod scores calculated for CLN3 at various positions by using multipoint analysis and a fixed map of the five marker loci is shown in figure 1. The analysis suggested that the CLN3 gene is most likely located between D16S148 and D16S67 (maximum lod score 12.50), with 5.0×10^6:1 odds favoring this location over the second-best location.

Somatic Cell Hybrid Analysis

Somatic cell hybrid analysis allowed (*a*) the probes to be physically mapped to six intervals within the

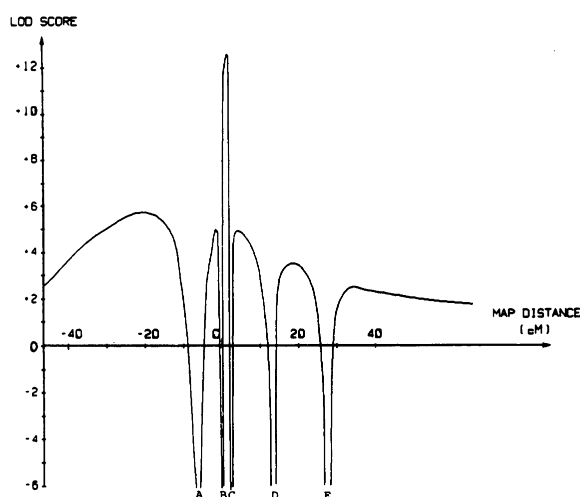

Figure 1 Location map summarizing lod scores calculated for CLN3 at various map positions on fixed marker map of chromosome 16. The loci used were D16S159 (A), D16S67 (B), D16S148 (C), D16S150 (D) and D16S151 (E). D16S67 (CRI-0391) was arbitrarily placed at 0. The sex-combined recombination fractions used were D16S159–.058–D16S67–.023–D16S148–.096–D16S150–.122–D16S151. Map distance in centimorgans was calculated using Haldane's formula.

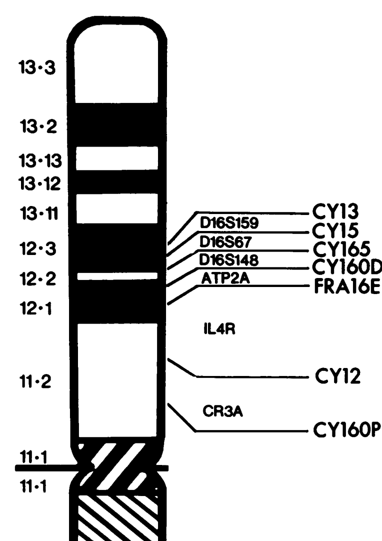

Figure 2 Ideogram of human chromosome 16p, showing approximate location of breakpoints and physical location of marker loci. The interstitial deletion of 16p is indicated by the distal breakpoint (CY160D) and the proximal breakpoint (CY160P).

proximal portion of chromosome 16p (see table 1) and (*b*) their order to be confirmed. The hybrid CY160 contains an interstitial deletion of the short arm of chromosome 16, a deletion which encompasses the genes ATP2A, IL4R, and CR3A, with a distal breakpoint between ATP2A and D16S148. The D16S148 locus is distal to this breakpoint, since it is distal to the CY12 breakpoint. These data enable six different intervals to be distinguished in the region 16p12.3→p11.1, as illustrated in figure 2. The FRA16E site provides an accurate anchor point for this physical map to the chromosome. Therefore, the two probes D16S148 and D16S67, which genetically flank CLN3, are clearly located in the region 16p12.1-p12.3. The hybrid CY165 possesses a breakpoint which is between these two probes. The other two probes, D16S150 and D16S151, which have been shown to be genetically linked to CLN3, map to the proximal half of the long arm of chromosome 16 (data not shown).

The gene ATP2A has been shown by MacLennan et al. (1987) to be localized on chromosome 16. The

Table 1

Results of Somatic Cell Hybrid DNA Analysis: Physical Mapping of Probes to Proximal Portion of Chromosome 16p

	Status at[a]					
Locus (probe)	CY13	CY15	CY165	FRA16E	CY12	CY160
D16S159 (pCJ52-94T1	+	−	−		−	+
D16S67 (pCRI-0391).......	+	+	−		−	+
D16S148 (pCJ52-95MI) ...	+	+	+		−	+
ATP2A	+	+	+	−	−	−
IL4R	+	+	+	+	−	−
CR3A	+	+	+		+	−

[a] A plus sign (+) indicates either that the probe hybridized to the hybrid DNA or that a positive result was obtained using PCR; a minus sign (−) indicates a negative result by either method. For FRA16E, investigated by in situ hybridization, the plus sign indicates hybridization proximal to FRA16E, and the minus sign indicates hybridization distal to this fragile site.

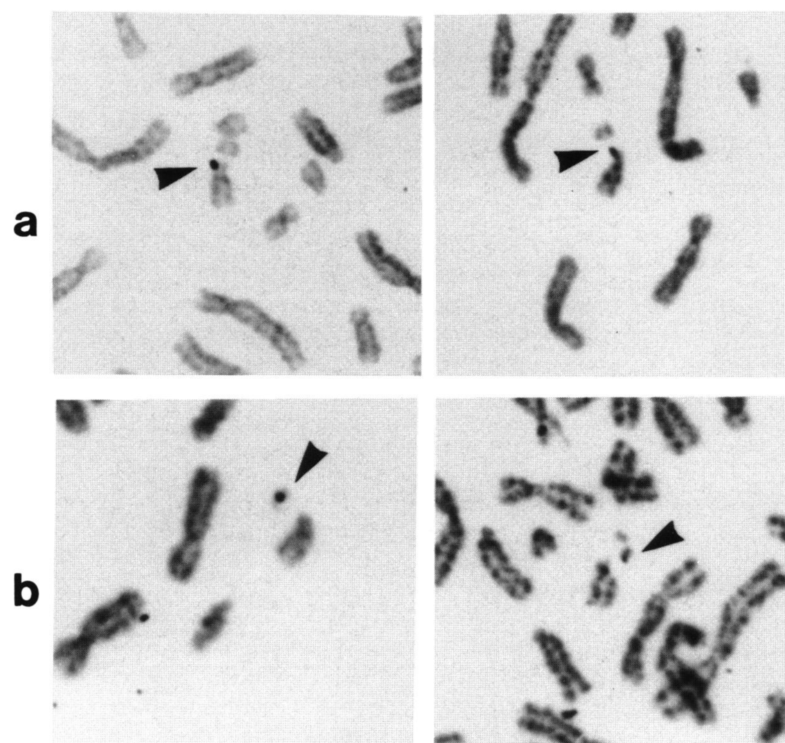

Figure 3 In situ hybridization of tritiated probes to FRA16E: partial metaphases showing expression of the fragile site FRA16E at 16p12.1. *a,* In situ hybridization with probe detecting IL4R locus, showing grains proximal to breakpoint of fragile site. The IL4R locus is proximal to FRA16E. *b,* In situ hybridization with probe detecting ATP2A locus, showing grains distal to breakpoint of fragile site. The ATP2A locus is distal to FRA16E.

present report allows the localization of ATP2A to be refined to the short arm of chromosome 16, in band p12.1-p12.2 just distal to the fragile site FRA16E (fig. 3*a*). The localization of the genes IL4R (fig. 3*b*) and CR3A is consistent with published data (Pritchard et al., in press; Richards et al., in press).

In Situ Hybridization

For all probes a specific signal appeared as twin spots on the two chromosomes 16, in 70%–80% of the metaphases analyzed. The accuracy of the localization is in part related to the degree of elongation of chromosomes, and the most probable localization was chosen in counting a sufficient number of metaphases (table 2). The two cosmid clones CJ52.95 and CRI.0391 which flank CLN3 are localized to the same region, 16p11.2-16p12.1. Simultaneous hybridization of these probes labeled with digoxigenin and biotin and visualized with different fluorophores was used to order them, but their order could not be determined with present data (data not shown). CJ52.161 (which

Table 2

Distribution of In Situ Hybridization Signals Observed in Metaphase Chromosomes by Using Four Cosmid Clones

Probe and Chromosomal Bands	No. of Spots Observed/ Total No. of Metaphase Chromosomes Studied
pCJ52-94:	
16p11.2	9/78 (12%)
16p11.2-p12.1	26/78 (33%)
16p12	43/78 (55%)
CRI-O.391:	
16p11.2	34/56 (61%)
16p11.2-p12.1	22/56 (39%)
CJ52-95:	
16p11.2	55/75 (73%)
16p11.2-p12.1	20/75 (27%)
CJ52-161:	
16q12.1-q12.2	16/65 (25%)
16q12.2	41/65 (63%)
16q13	8/65 (12%)

detects locus D16S150) mapped to the long arm in band 16q12.2.

Discussion

A combination of genetic and physical mapping has allowed CLN3 to be mapped to human chromosome 16p12. Linkage to the haptoglobin locus on the long arm of human chromosome 16 allowed initial localization of CLN3 to this chromosome (Eiberg et al. 1989), and additional analysis using markers for one of the published genetic maps of chromosome 16 (Gardiner et al. 1990) indicated that this disease locus was at a considerable genetic distance from HP. The physical localization of these markers was, however, unknown.

Genetic data have been further refined using additional families and markers from the other available chromosome 16 genetic map (Keith et al. 1990). The data presented here indicate that the most likely location of CLN3 is in the interval between D16S67 and D16S148. The physical location of these markers in the proximal region of the short arm of human chromosome 16 has now been established using somatic cell hybrid analysis and in situ hybridization.

The data summarized in table 1 enable six different regions to be distinguished in the region p11.1→p12.3. It is generally difficult to unequivocally locate breakpoints of chromosome translocations, because of the limitations in interpreting the G-banding pattern at the translocation breakpoint. However, the FRA16E does provide an accurate anchor point for the chromosome, since the position of fragile sites can be determined precisely. These data therefore clearly localize, to bands p121→p12.3, the two probes CRI-0391 (D16S67) and pCJ5295-M1 (D16S148), which flank CLN3.

In situ hybridization using these probes provides results consistent with these observations. Variations in the degree of elongation of chromosomes is, of course, one factor limiting resolution. The distribution of signals observed suggested a more proximal location but encompassed the region to which these probes map by somatic cell hybrid analysis. Together with the somatic cell hybrid data, inability to separate pCJ52-95 and CRI-0391 during simultaneous hybridization suggests that these DNA sequences are located within a region of approximately 3 Mb.

Work in progress is using additional markers mapped to this region, as well as newly available families, to further narrow the genetic interval encompassing CLN3, as a prelude to constitution of a physical map of the CLN3 region. The availability both of YAC libraries of the human genome and of a cosmid contig map covering half of the euchromatin content of chromosome 16 (Stallings et al. 1990) should facilitate work directed toward cloning a region of genomic DNA encompassing CLN3.

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