Short reports

Rapid diagnostic test for the major mutation underlying Batten disease

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Abstract

Batten disease is the most common progressive neurodegenerative disorder of childhood in western countries. A novel cDNA responsible for Batten disease has recently been identified. We have developed a rapid diagnostic solid phase minisequencing test to detect the major 1.02 kb deletion which is responsible for 81% of affected chromosomes in Batten disease worldwide. In Finland, 90% of Batten chromosomes carry the major deletion owing to the enrichment of the CLN3 gene in the isolated Finnish population.

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Key words: Batten disease; mutation; diagnostic test.

Batten disease (juvenile onset neuronal ceroid lipofuscinosis, Spielmeyer-Vogt) is the most common inherited neurodegenerative disorder of childhood with an incidence of 1:25 000 in western countries. The clinical features include visual failure, seizures, psychomotor deterioration, and premature death. To date, the presence of vacuolated lymphocytes and retinal degeneration have been the most reliable diagnostic criteria.[1] The gene, CLN3, underlying Batten disease is located on chromosome 16p12.1.[2] The cDNA which is defective in Batten patients was recently identified, and it encodes a protein with unknown function.[3] The major mutation underlying Batten disease is a 1.02 kb deletion which removes two exons, and it is carried by 81% of the affected chromosomes from 16 different populations analysed so far.

We have developed a solid phase minisequencing based diagnostic test to identify the major mutation. The method[4] originally developed to detect point mutations was devised to detect the 1.02 kb deletion. The strategy for the method is described in fig 1. Three PCR primers (table 1) were designed according to the previously determined genomic sequence of the CLN3 gene (H M Mitchison, personal communication). The result is expressed as the ratio (R) of radioactivity incorporated in the reaction with the ^{3}H-dCTP defining the mutated allele to that incorporated with the ^{3}3H-dTTP defining the normal allele (table 2). The genotype of each sample is unequivocally determined by the R values, which fall into three distinct categories (tables 2 and 3). The R values obtained were >6 for homozygotes for the mutation, 0.25-0.68 for heterozygotes, and <0.09 for normal subjects (table 3). All patients (n = 37) who were known to carry the "56" haplotype (as defined by the microsatellite markers D16S299 and D16S298 closely linked to the CLN3 gene[5]) carried the 1.02 kb deletion. In addition, two sets of sibs homozygous for the haplotype "46" and "66", respectively, and one patient with an unknown haplotype, were homozygous for the 1.02 kb deletion (table 4). The remaining patients showed R values identical to the carriers, indicating that they carry the major mutation on one of their affected chromosomes and other, rarer mutations on the other chromosome (table 4). Our results show that 80% of the Finnish Batten families carry the major mutation in homozygous form with the remainder being compound heterozygotes. In all, 90% of the Finnish CLN3 chromosomes carry the major deletion.

The test will be informative for more than 80% of cases worldwide.[3] The test also identifies carriers of the deletion and excludes healthy subjects as carriers of the disease. We have recently reported the prenatal diagnosis in a case of Batten disease, in which the 1.02 kb deletion was detected by PCR followed by size analysis of the products.[6] The two methods were compared by analysing 25 samples, representing homozygous and heterozygous genotypes and concordant results were obtained in each case (data not shown). The minisequencing method represents an improvement compared to previously used size based iden-

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Table 1 Primers

Name	Sequence 5' to 3'*	Position on the CLN3 gene
6081	CATTCTGTCACCCTTAGAAGCC	nt 407-387 upstream of the 5' deletion breakpoint
B-51332	GGCTATCAGAGTCCAGATTCCG	nt 51-31 downstream of the 3' deletion breakpoint
B-6302	TGCACCCTTGATGTCTCTGCC	nt 56-36 downstream of the 5' deletion breakpoint
6101	TGACAGGGCGAGACTCCGTC	nt 20-1 upstream of the 5' deletion breakpoint

* The primers were synthesised on an Applied Biosystems 392 DNA Synthesiser. The 5' ends of the primers B-6302 and B-51332 were biotinylated during the synthesis.

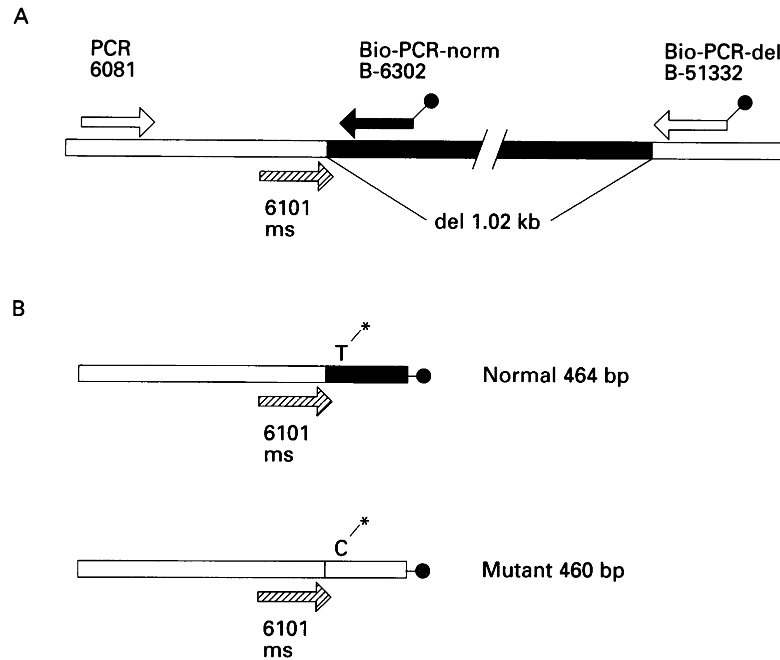

Figure 1 (A) Principle of the minisequencing test. The 5′ sense PCR primer (6081) was located upstream from the 1.02 kb deletion in intron 6 of the CLN3 gene. The biotinylated downstream primer for the normal allele (B-6302) was located within the deletion, and the biotinylated downstream primer for the mutant allele (B-51332) was located immediately 3′ of the deletion. Approximately 25 ng of DNA was amplified in a total volume of 100 µl with the primer B-51332 at 0.2 µmol/l, the primer B-6302 at 0.08 µmol/l, the non-biotinylated primer at 1 µmol/l, and the four dNTPs at 200 µmol/l concentration in 20 mmol/l Tris-HCl, pH 9.3, 1.5 mmol/l $MgCl_2$, 15 mmol/l $(NH_4)SO_2$, 0.1% TritonX-100, and 0.01% gelatin using 1 unit of Dynazyme II DNA polymerase (Finnzymes Ltd, Espoo, Finland). The PCR was initiated by a "hot start", and 33 cycles of one minute at 94°C, one minute at 56°C, and one minute at 72°C were carried out. (B) PCR products obtained from the normal (464 bp) and mutant (460 bp) alleles. Four 10 µl aliquots of the PCR product from each sample and 40 µl of 20 mmol/l sodium phosphate buffer, pH 7.5, 0.15 mol/l NaCl, and 0.1% Tween 20 were incubated at 37°C for 90 minutes in streptavidin coated microtitre wells with gentle shaking. The wells were then washed six times with 350 µl of 40 mmol/l Tris-HCl, pH 8.8, 1 mmol/l EDTA, 50 mmol/l NaCl, and 0.1% Tween20, at 20°C using an automated microtitre plate washer. The wells were treated with 100 µl of 50 mmol/l NaOH for three to five minutes, and washed as above. Fifty microlitres of the minisequencing reaction mixture containing 10 pmol of the detection step primer 6101, 0.1 µCi of either ^{3}H-dCTP (TRK625, 71 Ci/mmol, Amersham) or ^{3}H-dTTP (TRK576, 134 Ci/mmol) and 0.05 unit of Dynazyme II DNA polymerase in the PCR buffer were added to the wells, and the plates were incubated for 10 minutes at 50°C. The wells were washed as above, and 70 µl of 50 mmol/l NaOH were added to each well, and the plates were kept at 20°C for three to five minutes. The eluted radioactivity was measured in a liquid scintillation counter.

Table 2 Example of the detection of the 1.02 kb deletion in the CLN3 gene by solid phase minisequencing

Sample type	Incorporated radioactivity*		Ratio ^{3}dCTP/^{3}dTTP
	^{3}H-dCTP	^{3}H-dTTP	
Homozygous del 1.02 kb	2110	170	12.4
Heterozygous del 1.02 kb	1580	3080	0.51
Homozygous normal	92	3020	0.030

* Mean value of duplicate assays.

tification of the deletion mutation. Firstly, in the minisequencing test PCR products of a similar size are amplified from both alleles. This avoids the risk of preferential amplification of the significantly smaller allele which might result in the mistyping of a heterozygous sample as homozygous mutant.[7] Moreover, an identical minisequencing test can be devised for the detection of point mutations in the CLN3 gene, and hence practically all mutations causing Batten disease can be screened for by the same test. Secondly, the minisequencing method comprises simple manipulations without gel electrophoretic separation steps and the results are obtained as objective numerical cpm values. Thus, the method can easily be automated facilitating computer assisted interpretation and data storage which are technical prerequisites for large scale mutation screening programmes. In prenatal diagnosis, any maternal contamination in a sample using this techique will be detected, as the R value will fall outside the three distinct groups defining the genotypes. The test can be performed in one working day, and a large amount of the costs of demanding neurophysiological and electron microscopic investigations is avoided, which until recently have formed the diagnostic basis for Batten disease.

Table 3 Results of the solid phase minisequencing test of Finnish Batten patients homozygous for the major "56" haplotype, obligate carriers of the "56" haplotype, and controls*

Sample	Ratio ^{3}H-dCTP/^{3}H-dTTP (range of variation)
Patients (n=37)	6.0-31
Obligate carriers (n=50)	0.25-0.68
Controls (n=50)	0.029-0.090

* "56" haplotype formed by markers D16S299 and D16S298.[5]

Table 4 Results of the minisequencing test of Finnish Batten patients carrying other than the major "56" haplotype

Haplotype	Ratio ^{3}H-dCTP/ ^{3}H-dTTP*	Result
46/46	13	Homozygous for 1.02 del
46/46	9.9	Homozygous for 1.02 del
66/66	8.8	Homozygous for 1.02 del
66/66	8.9	Homozygous for 1.02 del
45/56	0.34	Heterozygous for 1.02 del
63/56	0.31	Heterozygous for 1.02 del
66/56	0.31	Heterozygous for 1.02 del
76/56	0.44	Heterozygous for 1.02 del
3 kb del/56	0.52	Heterozygous for 1.02 kb del
ND	0.51	Heterozygous for 1.02 del
ND	0.44	Heterozygous for 1.02 del
ND	6.1	Homozygous for 1.02 del

* Mean values of duplicate assays.
ND = haplotype not determined.

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