Eur J Hum Genet 1995;3:326–328

Exclusion Mapping of Classical Late Infantile Neuronal Ceroid Lipofuscinosis (Jansky-Bielschowsky Disease, CLN2)

Julie D. Sharp[a]
Ruth B. Wheeler[a]
Minna Savukoski[b]
Marjo Kestila[b]
Irma E. Järvelä[b]
Leena Peltonen[b]
Hans Eiberg[c]
R. Mark Gardiner[a]
Ruth Williams[a]

[a] Department of Paediatrics, University College London Medical School, The Rayne Institute, London, UK;
[b] Laboratory of Molecular Genetics, National Public Health Institute, Helsinki, Finland;
[c] University Institute of Medical Genetics, Copenhagen, Denmark

The neuronal ceroid lipofuscinoses (NCLs) are a group of neurodegenerative disorders characterised by the accumulation of ceroid- and lipofuscin-like material in neurons and other cell types. There are at least four childhood types, each displaying autosomal recessive inheritance: infantile (MIM 256730, Haltia-Santavuori disease, locus CLN1), classical late-infantile (MIM 204500, Jansky-Bielschowsky disease, locus CLN2), Finnish-variant late-infantile (MIM 256731, variant Jansky-Bielschowsky disease, locus CLN5), and juvenile (MIM 204200, Batten's disease, locus CLN3) NCL. The major clinical features include seizures and visual failure, with progressive mental and motor deterioration. Treatment is symptomatic and the underlying metabolic defect remains unknown. The genes for infantile (CLN1), juvenile (CLN3) and Finnish-variant late-infantile NCL (CLN5) have been mapped to 1p32, 16p12 and 13q, respectively [1–3]. The gene for classical late-infantile NCL is so far unmapped, but has been excluded from these regions [3, 4] and therefore represents a fourth genetic locus. In the absence of other likely candidate regions in which to look for the CLN2 gene, a systematic search of the genome was commenced using highly polymorphic microsatellite markers.

A total of 24 families were studied from eight different countries. There were 10 families with two affected children giving a total of 34 affected individuals with 25 normal siblings. The diagnosis was made according to established criteria [5]. In all cases histology was said to confirm the diagnosis of classical late infantile NCL, and in 22 cases (64%) details of the clinical history, neurophysiology and histology investigation results were available and were known to be typical. In two families, the diagnosis of the second affected child was made on histological evidence only before the onset of symptoms.

A set of highly polymorphic microsatellite markers (heterozygosities >70%) were chosen at 20- to 30-cM intervals across all 22 autosomes. Microsatellite loci were analysed using initial PCR amplification and one of three methods of allele detection. (1) Amplification was carried out with the incorporation of α^{32}P-dCTP and standard detection methods as described previously [4]. (2) 64 Généthon microsatellite markers were typed using the semi-automated method described by Vignal et al. [6]. Amplification and blotting of microsatellites was carried out at the Généthon laboratories and detection was carried out in London. Detection was by hybridisation with either γ^{32}P-dATP-labelled oligonucleotides or by chemiluminescence-based methods using a modification of the ECL procedure (Amersham). (3) Fluorescently labelled primers with an average spacing of 10–20 cM were chosen from the chromosome-specific sets described by Reed et al. [7] and used for further marker typing using an ABI 373A automated sequencer. Pairwise lod scores were calculated between the disease locus CLN2 and each marker locus using the MLINK option of LINKAGE [8].

Received:
July 14, 1995
Accepted:
July 26, 1995

Dr. Julie D. Sharp
Department of Paediatrics
University College London Medical School
The Rayne Institute, University Street
London WC1E 6JJ (UK)

1018–4813/95/
0035–0326$8.00/0

Table 1. Mapping results for CLN2

Chromosome	Number of loci tested	cM excluded	Chromosomal probability	Maximum likelihood
1	14	170	0.01	1.10
2	6	55	3.69	1.07
3	9	90	0.34	1.01
4	3	14	7.29	1.12
5	15	124	24.39	117.16
6	8	105	0.54	1.05
7	16	130	0.18	24.77
8	7	82	0.00	0.99
9	8	28	60.16	17.61
10	9	50	0.15	2.25
11	15	162	0.00	2.28
12	12	102	0.24	8.22
13	6	40	0.82	1.75
14	7	53	0.63	1.59
15	6	57	0.75	3.06
16	8	111	0.00	1.33
17	7	80	0.00	2.10
18	7	66	0.01	7.39
19	4	40	0.15	0.51
20	2	40	0.02	0.41
21	2	20	0.56	0.52
22	2	45	0.05	0.22

Results of these calculations were used as input for the FASTMAP [9] and EXCLUDE [10] programs.

A total of 173 markers have been typed, none of which provides significant evidence for linkage (data not given, but can be provided on request from the authors). Table 1 gives the number of loci typed for each chromosome together with the approximate genetic distance excluded using the standard criterion of $Z \leq -2$ from pairwise lod scores (total excluded: 1,664 cM) and the results of EXCLUDE. Analysis using the summary multipoint maps of each chromosome produced using FASTMAP increases the total genetic distance excluded to 2,460 cM, and suggests that chromosomes 4, 9, and 13 have the largest regions which have not yet been excluded, and that chromosomes 1, 5, 7, 8, 11, 12, 16, 17 and 22 have been almost entirely excluded. Chromosomes 5 and 9 are the most likely locations for CLN2 according to EXCLUDE as they were given high chromosomal probabilities and maximum likelihoods.

Our future strategy will be to investigate the regions which are indicated by FASTMAP and EXCLUDE, in particular those chromosomes which are known to be gene rich [11, 12].

Acknowledgment

This project would not have been possible without the helpful co-operation of the families and their physicians. The financial support of the following is acknowledged: The Wellcome Trust, Medical Research Council (UK), National Institutes of Health, The Research Trust for Metabolic Diseases in Childhood, Action Research, The Sigrid Juselius Foundation, The Academy of Finland, and the Child Brain Disease Foundation. We also thank Professors Pirkko Santavuori and Brian Lake for their clinical advice and Mr. Keith Parker for invaluable technical assistance with the ABI sequencer. Thanks go to Professor John Edwards for providing the EXCLUDE program and to Dr. Alan Young for providing the GAS computer program. We are also grateful to June Davies and John Todd for advice in the use of fluorescently labelled microsatellites.

References

1 Järvelä I, Scleutker J, Haataja L, Santavuori P, Puhakka L, Manninen T, Palotie A, Sandkuijl LA, Renlund M, White R, Aula P, Peltonen L: Infantile form of neuronal ceroid lipofuscinosis (CLN1) maps to the short arm of chromosome 1. Genomics 1991;9:170–173.

2 Callen DF, Baker E, Lane S, Nancarrow J, Thompson A, Whitmore SA, Maclennan DH, Berger R, Cherif D, Järvelä I, Peltonen L, Sutherland GR, Gardiner RM: Regional mapping of the Batten disease locus (CLN3) to human chromosome 16p12. Am J Hum Genet 1991;49: 1372–1377.

3 Savukoski M, Kestila M, Williams R, Jarvela I, Sharp J, Harris J, Santavuori P, Gardiner M, Peltonen L: Defined chromosomal assignment of CLN5 demonstrates that at least four genetic loci are involved in the pathogenesis of human ceroid lipofuscinoses. Am J Hum Genet 1994; 55:695–701.

4 Williams R, Vesa J, Jarvela I, McKay T, Mitchison H, Hellsten E, Thompson A, Callen D, Sutherland G, Luna-Battadano D, Stallings R, Peltonen L, Gardiner M: Genetic heterogeneity in neuronal ceroid lipofuscinosis (NCL): Evidence that the late infantile subtype (Jansky-Bielschowsky disease; CLN2) is not an allelic form of the juvenile or infantile subtypes. Am J Hum Genet 1993;53:931–935.

5 Santavuori P: Neuronal ceroid lipofuscinoses in childhood. Brain Dev 1988;10:80–83.

6 Vignal A, Gyapay G, Hazan J, Nguyen S, Dupraz C, Cheron N, Becuwe N, Tranchant M, Weissenbach J: A non-radioactive multiplex proceedure for genotyping of microsatellite markers; in Adolph KW (ed): Methods in Molecular Genetics. Vol 1: Chromosome and Gene Analysis. San Diego, Academic Press, 1993, pp 211–221.

7 Reed PW, Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough SCL, Kawaguchi Y, Cordell HJ, Balfour KM, Jenkins SC, Powell EE, Vignal A, Todd JA: Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 1994;7:390–395.

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

9 Curtis D, Gurling H: A procedure for combining two-point lod scores into a summary multipoint map. Hum Hered 1993;43:173–185.

10 Edwards JH: Exclusion mapping. J Med Genet 1987;24:539–543.

11 Antonarakis SE: Genome linkage scanning: Systematic or intelligent? Nat Genet 1994;8:211–212.

12 Craig JM, Bickmore WA: The distribution of CpG islands in mammalian chromosomes. Nat Genet 1994; 7:376–382.