Localization of CSNBX (CSNB4) Between the Retinitis Pigmentosa Loci RP2 and RP3 on Proximal Xp

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Purpose. Proximal Xp harbors many inherited retinal disorders, including retinitis pigmentosa (RP) and congenital stationary night blindness, both of which display genetic heterogeneity. X-linked congenital stationary night blindness (CSNBX) is a nonprogressive disease causing night blindness and reduced visual acuity. Distinct genetic loci have been reported for CSNBX at Xp21.1, which is potentially allelic with the RP3 gene, and at Xp11.23, which is potentially allelic with the RP2 gene. The study to identify the RP2 gene led to an extended study of families with potentially allelic diseases that include CSNBX.

Methods. Haplotype analysis of a family diagnosed with CSNBX was performed with 17 polymorphic markers on proximal Xp covering previously identified loci for CSNBX and XLRP. Two-point and multipoint lod scores were calculated.

Results. Informative recombinations in this family define a locus for CSNBX (CSNB4) with flanking markers DXS556 and DXS8080 on Xp11.4 to Xp11.3, an interval spanning approximately 5 to 6 cM. A maximum lod score of 3.2 was calculated for the locus order DXS556—1 cM—(CSNB4—DXS993)—2 cM—DXS1201.

Conclusions. The results describe a new localization for CSNBX (CSNB4) between the RP2 and RP3 loci on proximal Xp. CSNB4 is not allelic with any previously reported XLRP loci; however, the interval overlaps the locus reported to contain the cone dystrophy (C0D1) gene, and both diseases are nonrecombinant with DXS995. Because mutations in the RPGR gene to date account for disease in only a small proportion of RP3 families, the possibility that this new locus (CSNB4) also segregates with an as yet unidentified XLRP locus cannot be excluded. Invest Ophthalmol Vis Sci. 1997;38:2750–2755.

Congenital stationary night blindness (CSNB) denotes a series of nonprogressive retinal disorders inherited in an autosomal dominant (adCSNB), autosomal recessive (arCSNB), and an X-linked (CSNBX) form. CSNBX is associated with myopia and decreased visual acuity and is believed to result from defective neurotransmission between photoreceptors and bipolar cells.1–3 Night blindness is also a well characterized early clinical feature of the progressive degenerative retinal disease retinitis pigmentosa (RP), and the functional relationship between RP and CSNB has been highlighted in studies describing mutations in the same gene that result in an RP or a CSNB phenotype. Autosomal dominant CSNB and RP (adRP) are associated with mutations in the rhodopsin (RHO) gene on chromosome 3p,4,5 and mutations in the gene for the phosphodiesterase β-subunit (PDEβ) on chromosome 4p result in autosomal recessive congenital stationary night blindness (CSNB3) or in an autosomal recessive retinitis pigmentosa (arRP).6

Genetic analyses of CSNBX families during the past decade have clearly established the heterogeneity of this disorder, implicating at least two genes on the proximal short arm of the X chromosome (Fig. 1). The first descriptions of genetic mapping for CSNBX suggested close linkage with the locus DXS7, and no evidence for genetic heterogeneity,8–10 similar to the first reports of X-linked retinitis pigmentosa (XLRP).11,12 CSNBX displays phenotypic heterogeneity, with complete and incomplete forms defined by
the presence of slight rod function in the latter. Complete CSNBX (CSNB1) was mapped proximal to DXS7, and this locus was further refined by genetic analysis in families, without further clinical definition, to between the loci MAOA and DXS426 (Xp11.3 to Xp11.23).1314 The map position of this disease indicates potential allelism with one form of XLRP (RP2).15 Subsequently, a family clinically defined as incomplete CSNBX (CSNB2) was described with a key recombination placing the disease proximal to MAOB,16 consistent with the interval assignment for CSNB1 and also potentially allelic with RP2 (Fig. 1).

Intrafamilial phenotypic heterogeneity became apparent when Pearce et al described patients with complete and incomplete forms of the disease within the same pedigree.17 The report of disease in a family with CSNB1 mapping proximal to TIMP-1 (Xp11.23)18 suggested genetic heterogeneity, in that this genetically defined interval overlaps the previously defined CSNBX locus only by approximately 100 kb.19 More recently, evaluation of a single family places CSNBX between the markers OTC and DXS1003,20 a location that significantly overlaps the newly defined RP2 critical interval.21 Genetic heterogeneity was clearly established when the locus for CSNBX described in a clinically heterogeneous family placed the disease in Xp21.1.21 This interval is clearly distinct from other reported locations, bounded by the loci STR44 and DXS228, and is closely linked to the RP3 gene region, providing further evidence for a functional relationship between CSNBX and XLRP.

In this study, we report a new location on proximal Xp for CSNBX (CSNB4), in a single large family, which is not associated with previously described XLRP loci.

**PATIENTS AND METHODS**

**Clinical Assessment**

A diagnosis of CSNBX was based on detailed family history and comprehensive ophthalmic testing at Moorfields Eye Hospital, London, United Kingdom. Only men were affected. The phenotype was typical of the disorder with life-long symptoms of night blindness, reduction of central vision to between 6/12 and 6/36 and high degree of myopia. The electroretinogram (ERG) showed a negative potential without oscillatory potentials to bright white light and was small to dim blue light (Schubert–Bornschein-type ERG). Dark adaptation showed little evidence of a rod–cone break but spectral sensitivity testing demonstrated that rods mediated vision at short wavelength in the dark-adapted state. Thus, the detectable rod function was such that the condition would be classified as incomplete.

**DNA Analysis**

All investigations followed the tenets of the Declaration of Helsinki, and informed consent and full institutional review board approval were obtained. The forward primer for each microsatellite was end-labeled with 32P-γdATP at 37°C for 45 minutes with T4 polynucleotide kinase (New England Biolabs, Hertfordshire, UK). Polymerase chain reaction (PCR) was performed as previously described.22 Alleles were detected by electrophoresing the PCR products on 6% denaturing polyacrylamide gels (Promega, Southampton, UK). Details of primer sequences and PCR conditions for all microsatellites used in this study are available from Genome Database.

**Linkage Analysis**

Two-point linkage analysis for CSNBX and seven markers on proximal Xp was carried out with LINKAGE version 5.1 using MLINK (Columbia University, New York, NY). The frequency of the CSNBX gene in the general population was taken to be 0.0001. Penetrance values for carriers were set at 0.0000. Alleles at marker loci were assumed to have equal frequency, and alteration of frequencies did not affect significant results. ILINK was used to calculate Θmax and Zmax. Multipoint linkage analysis (LINKMAP) with loci order DXS556–DXS995–DXS1201 was performed, using genetic distances of 1 cM and 2 cM, respectively.2225
FIGURE 2. Pedigree of CSNBX family showing haplotypes constructed with 17 microsatellites on proximal Xp, localizing disease to between markers DXS556 and DXS8080. Disease-associated haplotypes are shown as dark bars, and the hatched bar represents markers that were uninformative. One woman of unknown clinical status is included in the pedigree (?), and recombination events are highlighted (*).

RESULTS

Haplotype Analysis

The family was analyzed with 17 polymorphic microsatellite marker loci on proximal Xp, spanning previously identified CSNBX and XLRP loci to create the haplotypes shown in Figure 2. Subject IV-2, an obligate carrier, is recombinant, relative to subjects IV-3, IV-4, and IV-5. This informative recombination locates the disease-associated haplotype proximal to DXS1110. The same crossover is observed in subject V-1, indicating that the recombination observed in IV-2 occurred in generation II of this branch of the family.

The distal boundary of CSNBX in this family is further refined by a recombination event in subject V-7, an unaffected man who has inherited the disease-associated haplotype at DXS556, placing the disease proximal to this locus. Subject V-7 does not have night blindness; his clinical status was reconfirmed recently at Moorfields.

Subject V-3 is recombinant, relative to his carrier mother (IV-2), between the loci DXS8080 and DXS1201 (MAOB and DXS7 are uninformative), with the disease-associated haplotype located distal to DXS8080. The data shown indicate that V-5, a woman of unknown clinical status, is evidently a carrier of CSNBX.

In summary, haplotype data clearly define a locus for CSNBX (CSNB4) between the loci DXS556 and DXS8080 on Xp11.4 to Xp11.3. Haplotypes also demonstrate that DXS1201 and DXS995 cosegregate with disease.

Linkage Analysis

Table 1 describes two-point lod scores between CSNB4 and key marker loci on proximal Xp. The informative
TABLE 1. Two-Point Linkage Analysis Between CSNB4 and Informative Xp Marker Loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>LOD Score at Recombination Fraction of</th>
<th>Z_max</th>
<th>( \theta_{max} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>DXS1242</td>
<td>-∞</td>
<td>-2.06</td>
<td>-0.67</td>
</tr>
<tr>
<td>DXS1110</td>
<td>-∞</td>
<td>0.36</td>
<td>0.91</td>
</tr>
<tr>
<td>DXS556</td>
<td>-∞</td>
<td>0.93</td>
<td>1.46</td>
</tr>
<tr>
<td>DXS993</td>
<td>2.51</td>
<td>2.47</td>
<td>2.29</td>
</tr>
<tr>
<td>DXS1201</td>
<td>1.25</td>
<td>1.25</td>
<td>1.21</td>
</tr>
<tr>
<td>DXS8080</td>
<td>-∞</td>
<td>-0.54</td>
<td>0.08</td>
</tr>
<tr>
<td>DXS1003</td>
<td>-∞</td>
<td>-0.46</td>
<td>0.21</td>
</tr>
</tbody>
</table>

loci chosen represent regions around CSNBX–RP3 (DXS1242,DXS1110), CSNBX–RP2 (DXS8080, DXS1003) and loci in the interval between these regions (DXS556,DXS993,DXS1201). The locus DXS1003, which lies within the RP2–CSNBX interval on Xp11.23 is not linked to CSNB4 (\( Z_{max} = 0.50; \theta_{max} = 0.17 \)). Instead, a significant lod score was obtained with DXS993 (\( Z_{max} = 2.51; \theta_{max} = 0 \)). DXS1110 showed considerably weaker linkage to CSNB4 (\( Z_{max} = 1.03; \theta_{max} = 0.11 \)).

Multipoint analysis was performed to determine the most likely location of CSNB4 in relation to DXS556, DXS993, and DXS1201. A maximum lod of 3.2 was scored for the locus order DXS556–1 cm–(CSNB4–DXS993)–2 cm–DXS1201. A one-lod confidence interval based on these data places the CSNB4 locus in the 24-cM interval proximal to DXS556.

DISCUSSION

CSNBX is known to be phenotypically and genetically heterogeneous, with three locations for the disease on proximal Xp. The most commonly reported locus seems to lie between MAOA and DXS426,14,15 overlapping other locations,16,29 and is consistent with the newly defined locus assignment for RP2.15 Bech-Hansen et al described another location proximal to TIMP-1,14 and a third locus for CSNBX linked to the RP3 gene region was reported by Bergen et al.24 Aland Islands eye disease (AIED), a clinically variant form of CSNBX, has a genetic interval defined by the loci DXS7 and DXS255,24,25,26 which is compatible with the most commonly reported locus assignment for CSNBX.

Haplotype analysis in this study using 17 microsatellites on proximal Xp has revealed a new location for CSNBX (CSNB4) between the loci DXS556 and DXS8080. The genetic interval is estimated to be 5 to 6 cm,22,23 and is not associated with localizations for RP327 or RP2.15

The genetic interval for CSNBX described by Bergen et al,21 combined with new data from a different family28 that show no recombination with the locus DXS1110, suggests the order DMD–DYS1–(DXS1110–CSNBX–XRPRP)–DXS7–DXS1003, which is indicative of potential allelism with RP3. Another group has since reported a mutation in the RP3 gene (RPGR) causing CSNBX, confirming allelism.29 In contrast, multipoint linkage analysis in this study suggests the order DXS556–1 cm–(CSNB4–DXS993)–2 cm–DXS1201 with a maximum lod score of 3.2.

These data, coupled with informative recombinations in the family described here, clearly distinguishes this disease from RP3, RP2, and their associated CSNBX loci.

CSNB4 appears to map to the same interval as X-linked cone-rod dystrophy (CODI).30,31 CODI was subsequently refined to an interval bounded by the loci DXS1058 and DXS1201 in Xp11.4 to Xp11.3,32 with DXS993 showing no crossovers. It should also be noted that DXS993 cosegregates fully without recombination with CSNB4, therefore raising the possibility that CODI and CSNB4 could be allelic variants. Because mutations in Peripherin–RDS can cause cone-rod dystrophy or RP, and as already discussed, mutations in RH O, PDE6, and RPGR can cause CSNB and RP, it seems reasonable to assume that further studies of families with XLRP may reveal a potentially allelic locus with this newly defined 5- to 6-cM interval.

It is particularly interesting to observe that many families with XLRP appear to segregate with the RP3 locus (that is, disease is distal to DXS7 and proximal to other XLRP loci), which is reportedly more common than RP2.33 However, relatively few mutations in the RPGR gene have been discovered to date.27,34 Because it is possible that another gene more proximal to RPGR is causing disease in these families, the potential for this new common interval for various forms of retinal dystrophy (CSNB4 and CODI) also segregating with an as yet undefined XLRP locus can not be excluded.

Key Words

allelic, haplotype analysis, heterogeneity, stationary night blindness, X-linked congenital, X-linked retinitis pigmentosa
Acknowledgments

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