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ORIGINAL INVESTIGATION

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Linkage analysis in a Dutch family with X-linked recessive congenital stationary night blindness (XL-CSNB)

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Abstract Linkage analysis has been performed in a large Dutch pedigree with X-linked recessive congenital stationary night blindness (CSNB) by utilizing 16 DNA markers from the proximal short arm of the human X chromosome (Xp21.1–11.2). Thirteen polymorphic markers are at least partially informative and have enabled pairwise and multipoint linkage analysis. For three loci, i. e. DXS228, the monoamine oxidase B gene and the Norrie disease gene (NDG), multipoint linkage studies have yielded maximum lod scores of >3.0 at a recombination fraction of zero. Analysis of recombination events has enabled us to rule out the possibility that the underlying defect in this family is allelic to RP3; the gene defect could also be excluded from the proximal part of the region known to carry RP2. Linkage data are consistent with a possible involvement of the NDG but mutations in the open reading frame of this gene have not been found.

Introduction

The X-linked (XL) form of congenital stationary night blindness (CSNB, McKusick no. 310500) is characterized by abnormal scotopic vision attributable to a presumptive defect of neurotransmission between rods and bipolar cells and is frequently associated with diminished visual acuity, and nystagmus, and with myopia or hyperopia. According to the classification of Miyake et al. (1986), there are two types of X-linked CSNB: (1) The complete form (CSNB1) is characterized by the absence of the b-wave on scotopic testing and, in most cases, is associated with myopia. (2) The incomplete form (CSNB2) is accompanied by either myopia or hyperopia and a reduced scotopic b-wave, indicating some residual rod function. Both types have been described within one family (Khouri et al. 1988, Pearce et al. 1990).

Linkage analysis has mapped the CSNB gene to the proximal short arm of the human X chromosome, in the vicinity of DXS7 and the monoamine oxidase A (MAOA) gene (Gal et al. 1989, Musarella et al. 1989, Bech-Hansen et al. 1990, 1992). Cross-overs with DXS426 and MAOA, respectively, observed in a British family, have defined the proximal and distal boundaries of the region carrying the CSNB gene (Aldred et al. 1992).

In order further to refine the mapping interval of this gene, we have performed linkage analyses in a Dutch pedigree with XL recessive CSNB by employing 16 markers from the Xp21–p11 segment. Moreover, we have searched for mutations in the Norrie disease gene (NDG) in one patient from this family to rule out the possibility that CSNB and ND are allelic.

Materials and methods

DNA analysis

Genomic DNA was extracted from 10 ml EDTA-anticoagulated frozen blood (Miller et al. 1988). DNA typing was performed by either restrictio fragment length polymorphisms or the polymerase chain reaction (PCR) of polymorphic dinucleotide/variable number of tandem repeat markers, derived from the GDB (Genome Database). For the DXS1368 marker, primer sequences were provided by A. Meindl, Munich, Germany. Southern blot analysis was carried out on GeneScreenPlus membrane (alkaline method) according to the manufacturer's instructions.

For all primers, the following PCR conditions were employed: 15 µl total reaction volume contained 10 m*M* TRIS pH 9.0, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% (w/v) gelatine, 0.1% Triton X-100, 0.06 µl ^{32}PdCTP (3000 Ci/mmol, Amersham), 0.2 m*M* each dATP, dGTP, dTTP, 2.5 M dCTP, 0.63 pmol of each primer and 0.012 µl Super*Taq* polymerase (HT Biotechnology. An initial denaturation for 7 min at 94° C was followed by 35 amplification cycles (1 cycle: 1 min at 94° C, 2 min at 55° C and 3 min at 72° C) and a final elongation for 6 min at 72C.

Direct sequencing of the NDG coding region and the splice sites between exons 2 and 3 was performed on PCR fragments 2B and 3 as described (Berger et al. 1992).

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I

1 2 3 4

AB	DXS1368
CE	DXS538
CB	DMD
BC	CYBB
BA	OTC
BA	DXS228
BB	MAOB
AA	NDG
CA	DXS1003
CB	DXS426
CA	DXS337
CD	DXS255
BA	ALAS2

II

1 2 3 4 5 6 7 8

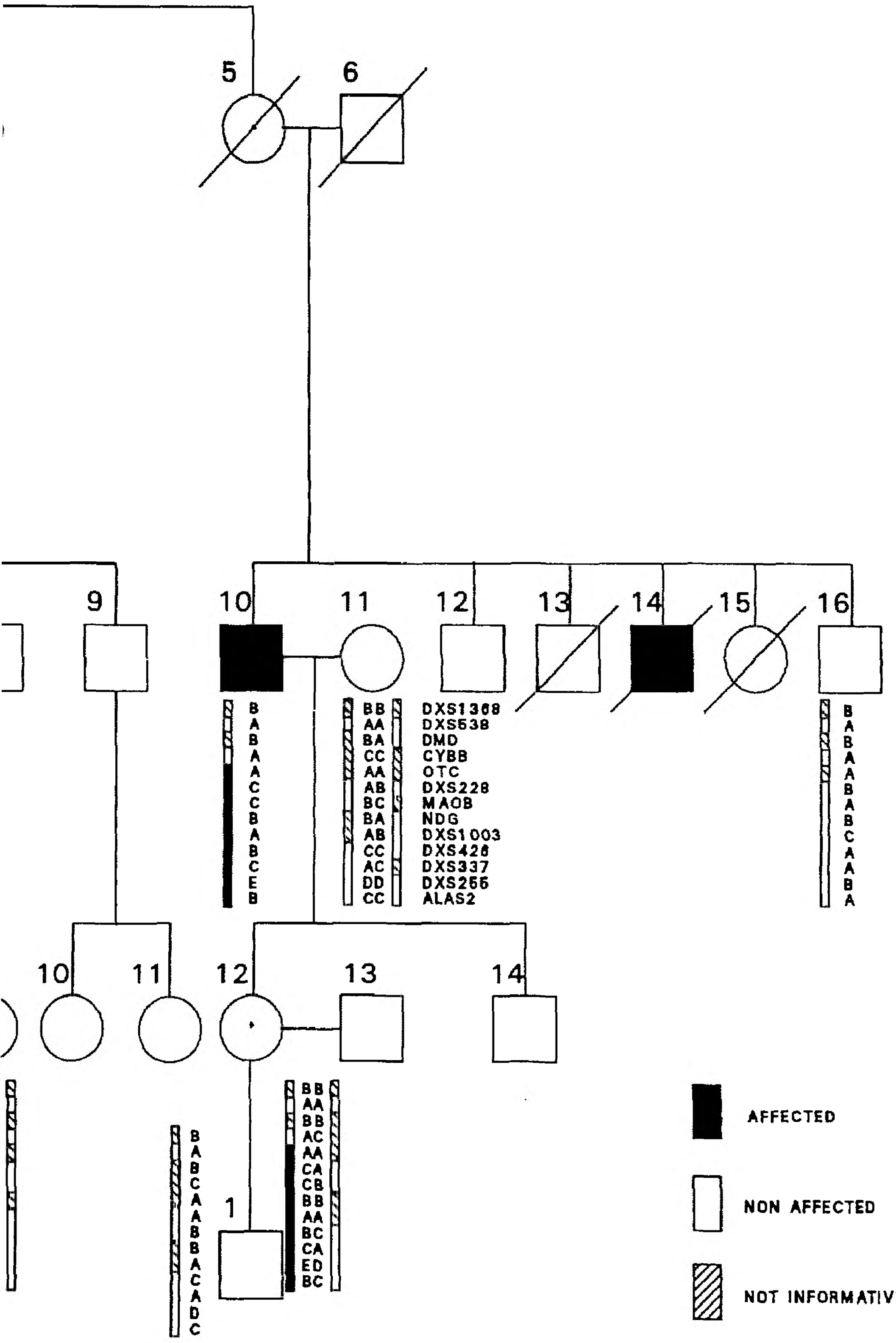
1	2	3	4	5	6	7	8
BA	BA	BA	BA	BB	BB	BB	BA
BC	BC	BC	BC	BE	BE	BE	BA
BC	CB	CB	CB	BB	BB	BB	DA
CB	AB	AB	AB	CC	CC	CC	AB
AB	CB	CB	CB	AA	AA	AA	BB
CB	CB	BA	BA	CA	CA	CA	CB
BA	BA	AC	AC	CB	CB	CB	DA
AC	AC	BC	BC	BA	BA	BA	AA
BC	BC	CC	CC	AA	AA	AA	BB
CC	CC	EC	EC	BB	BB	BB	AB
EC	EC	EB	EB	CA	CA	CA	AB
BB	BB			ED	ED	ED	BC
				BA	BA	BA	AD
							DA
							AC

III

1 2 3 4 5 6 7 8 9

IV

A	DXS1368	A	B	A	A	DXS1368	B	BD	B	BB
C	DXS538	C	B	C	C	DXS538	B	BC	B	EA
C	DMD	C	B	C	C	DMD	B	CA	B	BB
B	CYBB	B	A	B	B	CYBB	A	AA	A	CB
B	OTC	B	C	B	B	OTC	C	CB	C	AA
C	DXS228	B	C	B	B	DXS228	C	CA	C	AD
C	MAOB	B	B	B	B	MAOB	B	BB	B	BA
B	NDG	A	A	A	A	NDG	A	AA	A	AB
A	DXS1003	C	B	C	C	DXS1003	A	BC	B	AB
B	DXS426	C	A	C	C	DXS426	B	CD	C	BC
C	DXS337	C	C	C	C	DXS337	C	ED	E	AD
E	DXS255	C	E	C	C	DXS255	E	BA	B	DA
-	ALAS2	B	B	B	B	ALAS2	B		C	AC


5
6
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11
12
13
14
15
16
B A B A A C C B A B C E B
BB AA BA CC AA AB BC BA AB CC AC DD CC
DXS1368 DXS538 DMD CYBB OTC DXS228 MAOB NDG DXS1003 DXS428 DXS337 DXS255 ALAS2
B A B A B A B C A A B A
10
11
12
13
14
1
B A B C A A B B A C A D C
BB AA BB AC AA CA CB BB AA BC CA ED BC
AFFECTED
NON AFFECTED
NOT INFORMATIVE

Table 1 Peak lod scores of the CSNB locus versus XL markers

θ	0.00	0.01	0.05	0.10	0.20	0.30	0.40
DXS228a	2.19	2.15	2.00	1.81	1.41	0.98	0.51
DXS228b	3.14	3.09	2.88	2.62	2.05	1.43	0.76
MAOB	3.09	3.04	2.84	2.58	2.02	1.41	0.75
ND	2.11	2.08	1.95	1.79	1.43	1.02	0.55

Linkage analysis

Linkage analysis was performed with LINKAGE version 5.1 (MLINK and LINKMAP). The gene frequency was set at 0.00002 and the penetrance at 100%. A two-point linkage was established with MLINK for all informative markers, whereas multipoint linkage studies (with LINKMAP) were restricted to the following loci: OTC, DXS228, MAOB, NDG and DXS1003. The locus order and approximate distances were taken from the consensus map published elsewhere (Schlessinger et al. 1993).

Results

Linkage analysis was performed in a large Dutch pedigree with XL-CSNB by utilizing 16 markers from the proximal short arm of the human X chromosome. The polymorphisms for MAOA, DXS7 and DXS1110 were not informative. No recombination with CSNB was observed for DXS228, MAOB or NDG. Consequently, these loci yielded the highest lod scores (see Table 1). Two crossover events enabled us to define the distal and proximal boundaries of the region containing the CSNB gene. Assuming the locus order Xpter-DMD-CYBB-OTC-DXS228-MAOB-NDG-DXS1003-DXS426-DXS255-Xcen (Schlessinger et al. 1993 and references therein), the recombination between CSNB and the more distal markers OTC, CYBB, DMD, DXS538 and DXS1368 in patient III/1 (see Fig. 1) places the CSNB gene proximal to OTC. Patient III/1 was examined at the age of 15. He had diminished visual acuity and myopia. His electroretinogram (ERG) revealed a reduced b-wave and an absent b2-wave during dark adaptation (Pinckers et al. 1978). The recombination between CSNB and the markers DXS1003, DXS426 and DXS255 in individual III/5 (see Fig. 1) maps the CSNB gene distal to DXS1003. Individual III/5 was born in 1968 and was examined in December 1993. His ERG showed no alterations and he has no visual problems. Recombination was also observed between OTC and (CYBB-DXS538) in patient II/10. Ophthalmological examination, performed in December 1993, of this 72-year-old male revealed nystagmus and myopia. No b2-wave was observed in the ERG during dark adaptation.

Six-point linkage analysis with the markers OTC, DXS228, MAOB, NDG, DXS1003 and the CSNB gene revealed maximum lod scores of 3.29, 3.29 and 3.28 for DXS228, MAOB and NDG, respectively (Fig. 2). Thus, our data establish that the CSNB gene is linked to DXS228, MAOB and NDG. In order to exclude the latter as a possible candidate for XL-CSNB, we have sequenced its protein coding portion in patient III/1. This analysis failed to detect any sequence alterations within the open reading frame (data not shown).

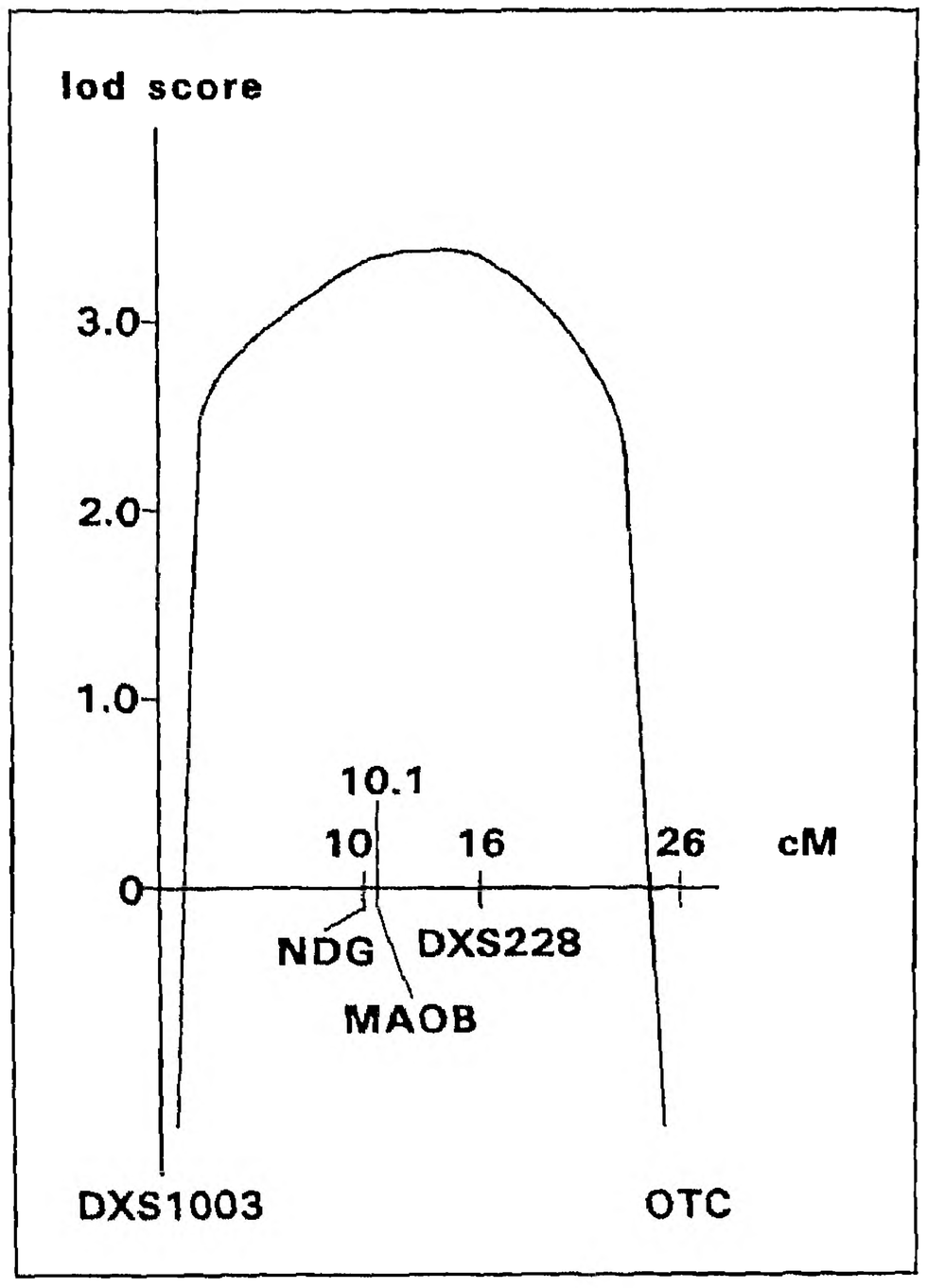

Fig. 2 Curve of the six-point linkage results. Maxima were obtained for the region between DXS228 and NDG

Discussion

Former linkage studies have mapped the sex-linked form of CSNB to the proximal short arm of the human X chromosome (Musarella et al. 1989, Bech-Hansen et al. 1990, 1992, Aldred et al. 1992). In order to refine this localization and to test for heterogeneity, we have performed linkage analysis in a large Dutch pedigree. Peak lod scores at DXS228, MAOB and NDG are consistent with previously reported linkage data, that have defined MAOA and DXS426 as the markers flanking the CSNB gene (Aldred et al. 1992). In our family, crossing over events indicate that the distal and the proximal boundaries of the CSNB gene region are OTC and DXS1003, respectively. Taken together and assuming that our studies and previous reports deal with the same gene, the CSNB locus is flanked distally by MAOA and proximally by the marker DXS1003. Thus, the relevant interval overlaps with the

◀ **Fig. 1** Segregation of DNA haplotypes in a Dutch CSNB family. Recombinations have been detected as follows: patient III/1 with OTC,CYBB,DMD,DXS538 and DXS1368, individual III/5 with DXS1003,DXS426 and DXS255, and patient II/10 with CYBB and DXS538

distal part of the RP2 gene region, which is confined by MAOA and DXS255 (Schlessinger et al. 1993).

Recently, two forms of autosomal CSNB have been shown to be allelic to RP: both CSNB and RP can result from mutations in (1) the rhodopsin gene (Dryja et al. 1993, Rao et al. 1994) and (2) the gene encoding the β-subunit of the rod cGMP phosphodiesterase (Gal et al. 1994). In the light of these findings, it is tempting to speculate that XL-CSNB and RP2 are also allelic. Therefore, the fine mapping of CSNB to the MAOA-DXS1003 interval may have implications for the localization of the RP2 gene.

According to our results, NDG is located in the middle of the CSNB interval. Therefore, at least formally, it is a possible candidate gene for CSNB. Clinically, the two disorders are different but, on the other hand, the clinical picture of patients with mutations in NDG is by no means uniform (Chen et al. 1993) and mild forms of the disease may exist. However, no sequence alterations have been detected within the open reading frame of NDG. Although this result does not entirely exclude the possibility that NDG and CSNB are allelic, these diseases are probably caused by defects in different genes that map to almost the same region of the X chromosone.

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