Localization of the Mouse nob (no b-wave) Gene to the Centromeric Region of the X Chromosome

Sophie I. Candille,1 Macbelle T. Pardue,2,3 Maureen A. McCall,4,5 Neal S. Peachey,2,3,6 and Ronald G. Gregg1,5

Purpose. To determine the position on the X chromosome of the gene responsible for a spontaneous mouse mutation, nob (no b-wave), which matches the phenotype of complete X-linked congenital stationary night blindness (CSNB) type 1 in human.

Methods. Inter- and intraspecific pedigrees were generated, and the phenotype of each mouse was scored on the basis of either the presence or the absence of an electroretinographic b-wave. DNA was isolated from a tail biopsy from each mouse and was used to determine the genotype at various polymorphic markers on the X chromosome. LOD scores (Z) between the nob phenotype and each marker were calculated to determine the most probable location of the nob gene.

Results. A total of 174 informative offspring were analyzed. The nob gene is tightly linked to DXMit103 with a maximum LOD score of 25.9 at a recombination fraction of zero. This marker is located at 4.2 cM on the X chromosome of the mouse map. Haplotype analyses of several recombinant chromosomes in the region indicates that the nob gene maps between DXMit54 (3.8 cM) and Ube1x (5.7 cM).

Conclusions. The genetic position of the mouse nob gene overlaps the homologous region in human that contains the locus for CSNB1 and excludes the region of CSNB2. Further studies are planned to identify the mouse nob gene and to evaluate it as a candidate for CSNB1. (Invest Ophthalmol Vis Sci. 1999;40:2748–2751)

The first steps in vision depend on the transduction of light energy into electrical activity in the photoreceptors and the synaptic transmission of that information to the bipolar cells. Considerable progress regarding the biochemistry and the neurobiology of this process has been made, particularly with respect to the transduction events occurring in the photoreceptors.1 This effort has been accelerated by the study of genetic diseases of the retina.

Congenital stationary night blindness (CSNB) refers to a family of retinal disorders that produce a profound loss of rod photoreceptor mediated visual sensitivity. These disorders can be inherited as an autosomal dominant, an autosomal recessive, or an X-linked trait.2 Some forms of CSNB have been shown to result from mutations in genes involved in the rod phototransduction cascade and are characterized by the absence of a rod a-wave in the electroretinogram.3,4 In other forms of CSNB, the electroretinogram (ERG) a-wave is normal; however, the ERG b-wave is selectively diminished.5 Within this latter group, two distinct X-linked forms, complete and incomplete, have been identified in humans.5 In patients with complete X-linked CSNB, it is not possible to measure postreceptoral rod photoreceptor function in the ERG. In comparison, patients with the incomplete form of X-linked CSNB have both modest rod-mediated vision, and postreceptor components are present in the ERG. The complete and incomplete forms of X-linked CSNB also may be distinguished by a number of other clinical characteristics, including nystagmus, myopia, and impaired visual acuity.5 Recent linkage analysis of human X-linked pedigrees indicate that different genes are responsible for the complete and incomplete forms of CSNB.6 In humans, the locus for the incomplete form (CSNB2) is localized to Xp11.23 and is due to mutations in a putative L-type calcium channel.7,8 The locus for the complete form of CSNB (CSNB1), which is located more distally between Xp11.3 and 11.4,6 is yet to be cloned.

Recently, we described a spontaneous X-linked mouse mutation (no b-wave [nob]) that may provide insight into the process of synaptic transmission from photoreceptors to bipolar cells. These mice have normal retinal architecture and a
normal ERG a-wave, but lack the ERG b-wave. Therefore, the phenotype in these mice closely resembles the complete type of X-linked CSNB in humans. Here we report that the nob gene is located near the centromere of the X chromosome in mouse. The minimal region containing the nob gene is homologous to the region in human containing the CSNB1 gene.

**MATERIALS AND METHODS**

**Breeding**

Intra- and interspecific breeding strategies were used to generate mouse pedigrees for linkage analyses. Affected BALB/cByJ nob males were crossed to normal C57BL/6J females purchased from the Jackson Laboratory (Bar Harbor, ME). F1 females, which must be heterozygous (nob/+) for the nob mutation, were then backcrossed to BALB/cByJ nob males. A total of 88 mice from the intraspecific backcross were used in the linkage analysis. To increase the number of polymorphic markers that were informative for linkage analysis, we also used an interspecific cross. Figure 1A illustrates the interspecific pedigree between a BALB/cByJ nob/nob (Mus musculus) female and SPRET/Ei (M. spretus, Jackson Laboratory) male. Backcrossing the F1 heterozygous females to affected BALB/cByJ males yielded 86 interspecific backcross mice.

**Electroretinogram**

Mice were phenotyped at 4 to 5 weeks of age, using the ERG. At this age the ERG has achieved the adult configuration (unpublished observations). Further, the nob defect is apparent as early as postnatal day 18, and mortality associated with the administration of anesthetic agents and the other procedures involved in ERG recording is minimized. After overnight dark adaptation, mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg), and the pupils were dilated. ERGs were recorded (1–1000 Hz) to a high-intensity strobe flash presented in a ganzfeld. Figure 1B shows representative examples of dark-adapted ERGs from a normal and a nob mouse. Affected animals exhibit a normal ERG a-wave and lack the ERG b-wave. The presence of reproducible a- and b-waves was taken as evidence of a normal male or a nob/1 female (Fig. 1B). The ERGs were repeated on all mice that had a recombination event in the region of interest. While the mice were still anesthetized, a small tail biopsy was obtained and frozen for later isolation of DNA. All procedures were approved by the local Institutional Animal Care and Use Committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Mouse Genotyping**

DNA was extracted from each tail biopsy using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Samples were incubated overnight in 400 µl cell lysis solution containing 250 µg/ml proteinase K. The remainder of the protocol was according to the manufacturer’s instructions. For PCR purposes, these DNA solutions were diluted to a concentration of 25 ng/µl.

Thirty-six polymorphic markers, all short, simple sequence repeats of the dinucleotide (CA)_n type, were se-

<table>
<thead>
<tr>
<th>Locus</th>
<th>cM</th>
<th>Recombination fraction (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
<td>0.05</td>
</tr>
<tr>
<td>DXMit55</td>
<td>1.4</td>
<td>–∞</td>
</tr>
<tr>
<td>DXMit26</td>
<td>1.5</td>
<td>–∞</td>
</tr>
<tr>
<td>DXMit161</td>
<td>2.4</td>
<td>–∞</td>
</tr>
<tr>
<td>DXMit103</td>
<td>4.2</td>
<td>25.9</td>
</tr>
<tr>
<td>Ube1x</td>
<td>5.7</td>
<td>–∞</td>
</tr>
<tr>
<td>DXMit50</td>
<td>12.7</td>
<td>–∞</td>
</tr>
<tr>
<td>DXMit140</td>
<td>19</td>
<td>–∞</td>
</tr>
<tr>
<td>DXMit64</td>
<td>45</td>
<td>–∞</td>
</tr>
</tbody>
</table>
lected from the publicly available mouse genetics map (http://www.informatics.jax.org) and primers for each purchased (Research Genetics, Inc., Huntsville, AL). Primers flanking a (CT)$_{23}$(AT)$_{28}$ polymorphic repeat within the Ube1x cDNA (Accession no. U09051) were designed (5' CCCTGGAGCCTAGTTCAGTG 3' and 5' GGAGTCTCTGT-TAGGGAGTA 3'). Polymerase chain reaction (PCR) conditions were optimized for each primer pair. PCR reactions contained (in 25 μl) either 10 pmols (for Ube1x primer pair) or 3.3 pmols (for Research Genetics primer pairs) of each primer, 5 nmoles of each dNTP, 50 ng of genomic DNA, 1.25 U of Taq polymerase, and 1X Taq assay buffer A (Fisher Scientific, Itasca, IL). To genotype the mice, the PCR products were separated by electrophoresis either on 3% agarose gels or, when the allele sizes between the strains were less than 14 bp, on a 6% denaturing polyacrylamide gel. For detection of the products on polyacrylamide gels, one of the primers was end-labeled using [γ-32P]ATP (Amersham, Haywood, CA) and T4 polynucleotide kinase (Promega, Madison, WI).

Two-point LOD scores (Z) were calculated between each marker and the nob phenotype, using the formulas for phase

---

**FIGURE 2.** Haplotypes of recombinant chromosomes. The open and filled boxes represent recombinant and nonrecombinant alleles, respectively, at the markers indicated. These data indicate that the nob gene is located between DXMit54 and Ube1x.

**FIGURE 3.** Homologous regions of the mouse (left) and human X (right) chromosomes, in the region containing the mouse nob gene. Other genes mapped into this region also are indicated. CSNB1 and CSNB2, congenital stationary night blindness type 1 and 2; RP2 and RP3, retinitis pigmentosa 2 and 3; COD1, X-linked cone-rod dystrophy; mRPGR, mouse retinitis pigmentosa GTPase regulator. The marker positions for the mouse and human maps were obtained from databases accessible via the Internet. (mouse, http://www.informatics.jax.org; and human, http://www.ibc.wustl.edu/cgm/cgm.html). The large arrows on the vertical lines representing the chromosomes show the position and orientation of the homologous regions between the two species. The vertical double headed arrows indicate the minimal region containing the locus indicated. The horizontal arrows indicate the position of cloned genes. The numbers next to each vertical line represent the position, in centimorgans for mouse and in megabases for human, of the various markers.
known pedigrees. When either $\theta > 0$ or $\theta = 0$ and $R \neq 0$, we used $Z(\theta) = N(\log 2) + NR[\log(1-\theta)] + R(\log \theta)$, where $\theta$ = the recombination fraction, $N$ = the total number of offspring, and $R$ and $NR$ are the number of recombinant and nonrecombinant chromosomes, respectively. When $\theta = 0$ and $R = 0$, we used $Z(0) = N(\log 2)$. Haplotypes were constructed for chromosomes that had recombination events near the nob critical region.

RESULTS AND DISCUSSION

The intraspecific pedigree, BALB/cByJ $\times$ C57BL/6J, was fully informative for markers DXMit64, DXMit140, and DXMit55, and 38 of 88 offspring were informative for the Ube1x marker. All the markers tested were fully informative for the interspecific (BALB/cByJ $\times$ SPRET/Ei) pedigree.

Linkage was first detected between nob and Ube1x ($Z = 34.8, \theta = 0.01$), which is located at 5.7 cM on the X-chromosome map. Table 1 shows the composite LOD score data for informative markers between 1.4 and 45 cM on the mouse map. The most tightly linked marker is DXMit103 ($Z = 25.9, \theta = 0$), which is positioned at 4.2 cM on the mouse genetic map. To refine the location of the nob locus further, haplotypes for all mice that contained recombinant chromosomes between DXMit26 and DXMit81 were constructed. The results of the recombination events are shown in Figure 2. These data indicate that the nob gene is localized to a 1.9 cM interval between DXMit54 at 3.8 cM and Ube1x at 5.7 cM.

Figure 3 presents a schematic diagram of the region of the mouse X chromosome (left) that contains the nob gene and the homologous region on the human X chromosome (right). This region contains genes for several human retinal disorders, including CSNB1, CSNB2,–8 RP3,10,11 RP2,12,13 (right). This region contains genes for several human retinal disorders, including CSNB1,6 CSNB2,6–8 RP3,10,11 RP2,12,13 (right). This region contains genes for several human retinal disorders, including CSNB1,6 CSNB2,6–8 RP3,10,11 RP2,12,13

The intraspecific pedigree, BALB/cByJ $\times$ C57BL/6J, was fully informative for markers DXMit64, DXMit140, and DXMit55, and 38 of 88 offspring were informative for the Ube1x marker. All the markers tested were fully informative for the interspecific (BALB/cByJ $\times$ SPRET/Ei) pedigree.

Linkage was first detected between nob and Ube1x ($Z = 34.8, \theta = 0.01$), which is located at 5.7 cM on the X-chromosome map. Table 1 shows the composite LOD score data for informative markers between 1.4 and 45 cM on the mouse map. The most tightly linked marker is DXMit103 ($Z = 25.9, \theta = 0$), which is positioned at 4.2 cM on the mouse genetic map. To refine the location of the nob locus further, haplotypes for all mice that contained recombinant chromosomes between DXMit26 and DXMit81 were constructed. The results of the recombination events are shown in Figure 2. These data indicate that the nob gene is localized to a 1.9 cM interval between DXMit54 at 3.8 cM and Ube1x at 5.7 cM.

Figure 3 presents a schematic diagram of the region of the mouse X chromosome (left) that contains the nob gene and the homologous region on the human X chromosome (right). This region contains genes for several human retinal disorders, including CSNB1,6 CSNB2,6–8 RP3,10,11 RP2,12,13 and COD1.14 Several other genes also have been mapped to this region in both human and mouse, which allows the two regions to be aligned. The order of genes between CYBB and TIMP1 in humans is retained in mouse. This allows us to exclude RP2 and RP3 as candidates for nob (Fig. 3). A second, slightly more proximal region in human contains a putative voltage-gated calcium channel gene (CACNA1F), which corresponds to the CSNB2 locus. This gene is 5 kb proximal to the SYP gene.7,8 The homologous location in mouse would place this gene between the centromere and Syp, excluding it as a candidate for nob. In comparison, the intervals that contain the genes for CSNB1 in human and nob in mouse overlap. These data, combined with the electrophysiological and anatomic data reported previously,9 strongly suggest that nob and CSNB1 may result from mutations within the same gene.

The results presented in this report indicate that the nob gene in mouse and the CSNB1 gene in humans are likely to be one and the same. Our ability to produce large numbers of informative meioses should allow us to refine the genetic location of the nob gene, which will facilitate its cloning. The identification of the nob gene will let us investigate whether the nob and CSNB1 phenotypes are due to mutations in the same gene. In addition, the nob mice provide a valuable resource, which will provide insight into another important component of visual transmission between the photoreceptors and the bipolar cells. Finally, nob mice should be a useful model with which to investigate the pathophysiological mechanisms underlying CSNB1.

References