Am. J. Hum. Genet. 54:88–94, 1994

Linkage Disequilibrium between the Juvenile Neuronal Ceroid Lipofuscinosis Gene and Marker Loci on Chromosome 16p12.1

Terry J. Lerner,*,† Rose-Mary N. Boustany,‖ Kathleen MacCormack,* Jennifer Gleitsman,* Karen Schlumpf,* Xandra O. Breakefield,*,§ James F. Gusella,*,‡ and Jonathan L. Haines*,†

*Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown; †Department of Neurology, ‡Department of Genetics, and §Program in Neuroscience, Harvard Medical School, Boston; and ‖Division of Pediatric Neurology, Duke University Medical Center, Durham, NC

Summary

The neuronal ceroid lipofuscinoses (NCL; Batten disease) are a collection of autosomal recessive disorders characterized by the accumulation of autofluorescent lipopigments in the neurons and other cell types. Clinically, these disorders are characterized by progressive encephalopathy, loss of vision, and seizures. *CLN3*, the gene responsible for juvenile NCL, has been mapped to a 15-cM region flanked by the marker loci *D16S148* and *D16S150* on human chromosome 16. *CLN2*, the gene causing the late-infantile form of NCL (LNCL), is not yet mapped. We have used highly informative dinucleotide repeat markers mapping between *D16S148* and *D16S150* to refine the localization of *CLN3* and to test for linkage to *CLN2*. We find significant linkage disequilibrium between *CLN3* and the dinucleotide repeat marker loci *D16S288* ($\chi^2(7) = 46.5$, $P < .005$), *D16S298* ($\chi^2(6) = 36.6$, $P < .005$), and *D16S299* ($\chi^2(7) = 73.8$, $P < .005$), and also a novel RFLP marker at the *D16S272* locus ($\chi^2(1) = 5.7$, $P = .02$). These markers all map to 16p12.1. The *D16S298/D16S299* haplotype "5/4" is highly overrepresented, accounting for 54% of *CLN3* chromosomes as compared with 8% of control chromosomes ($\chi^2 = 117$, df = 1, $P < .001$). Examination of the haplotypes suggests that the *CLN3* locus can be narrowed to the region immediately surrounding these markers in 16p12.1. Analysis of *D16S299* in our LNCL pedigrees supports our previous finding that *CLN3* and *CLN2* are different genetic loci. This study also indicates that dinucleotide repeat markers play a valuable role in disequilibrium studies.

Introduction

Neuronal ceroid lipofuscinosis (NCL) is the most common neurodegenerative disorder of childhood. Clinically, this disorder is characterized by progressive encephalopathy, loss of vision, and seizures. Histochemically, this group of diseases is characterized by the accumulation of autofluorescent lipopigments within cytosomes found in the neurons and other cell types (Dyken 1988). These cytosomes are generally visualized as hallmark fingerprint or curvilinear bodies (Wisniewski et al. 1988).

Marked clinical heterogeneity is observed in NCL. Four major subtypes are now recognized on the basis of age at onset, clinical presentation, and ultrastructural morphology: infantile NCL (INCL; Haltia-Santavuori disease), found almost exclusively in Finland; late-infantile NCL (LNCL; Jansky-Bielschowsky disease); juvenile NCL (JNCL; Batten disease, Spielmeyer-Vogt disease); and adult NCL (ANCL; Kufs disease). The mean age at onset is 1 year for INCL, 3 years for LNCL, 8 years for JNCL, and 25 years for ANCL (Boustany et al. 1988). Approximately 10% of cases diagnosed as NCL are variant or atypical forms and may represent specific disease entities.

The childhood forms of NCL are inherited as autosomal recessive traits. Based on epidemiological studies, the incidence rate is estimated to be 1–5/100,000 (Rider and Rider 1988; Hofman 1990). This leads to an estimated gene frequency of approximately 1/250 and a carrier frequency of approximately 1/125.

DNA linkage studies have mapped the INCL locus

Received March 23, 1993; revision received September 7, 1993.

Address for correspondence and reprints: Terry Lerner, Ph.D., Molecular Neurogenetics Unit, Massachusetts General Hospital, Building 149, 13th Street, Charlestown, MA 02129.

0002-9297/94/5401-0012$02.00

CLN1 to chromosome 1 in Finnish families (Jarvela et al. 1991) and the JNCL locus *CLN3* to chromosome 16 in European (Eiberg et al. 1989; Gardiner et al. 1990; Callen et al. 1991) and North American (Yan et al. 1993) pedigrees. The LNCL locus *CLN2* has not yet been mapped. Elsewhere (Yan et al. 1993), we have presented data suggesting exclusion of the LNCL defect from the *CLN3* locus.

The fine localization of *CLN3* on chromosome 16 has proved problematic. Linkage with the classical protein marker haptoglobin (16q22) was found with a LOD score of 3 at $\theta = .00$ in males and $\theta = .26$ in females (Eiberg et al. 1989). Multipoint analysis with DNA RFLP markers confirmed the linkage to chromosome 16 (Gardiner et al. 1990) and assigned the gene to a broad region of 15 cM, flanked by *D16S150* and *D16S148*, spanning the centromere. In a subsequent fine mapping study, the same laboratory reported a new localization for the gene, outside this region, in the 2.3-cM interval between *D16S148* and *D16S67* on the short (p) arm (Callen et al. 1991). Using a novel highly polymorphic dinucleotide repeat marker *D16S285* (Konradi et al. 1991), we placed *CLN3* back between *D16S148* and *D16S150* (Yan et al. 1993) and rejected the finer localization suggested by Callen et al. (1991).

The absence both of recombination events and of allelic association between *D16S285* and the disease gene motivated us to type other highly polymorphic markers mapping between *D16S148* and *D16S150*, to refine the localization of *CLN3*. The data set yields significant evidence for strong allelic association (linkage disequilibrium) between *CLN3* and the microsatellite markers at *D16S288* (Shen et al. 1991), *D16S298*, and *D16S299* (Thompson et al. 1992) and an RFLP marker at the *D16S272* locus (Lerner et al. 1992), all residing in 16p12.1 (Callen et al. 1992). We also extend our exclusion of *CLN2* from this region.

Subjects and Methods

NCL Pedigrees

Patients with JNCL and LNCL were identified as described elsewhere (Yan et al. 1993). In all cases, diagnosis was confirmed by a single physician (R.-M.N.B.) using standard criteria (Boustany et al. 1988). A total of 20 JNCL and 8 LNCL pedigrees (as described in Yan et al. 1993), along with 2 additional LNCL pedigrees, were used in the analyses. Seven of the JNCL and five of the LNCL families had at least two affected children. For disequilibrium studies, an additional 16 unrelated affected individuals were included. Eighty-six percent of the JNCL families and 79% of the LNCL families were of northern European origin.

Blood from the patients and all available family members was used to initiate lymphoblastoid cell lines (Anderson and Gusella 1984). DNA was extracted from cell lines or directly from blood samples by using standard methods.

DNA Marker Typing

Analysis of the dinucleotide repeat polymorphisms for *D16S298*, *D16S299*, and *D16S300* was carried out using oligonucleotide primers previously described by Thompson et al. (1992). Primers for the *D16S288* locus were described by Shen et al. (1991), and those for *D16S285* locus were described by Konradi et al. (1991). Reaction volumes were 10 μl and contained 5 ng lymphoblast cell line DNA, 0.2 mM each dATP, dCTP, and dTTP; 2.5 μM dGTP; 4 ng each primer; 0.08 μl (α-^{32}P)dGTP (3,000 mCi/mM); 0.05 μl *Taq* polymerase (Boehringer-Mannheim); and 1 × reaction buffer (Boehringer-Mannheim). For *D16S288*, *D16S298*, *D16S299*, and *D16S300*, the reaction mixture also included 0.1 μl Perfect Match (Stratagene). For all primer sets, annealing was carried out at 55°C. To ensure consistent allele assignment from film to film, three DNA samples exhibiting different alleles for a given marker were included on each gel as controls.

Analysis of the *D16S272* RFLP was done using conventional procedures (Sambrook et al. 1989). The two-allele *Pvu*II RFLP (PIC = .49) at *D16S272* and the corresponding probe 16-129 have been reported to the Genome Data Base (Lerner et al. 1992). The order of the relevant markers is shown in the ideogram of chromosome 16 in figure 1 (Callen et al. 1992).

Linkage Analysis

All linkage analyses were performed using the LINKMAP subprogram of the LINKAGE (version 4.9) package (Lathrop et al. 1984). *CLN3* was modeled as a completely penetrant autosomal recessive trait with an allele frequency of .003. No new-mutation rate was assumed. All individuals used in the analysis were either clearly affected or normal and beyond risk of affection. We had previously observed no recombination events with *D16S285* in these families (Yan et al. 1993), and we observe no new ones here. Since all these markers have been mapped by somatic cell hybrids within the *D16S150–D16S148* interval (Callen et al. 1992), multipoint analysis was unnecessary and was not performed.

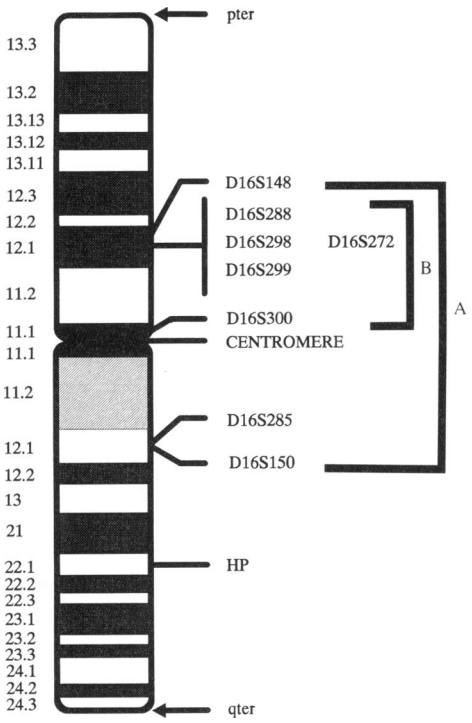

Figure 1 Ideogram of human chromosome 16, showing localization of markers used in this study. The relative order of the markers is based on the genetic linkage map (J. Mulley, personal communication) and the cytogenetic map (Callen et al. 1992) of chromosome 16. *D16S272* has not been genetically mapped but maps to the same subregion of 16p12.1 as do *D16S288*, *D16S298*, and *D16S299*. "A" indicates the *CLN3* candidate region based on recombination analysis, and "B" indicates the *CLN3* candidate region based on disequilibrium analysis.

Linkage Disequilibrium

Linkage disequilibrium was calculated as a χ^2 with correction for continuity. The expected observations were based on the allele or haplotype frequencies of 56 non-*CLN3*-bearing chromosomes in the families, as well as of a set of 60 unrelated control chromosomes (CEPH parents) in the case of *D16S299*. Alleles that were not present in the control population were not included in the χ^2 analysis. The calculations were carried out in two ways: the first included every allele class separately, while the second grouped rare alleles into classes so that no fewer than five observations were expected in any one class. There was little difference in the significance of the results. No differences between the two control populations were observed for *D16S299*, either when all alleles were used ($\chi^2(7) = 6.0$, $P > .50$) or when the rare alleles were collapsed into a single category ($\chi^2(4) = 1.57$, $P = .85$) (table 1).

Table 1

Comparison of Control Populations for *D16S299*

Allele	CEPH Chromosomes	Non-*CLN3* Chromosomes
1	2	4
2	1	2
3	10	12
4	12	8
5	21	22
6	9	8
7	2	0
8	3	0
Total	60	56

For haplotype frequencies, only chromosomes where phase could be determined unambiguously were used.

Results

Linkage Studies with CLN3

We analyzed 20 families with a total of 115 individuals, including 30 patients with JNCL. Table 2 shows the two-point LOD scores for these five markers and for *D16S285* (Yan et al. 1993), with respect to *CLN3*. All markers display significant linkage with *CLN3*, with no recombination (LOD > 3 at $\theta = 0$).

Four marker loci, *D16S288*, *D16S298*, *D16S299*, and *D16S272*, showed significant allelic association with *CLN3* (table 3). For *D16S298*, the "5" allele (180 bp) was overrepresented, despite being the most common allele in the control population. For *D16S299*, the "4" allele (118 bp) was strikingly overrepresented, and

Table 2

Pairwise LOD Scores of Chromosome 16 Markers with NCL

Disease and Marker	LOD Score at θ = .00	.05	.10	.20	.30	.40
JNCL:						
D16S288	8.06	6.95	5.79	3.68	1.81	.50
D16S298	3.98	3.37	2.72	1.62	.78	.20
D16S299	7.50	6.67	5.76	3.74	1.87	.52
D16S272	3.51	3.05	2.54	1.65	.82	.21
D16S300	6.51	5.72	4.91	3.26	1.69	.48
D16S285	7.41	6.35	5.32	3.40	1.72	.47
LNCL:						
D16S299	$-\infty$	−3.25	−1.76	−.60	−.18	−.01
D16S285	$-\infty$	−1.75	−.81	−.15	.02	.02

Table 3

Allelic Association of Chromosome 16 Markers with *CLN3*

	D16S288			*D16S298*			*D16S299*			*D16S272*			*D16S300*			*D16S285*		
		Frequency			Frequency			Frequency			Frequency			Frequency			Frequency	
Allele	No.	Observed	Expected	No.	Observed	Expected	No.	Observed	Expected	No.	Observed	Expected	No.	Observed	Expected	No.	Observed	Expected
0																6	.10	.07
1	1	.01	0	2	.03	.04	4	.05	.05	28	.82	.61						
2				2	.03	.19	1	.01	.02	6	.18	.39				2	.03	.06
3	6	.09	.02	5	.07	.17	8	.11	.19				0	0	.02			
4	16	.24	.41	5	.07	.10	40	.56	.17				3	.04	.04			
5	16	.24	.20	54	.75	.40	17	.24	.37				14	.20	.16			
6	6	.09	.20	3	.04	.06	2	.03	.15				7	.10	.05			
7	9	.13	.07	0	0	.04	0	0	.02				40	.59	.54			
8	2	.03	.02				0	0	.02				1	.01	.04	3	.05	.11
9													3	.04	.13			
10	1	.01	.04	1	.01	0										14	.23	.11
11																		
12	11	.16	.04										0	0	.02	15	.25	.35
13																		
14																8	.13	.10
15																		
16																6	.10	.10
17																		
18																3	.05	.06
19																		
20																3	.05	0
Total	68			72			72			34			68			60		

Table 4

Summary of Allelic Association Data

Marker	χ^2	df	*P*
D16S288	46.5	7	<.005
D16S298	36.6	6	<.005
D16S299	73.8	7	<.005
D16S272	5.7	1	.02
D16S300	8.5	7	.30
D16S285	11.2	8	.20

for *D16S288*, the "12" (142 bp) allele was overrepresented. Although only a simple two-allele system, the RFLP marker at *D16S272* also shows significant disequilibrium with the "1" allele (8 kbp). There was no evidence of allelic association between *CLN3* and alleles at the marker loci *D16S300* and *D16S285*. These data are summarized in table 4.

Further investigation of disequilibrium was performed by examining the haplotypes. As expected, the *D16S298/D16S299* haplotype "5/4" was very highly overrepresented, accounting for 25/46 (54%) of *CLN3* chromosomes as compared with 8% of control chromosomes. The $\chi^2(1)$ value of 117 is highly significant ($P < .0001$). No other haplotype was overrepresented. There is no evidence of linkage disequilibrium between *D16S298* and *D16S299* in the control population, as the predicted equilibrium frequency is 6%. When we extend the haplotype to include *D16S288*, 9 of 42 *CLN3* chromosomes carry the "12/5/4" haplotype. The "12" allele is not observed on any other *CLN3* haplotype.

Exclusion of CLN2 *from the* CLN3 *Candidate Region*

Analysis of *D16S299* in our 10 pedigrees with LNCL excludes linkage of this marker and *CLN2* to 7 cM (LOD = −2.00) (table 2).

Discussion

Localization of CLN3

Numerous "positional cloning" techniques have been developed to move from a linked DNA marker to a gene, and these have now been employed to clone many inherited diseases. With the notable exceptions of cystic fibrosis (Kerem et al. 1989), myotonic dystrophy (Aslanidis et al. 1992), and Huntington disease (Huntington's Disease Collaborative Research Group 1993), these cloning efforts have been aided by the presence of detectable chromosomal deletions or rearrangements in the affected individuals, which served to delineate the target candidate region. In the absence of chromosomal aberrations, genetic recombination mapping has been the primary source of localization information. Since finer localization of *CLN3*, based on classical recombination analysis, has proved not to be feasible in our current collection of families, we have pursued an alternative strategy based on the study of linkage disequilibrium and haplotype analysis.

The strong linkage disequilibrium we observe with the *D16S298/D16S299* haplotype suggests that *CLN3* lies closer to these markers in 16p12.1 than to *D16S288*. *D16S298* and *D16S299* lie approximately 0.2 cM (sex-averaged) apart on the genetic map and about 1 cM from *D16S288* (J. Mulley, personal communication), but their relative order is still unknown. The fact that no disequilibrium exists with *D16S300* indicates that it is an effective proximal boundary for the *CLN3* gene.

Although we cannot draw definitive conclusions, we can speculate that *CLN3* resides most closely to *D16S298*. Since the rare "12" allele at *D16S288* occurs on only the disequilibrium "5/4" haplotype but is seen on only a minority of those chromosomes, it is likely to have recombined, on or off this chromosome, early in the dissemination of *CLN3*. When the non-"5/4" JNCL chromosomes are examined, no suggestion of disequilibrium remains with *D16S288* or *D16S299*, but a nonsignificant increase in the "5" allele is seen with *D16S298*, suggesting that it resides closest to the *CLN3* mutation. Our approach may be complicated by two factors. The absence of linkage disequilibrium between *D16S298* and *D16S299* in the control population suggests that these polymorphisms have been around a long time and that they are relatively stable. The finding of disequilibrium between JNCL and these and other markers in the region supports the notion that the *CLN3* mutation is a relatively recent event and that the dilution of linkage disequilibrium is still minimal. Consequently, the target *CLN3* region may be significantly more than the 500 kbp found, e.g., in Huntington disease (MacDonald et al. 1992), and may encompass 2–3 cM. Although the genetic/physical distance in this region is not well characterized, recombination does seem to be repressed, since the markers *D16S148* and *D16S150* are only 15 cM apart yet span approximately one-third of the chromosome. Physical mapping studies using pulsed field gel electrophoresis are in progress to determine the actual physical distance between the markers *D16S298*, *D16S299*, and *D16S288*.

The detection of allelic and haplotype association between the *CLN3* locus and closely linked markers is consistent with a small number of mutations in JNCL. Our families were collected throughout the United States, but the majority (89%) are of northern European origin and may have arisen from a common founder(s). In our study, all the families with the "5/4" haplotype are of northern European descent. No specific northern European subgroup is overrepresented in our sample.

After we submitted the present paper, Mitchison et al. (1993) reported linkage disequilibrium between *CLN3* and the markers *D16S288, D16S298,* and *D16S299,* in their collection of 70 JNCL families. Our 142-bp *D16S288* "12" allele corresponds to their 142-bp "7" allele; our 180-bp *D16S298* "5" allele to their 180-bp "6" allele; and our 118-bp *D16S299* "4" allele to their 118-bp "5" allele.

The finding of linkage disequilibrium between *CLN3* and the *D16S298/D16S299* haplotype allows us to narrow our search for the JNCL gene. We plan to evaluate linkage disequilibrium between *CLN3* and novel microsatellite markers within this new target region. The generation of an extended haplotype encompassing additional highly informative markers will allow us to localize more precisely the *CLN3* gene.

Because of the somewhat higher mutation rate with microsatellite markers (Kwiatkowski et al. 1993) and the large number of alleles, the usefulness of these markers in disequilibrium studies has been in question. Our results demonstrate that genetic disequilibrium analysis based on microsatellite markers is not only feasible but can also lead to a substantial reduction in the candidate gene region despite a relatively small family set.

Exclusion of CLN2 *from the* CLN3 *Candidate Region*

It had been suspected that LNCL and JNCL might be allelic forms of the same disorder because they share many clinical similarities. In an earlier linkage study of North American LNCL pedigrees with the dinucleotide marker at *D16S285* (Yan et al. 1993), we were able to exclude *CLN2* from most of the *D16S150–D16S148* candidate region, with a small region at the distal end not formally excluded. Since our refined localization of *CLN3* lies at the distal end of this region, we sought to strengthen our result by analysis of a marker in strong disequilibrium with *CLN3*. Our results further support nonallelic heterogeneity for *CLN2* and *CLN3*. A genomewide search is currently underway to localize *CLN2*.

Acknowledgments

This work was supported by the Children's Brain Diseases Foundation, NINDS grant NS24279 (to J.F.G., X.O.B., and J.L.H.), and NIH grant HG00324 (to J.L.H.). We gratefully acknowledge Dr. John Mulley (Adelaide Children's Hospital) for providing genetic map information. We thank Mary Ann Anderson, Heather McFarlane, and Barbara Jenkins for establishing transformed cell lines. We also thank the families and their physicians for their help and cooperation.

References

Anderson MA, Gusella JF (1984) Use of cyclosporin A in establishing Epstein-Barr virus-transformed human lymphoblastoid cell lines. In Vitro Cell Dev Biol 20:856–858

Aslanidis C, Jansen G, Amemiya C, Shutler G, Mahadevan M, Tsifidia C, Chen C, et al (1992) Cloning of the essential myotonic dystrophy region and mapping of the putative defect. Nature 355:548–551

Boustany R-M, Alroy J, Kolodny EH (1988) Clinical classification of neuronal ceroid lipofuscinosis subtypes. Am J Med Genet Suppl 5:47–58

Callen DF, Baker E, Lane S, Nancarrow J, Thompson A, Whitmore SA, MacLennan DH, et al (1991) Regional mapping of the Batten disease locus (CLN3) to human chromosome 16p12. Am J Hum Genet 49:1372–1377

Callen DF, Doggett NA, Stallings RL, Chen LZ, Whitmore SA, Lane SA, Nancarrow JK, et al (1992) High-resolution cytogenetic-based physical map of human chromosome 16. Genomics 13:1178–1185

Dyken PR (1988) Reconsideration of the classification of the neuronal ceroid lipofucinosis. Am J Med Genet Suppl 5:69–84

Eiberg H, Gardiner RM, Mohr J (1989) Batten disease (Spielmeyer-Sjogren disease) and haptoglobin (HP): indication of linkage and assignment to chromosome 16. Clin Genet 36:217–218

Gardiner RM, Sandford A, Deadman M, Poulton J, Cookson W, Reeders S, Jokiaho I, et al (1990) Batten disease (Spielmeyer-Vogt disease, juvenile onset neuronal ceroid lipofuscinoses) gene (*CLN3*) maps to human chromosome 16. Genomics 8:387–390

Hofman IL (1990) The Batten-Spielmeyer-Vogt disease. Bartimeus Foundation Series. Bartimeushage, Doorn, The Netherlands

Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983

Jarvela I, Scheutker J, Haataja L, Santavuori P, Puhakka L, Manninen T, Palotie A, et al (1991) Infantile form of neuronal ceroid lipofuscinosis (*CLN1*) maps to the short arm of chromosome 1. Genomics 9:170–173

Kerem B-S, Rommens JM, Buchanan JA, Markiewicz D, Cox

TK, Chakravarti A, Buchwald M, et al (1989) Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073–1080

Konradi C, Ozelius L, Yan W, Gusella JF, Breakefield XO (1991) Dinucleotide repeat polymorphism on human chromosome 16. Nucleic Acids Res 19:5449

Kwiatkowski DJ, Dib C, Slaugenhaupt SA, Povey S, Gusella JF, Haines JL (1993) An index marker map of chromosome 9 provides strong evidence for positive interference. Am J Hum Genet 53:1279–1288

Lathrop GM, Lalouel JM, Julier J, Ott J (1984) Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Lerner TJ, Wright G, Leverone B, Dackowski W, Shook D, Anderson MA, Klinger K, et al (1992) Molecular analysis of human chromosome 16 cosmid clones containing NotI sites. Mammalian Genome 3:92–100

MacDonald ME, Novelletto A, Lin C, Tagle D, Barnes G, Bates G, Taylor S, et al (1992) The Huntington's disease candidate region exhibits many different haplotypes. Nature Genet 1:99–103

Mitchison HM, Thompson AD, Mulley JC, Kozman HM, Richards RI, Callen DF, Stallings RL, et al (1993) Fine genetic mapping of the Batten disease locus (*CLN3*) by haplotype analysis and demonstration of allelic association with chromosome 16p microsatellite loci. Genomics 16:455–460

Rider JA, Rider DL (1988) Batten disease: past, present, and future. Am J Med Genet Suppl 5:21–26

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Thompson AD, Shen Y, Homan K, Sutherland GR, Callen DF, Richards RI (1992) Isolation and characterization of $(AC)_n$ microsatellite genetic markers from human chromosome 16. Genomics 13:402–408

Wisniewski KE, Rapin I, Heaney-Kieras J (1988) Clinicopathological variability in the childhood neuronal ceroid lipofuscinoses and new observations on glycoprotein abnormalities. Am J Med Genet Suppl 5:27–46

Yan W, Boustany R-MN, Konradi C, Ozelius L, Lerner T, Trofatter JA, Julier C, et al (1993) Localization of juvenile, but not late-infantile, neuronal ceroid lipofuscinosis on chromosome 16. Am J Hum Genet 52:89–95