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Evidence for Genetic Heterogeneity in X-Linked Congenital Stationary Night Blindness

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Summary

X-linked congenital stationary night blindness (CSNB) is a nonprogressive retinal disorder characterized by disturbed or absent night vision; its clinical features may also include myopia, nystagmus, and impaired visual acuity. X-linked CSNB is clinically heterogeneous, and it may also be genetically heterogeneous. We have studied 32 families with X-linked CSNB, including 11 families with the complete form of CSNB and 21 families with the incomplete form of CSNB, to identify genetic-recombination events that would refine the location of the disease genes. Critical recombination events in the set of families with complete CSNB have localized a disease gene to the region between DXS556 and DXS8083, in Xp11.4-p11.3. Critical recombination events in the set of families with incomplete CSNB have localized a disease gene to the region between DXS722 and DXS8023, in Xp11.23. Further analysis of the incomplete-CSNB families, by means of disease-associated–haplotype construction, identified 17 families, of apparent Mennonite ancestry, that share portions of an ancestral chromosome. Results of this analysis refined the location of the gene for incomplete CSNB to the region between DXS722 and DXS255, a distance of 1.2 Mb. Genetic and clinical analyses of this set of 32 families with X-linked CSNB, together with the family studies reported in the literature, strongly suggest that two loci, one for complete (CSNB1) and one for incomplete (CSNB2) X-linked CSNB, can account for all reported mapping information.

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Introduction

X-linked congenital stationary night blindness (CSNB) is a nonprogressive retinal disorder; its clinical features include impaired night vision, variable myopia, reduced visual acuity, and congenital nystagmus (Carr 1974; Krill 1977; Khouri et al. 1988; Pearce et al. 1990; Héon and Musarella 1994; Miyake et al. 1994). Clinical heterogeneity among families with CSNB has led to the suggestion that the X-linked variety of CSNB should be classified as either complete (CSNB1 [MIM 310500]) or incomplete (CSNB2 [MIM 300071]) (Miyake et al. 1986). This classification is based on abnormalities both in the electroretinogram (ERG) (e.g., the scotopic b wave and the oscillatory potentials) and in the psychophysical dark-adaptation thresholds. In the complete form of CSNB, both the scotopic b wave that is evoked by dim blue flashes and the early oscillatory-potential wavelets are nonrecordable, and the night blindness is profound, as documented by the lack of rod adaptation to darkness. In the incomplete form of CSNB, the scotopic b wave is recordable, although it is subnormal in amplitude; the scotopic oscillatory potentials are often recordable, but they may show abnormal implicit times; and the psychophysical dark-adaptation thresholds are only slightly elevated above normal (for a review, see Héon and Musarella 1994).

Since the original localization of X-linked CSNB to Xp11, by linkage analysis (Gal et al. 1989; Musarella et al. 1989; Bech-Hansen et al. 1990), there has been uncertainty about whether the mapping information for X-linked–CSNB families supports genetic heterogeneity or whether it supports, instead, a single locus that exhibits wide variation in clinical phenotype (Khouri et al. 1988; Pearce et al. 1990). Early studies that used linkage and multipoint analyses of complete-CSNB and incomplete-CSNB families mapped these conditions to the same region (Musarella et al. 1992). Furthermore, recombinant chromosomes in families that have been ex-

amined to date have provided location information for X-linked CSNB, but seldom have individual families provided both close proximal and distal limits for a minimal genetic region that contains the disease gene. Data from the few families that are highly informative indicate that a minimum of two loci for X-linked CSNB are possible: one proximal to DXS426 (Bech-Hansen et al. 1991) and one in the region distal to DXS1003-TIMP1-DXS426 (Musarella et al. 1989; Aldred et al. 1992; Berger et al. 1995) or DXS228 (Bergen et al. 1995).

In addition to use of recent crossover events to localize a disease gene, ancestral crossovers can be investigated by disease-associated haplotype mapping in isolated populations. The assumption is that a distinct haplotype that reflects the specific allele combination of the ancestral chromosome should be present, in the immediate vicinity of a disease gene, in the descendants (for a review, see de la Chapelle 1993). Such studies have refined the location of several disease genes, including that for cystic fibrosis (Kerem et al. 1989); the cystic fibrosis gene is one of the first to be isolated by means of a positional cloning strategy.

To resolve the question of genetic heterogeneity in X-linked CSNB, we characterized 25 crossover events that were identified in 32 families affected by either the complete or the incomplete form of the disease. In addition, disease-associated–haplotype analysis identified a subset of families, of apparent Mennonite ancestry, with incomplete X-linked CSNB. These families possess a common haplotype that provides additional information on the location of the gene for incomplete CSNB. We present these data in the context of information published elsewhere, and we propose that two loci in Xp11 can account for the current mapping information on X-linked CSNB.

Subjects and Methods

Families

Thirty-two families that segregate X-linked CSNB were ascertained from a number of ophthalmologic centers in Canada, Japan, and the United States. Members of each family included in the present study underwent ophthalmologic examination and diagnosis according to procedures outlined elsewhere (Miyake et al. 1986; Khouri et al. 1988; Musarella et al. 1989; Pearce et al. 1990; Ruttum et al. 1992; Bech-Hansen and Pearce 1993). Family structure and clinical findings for 14 of the 32 families have been reported elsewhere; they include families 50(B), 70(A), 80(I), and 100(D) (Pearce et al. 1990); family 60(C) (Pearce et al. 1990; Bech-Hansen and Pearce 1993); families 9, R005, R007, Y001, Y002, and Y003 (Musarella et al. 1989); family 21 (Khouri et al. 1988; Musarella et al. 1989); and family 260 (Ruttum et al. 1992).

Of the 32 families, 11 (families 9, 260, 270, B1, B2, R005, R006, R007, Y001, Y002, and Y003) had absent rod scotopic vision, as determined from ERGs generated in response to a dim blue flash; absent oscillatory-potential wavelets; and/or profound night blindness (the absence of the rod branch in the dark-adaptation curve); these features are consistent with the complete form of X-linked CSNB. The remaining 21 families (21, 50, 60, 60B, 70, 80, 100, 130, 140, 150, 160, 170, 180, 190, 200, 230, 240, 250, 330, 340, and M10) had reduced but not absent rod scotopic vision, determined by means of ERG generated with a dim blue flash, and/or at least one member from each family had reduced but not absent rod psychophysical dark-adaptation findings; these characteristics were compatible with the incomplete form of the disease.

Families 21 and 60 were initially reported to contain both complete-CSNB and incomplete-CSNB patients. One of the four affected males in the family reported by Khouri et al. (1988) (designated "21" in the report by Musarella et al. [1989]) had both myopia and a relative lack of oscillatory potentials, consistent with complete CSNB, and two of nine affected males in a family reported by Pearce et al. (1990) ("family C"; referred to as "family 60" in the present report) had a dark-adaptation reduction that was severe enough to be considered an indication of complete CSNB, in accordance with the original definition (Miyake et al. 1986). These two families are included here as having incomplete CSNB, on the basis of both reevaluation of data (Miyake 1989; Weleber et al. 1989) and further clinical analyses (Bech-Hansen et al., in press).

Marker Analysis

Informed consent was obtained and blood was collected from family members. DNA was extracted, and RFLP and microsatellite polymorphism analyses were performed as described elsewhere (Musarella et al. 1989; Bech-Hansen et al. 1990; Bech-Hansen and Pearce 1993). Details on the RFLP (TIMP1) and the VNTR (DXS255) analyses used in this study can be found elsewhere (Musarella et al. 1989; Bech-Hansen and Pearce 1993). The microsatellite polymorphisms used in the present study include DXS8026, DXS556, DXS1368, DXS993, DXS228, DXS6810, DXS7, MAOA, MAOB, and DXS8083 (redesigned primers are L, 5′-CTT CTG CAC AGC AAA GGA AA; and R, 5′-ACT CCA GGA GGC CGT ATG TC); EC8058 (DXS9931); DXS1055, DXS1003, SYN1, and DXS426 (Boycott et al. 1997); DXS6849 (Boycott et al. 1997); DXS722 (Boycott et al. 1996); DXS6940, DXS1126, DXS1240, and DXS1470 (Boycott et al. 1997); DXS573, DXS8023, DXS1000,

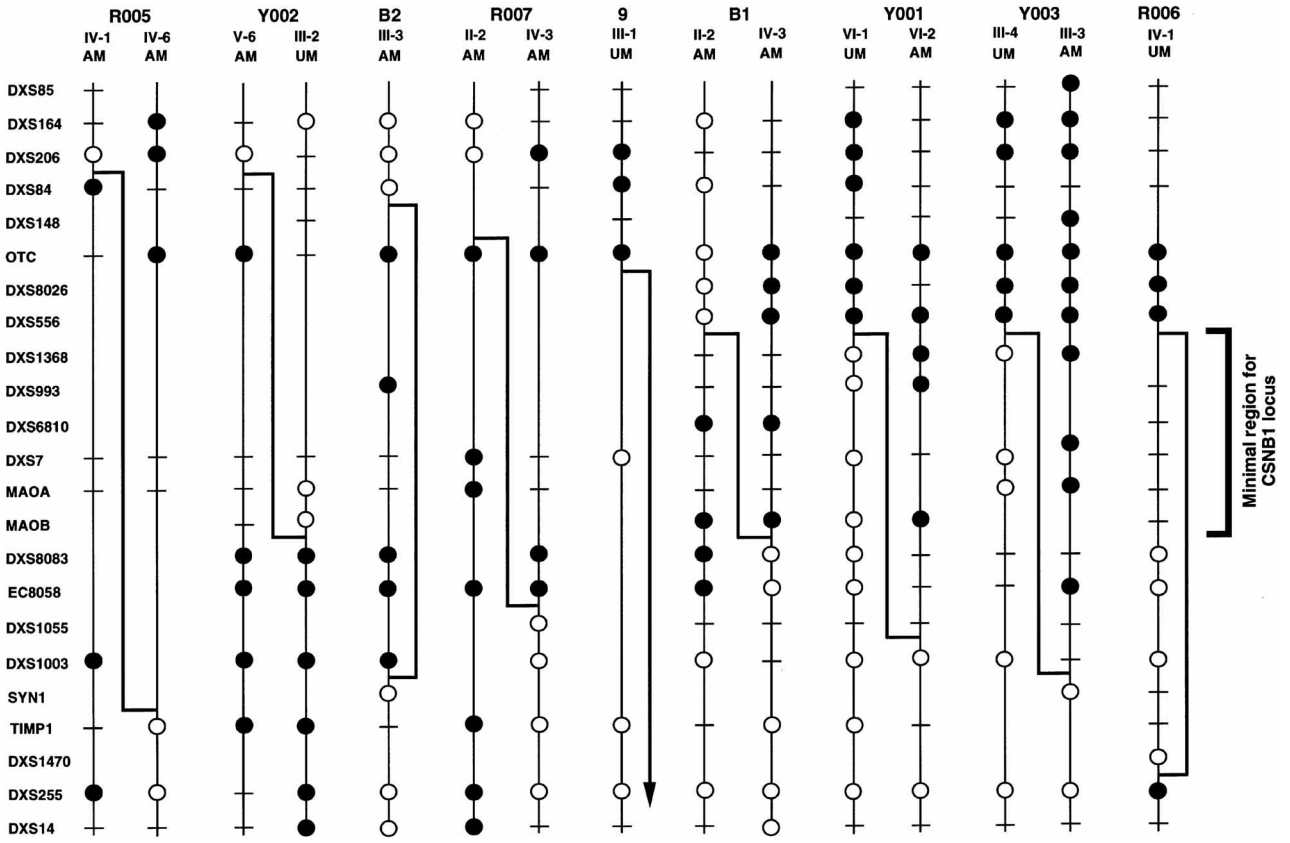

Figure 1 Fifteen recombination events in X chromosomes from nine families that segregate the complete form of X-linked CSNB. Polymorphic marker loci are shown on the far left, and the family and individual designations are shown along the top of the panel. The status of the person carrying the depicted chromosome is also indicated, along the top: AF = affected female; AM = affected male; CF = carrier female; and UM = unaffected male. Blackened circles indicate the loci that are in phase with the disease gene, unblackened circles indicate the loci that are out of phase, and short horizontal lines indicate uninformative markers. The downward-pointing arrow indicates the location of the disease gene in a family in which only one crossover has been identified, and brackets indicate the locations of the disease genes in families in which two crossovers or double crossovers have been identified that give both the distal and proximal limits of the disease gene.

DXS988, and DXS991; and the relevant information can be found in the Human Genome Database (GDB), unless stated otherwise. The allele sizes that we detected for the microsatellites DXS6940 (163–167 bp) and DXS1126 (239–261 bp) were slightly outside the range reported in the GDB. The map order of all the markers either was based on the X-chromosome consensus map (Nelson et al. 1995) or was determined by use of a panel of somatic-cell hybrids (Boycott et al. 1997) and detailed YAC contigs (Boycott et al. 1996; K. Stoddart and N.T. Bech-Hansen, unpublished data) within the region.

Results

Evaluation of Recombinant Chromosomes in X-Linked CSNB Families

Thirty-two families with X-linked CSNB were assessed for crossovers, on the X chromosome, that would localize the segregating disease gene. Crossovers identified in six complete-CSNB families (Musarella et al. 1989) and in three incomplete-CSNB families had been reported elsewhere (Musarella et al. 1989; Bech-Hansen et al. 1992; Bech-Hansen and Pearce 1993).

Complete X-linked CSNB.—Of the 11 families with complete CSNB, 6 had been reported to have crossovers in Xp11 (Musarella et al. 1989): In family 9, the disease gene was localized proximal to OTC; in family R005, between DXS206 and TIMP1; in family R007, between DXS206 and TIMP1; in family Y001, between OTC and DXS255; in family Y002, between DXS206 and TIMP1; and, in family Y003, between OTC and DXS255. To further refine the position of the gene for complete CSNB (designated "CSNB1"), we investigated the recombinant chromosomes in these families, using new microsatellite markers, and we identified and investigated crossovers in five new complete-CSNB families.

Results of these analyses are presented in figure 1.

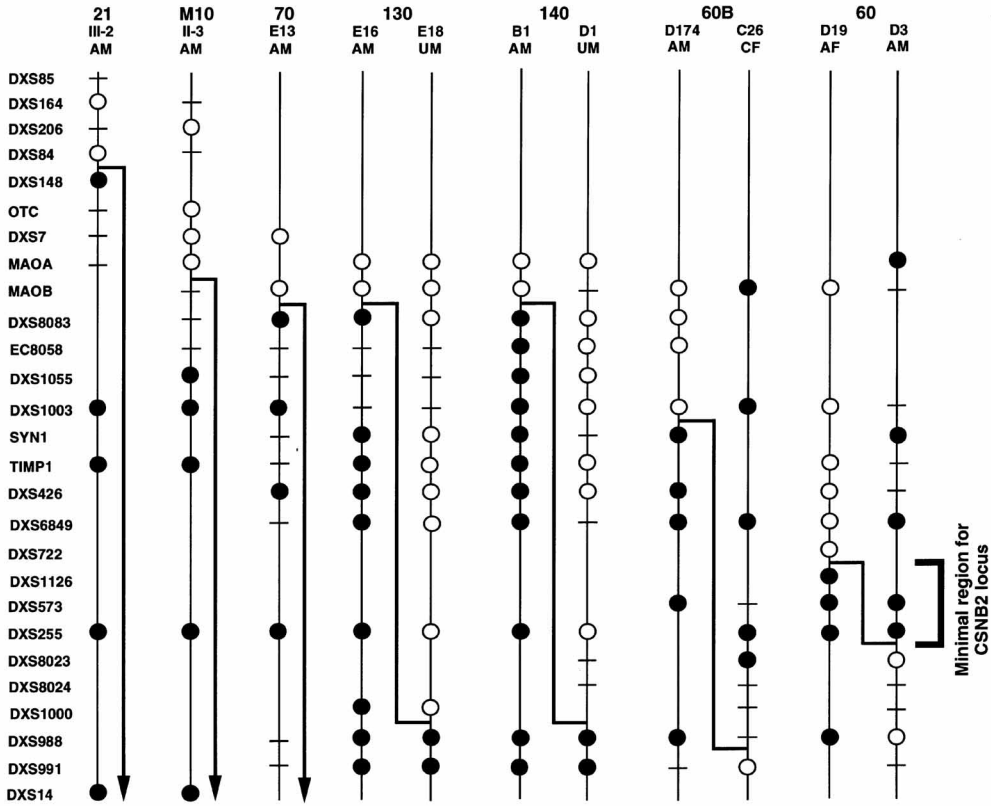

Figure 2 Eleven recombination events in X chromosomes from seven families that segregate the incomplete form of X-linked CSNB. Symbols are as described in legend to figure 1.

Informative crossovers were identified in three of the five new families: In family B2, one individual carried a double-recombinant chromosome that localized the CSNB1 gene to the region between DXS84 and TIMP1; in family B1, two recombinant chromosomes were identified that placed the CSNB1 gene in the region between DXS556 and DXS8083; and, in family R006, crossovers placed the CSNB1 gene between DXS556 and DXS255. In four of the six families reported elsewhere, the location of the CSNB1 gene was refined (fig. 1): In family Y002, the location of the gene was refined to the interval between DXS206 and DXS8083; in family Y001, to the region between DXS556 and DXS1003; in family Y003, to the region between DXS556 and EC8058; and, in family R007, to the region between DXS206 to DXS1055. In summary, the location of the gene for CSNB1, between the markers DXS556 and DXS8083 (the distal and proximal limits defined by family B1), is consistent with all the recombinant-chromosome mapping information for our set of 11 families with complete CSNB.

Incomplete X-linked CSNB.—Of the 21 incomplete-CSNB families, 4 (families 60, 60B, 70, and 21) had been reported to have crossovers that sublocalized the gene for incomplete CSNB (designated "CSNB2") on the X chromosome. An affected female in family 60 carried a recombinant chromosome that successively positioned the CSNB2 gene proximal to TIMP1 (Bech-Hansen and Pearce 1993), DXS426 (Bech-Hansen et al. 1991), and DXS6849 (Bech-Hansen et al., in press). This female is believed to have incomplete CSNB, because she has inherited two copies of the same mutant CSNB2 gene—one from her father (a member of family 60) and the other from her mother (a member of family 60B). Although there is no known common ancestor, there is evidence for homozygosity by descent (Bech-Hansen and Pearce 1993; Bech-Hansen et al., in press). Other crossovers identified in family 60 have mapped the CSNB2 gene distal to DXS988 (Boycott et al. 1994) and DXS8023 (Bech-Hansen et al., in press). In addition, clinical and genetic analysis of the extended family (60B) has been performed, and the CSNB2 gene in this family has been mapped to the region between DXS1003 and DXS991 (Bech-Hansen et al., in press). Furthermore, an individual from family 70 was reported to carry a recombinant chromosome that placed the CSNB2 gene proximal to DXS7 (Bech-Hansen et al. 1992), whereas an individual from family 21 was reported to carry a

Table 1

Haplotype Analysis of 17 Families with Incomplete X-Linked CSNB

	LOCUS																
FAMILY/ FAMILIES	MAOB	DXS 8083	PEC 8058	DXS 1055	DXS 1003	SYN1	DXS 426	DXS 6849	DXS 722	DXS 6940	DXS 1126	DXS 1240	DXS 1470	DXS 573	DXS 1039	DXS 255	DXS 8023
150, 160, 180, 20	184	240	313	87	181	195	220	245	190	163	257	169	206	139	93	D	132
340	184	240	313	87	181	195	222	245	190	163	257	169	206	139	93	D	132
190	178	240	313	87	181	195	220	245	190	163	257	169	206	139	93	D	132
250	176	240	313	87	181	195	220	245	190	163	257	169	206	139	93	D	132
170	180	232	313	87	181	195	220	245	190	163	257	169	206	139	93	D	132
130[a]	180	232	317	87	181	195	220	245	190	163	257	169	206	139	93	D	132
200	180	244	315	87	181	195	220	245	190	163	257	169	206	139	93	D	132
60B[a]	174	232	315	93	185	195	220	245	190	163	257	169	206	130	93	D	132
80	182	232	313	87	191	195	220	245	190	163	257	169	206	139	93	D	132
21	178	238	313	91	185	203	220	245	190	163	257	169	206	139	93	C	144
50	176	244	317	93	187	195	222	245	190	163	257	169	206	139	93	D	132
330	182	232	315	91	187	197	222	245	190	163	257	169	206	139	93	D	132
60-P[a]	182	230	317	95	187	205	222	239	190	163	257	169	206	139	93	D	140
70[a]	170	242	317	83	183	203	226	239	190	163	257	169	208	139	93	D	132
60-D[a]	174	232	315	93	185	203	240	241	192	163	257	169	206	139	93	D	132

NOTE.—Lines in the table delineate the portion of the haplotypes shared between families.

[a] Haplotype is from a recombinant individual.

recombinant chromosome that placed the CSNB2 gene proximal to DXS84 (Musarella et al. 1989). To further refine the map position of CSNB2, we continued to investigate the recombinant chromosomes in these families, using new microsatellite markers, and we identified and investigated crossovers in 16 new incomplete-CSNB families.

Results of these analyses are presented in figure 2. Crossovers were identified in 3 of the 16 new families: In family M10, a crossover in an affected male placed the CSNB2 gene proximal to MAOA, and, in families 130 and 140, crossovers placed the gene proximal to MAOB and distal to DXS988. In two of the three families reported elsewhere, the location of the CSNB2 gene was refined (fig. 2): In family 70, the location of the CSNB2 gene was refined to the region proximal to MAOB, and, in family 60, the location of the CSNB2 gene was refined to the interval between DXS722 and DXS8023. In summary, the location of the gene for CSNB2, between the markers DXS722 and DXS8023 (the distal and proximal limits of the gene, in family 60), is consistent with all the recombinant-chromosome–mapping information for our set of 21 incomplete-CSNB families.

Of the 21 families with incomplete X-linked CSNB, the majority did not contain any crossovers that would help to localize the CSNB2 gene. Eighteen of the 21 families were from Alberta, and 4 of these were known to be of Mennonite ancestry. To determine whether the apparent high incidence of incomplete CSNB in Alberta was due to an ascertainment bias or to a common-ancestor effect, we performed disease-associated–haplotype analysis.

Haplotype Analysis

Identification of disease-associated haplotypes provides a powerful approach for localization of a disease gene, in relation to DNA markers, and for identification of descendants of a founder population. Using 24 DNA markers that span most of Xp11, we constructed haplotypes for one affected male from each incomplete-CSNB family. Recombinant chromosomes that resulted from crossovers between any of the markers tested were included in the haplotype analysis. Specifically, in family 60, chromosomes recombinant for the markers used in haplotype construction are carried by an affected male and an affected female (60P and 60D, respectively), so both of these recombinant chromosomes were included in the analysis. Of the 21 families with incomplete CSNB, 17 retained various portions of a common haplotype that spans the region between marker DXS8026 and the centromere; haplotypes that span the region between the markers MAOB and DXS8023 are shown in table 1. These haplotypes suggest that 85% of the disease-associated chromosomes in our incomplete-CSNB

families are separated by single recombination events from a putative ancestral founding-mutation haplotype that spans the proximal portion of Xp11. Greater departures from the ancestral haplotype were seen with the distal set of DNA markers.

Although no new distal-limit information on the location of the CSNB2 gene was gained (it remains at DXS722), the affected haplotype of family 21 potentially moves the proximal limit of this gene from DXS8023 to DXS255. All 17 families, with the exceptions of families 70 and 340, share the entire haplotype between the markers DXS722 and DXS255. The haplotypes in families 70 and 340 deviate from the common haplotype, at markers DXS1470 and DXS426, respectively; this is probably due to a recent spontaneous mutation at this locus, which is not unexpected at a dinucleotide-repeat marker (Weber 1990).

Of the 17 families that share the common haplotype, 4 are of known Mennonite ancestry. Further investigation of the genealogies of the other 13 families suggested that they are all of Mennonite ancestry. Isolated populations, such as the Mennonites, can potentially have allele frequencies that are significantly different from those of the general Caucasian population. Therefore, the allele frequencies of all markers within the common haplotype were determined for a set of 31 Mennonites, chosen at random. Our results indicate that the allele frequencies in the Mennonite population are not significantly different from the allele frequencies in the Caucasians reported in the GDB, for any of the markers (data not shown). Therefore, the common Mennonite haplotype that contains the CSNB2 gene is apparently not the result of preferentially high frequencies of this set of alleles in the Mennonite population studied. This haplotype information, combined with the crossover in family 60 that defined the distal limit, suggests that the CSNB2 gene is located in the region between DXS722 and DXS255.

Discussion

Eleven complete-CSNB and 21 incomplete-CSNB families were studied, to refine the location of the locus or loci responsible for these two entities and, in turn, to evaluate the role of genetic heterogeneity in X-linked CSNB. In summary, our findings establish separate loci for the two forms of X-linked CSNB. The crossovers that we observed in family B1 and family 60 currently provide the most refined localizations, within individual families, for the CSNB1 and CSNB2 loci. Our data indicate that a gene (CSNB1) that is responsible for the complete form of X-linked CSNB lies between the markers DXS556 and DXS8083 (family B1), in Xp11.4-p11.3. This finding is entirely consistent with the mapping information that we have presented for the other complete-CSNB families. The results of our crossover-mapping studies also indicate that a gene (CSNB2) that is responsible for the incomplete form of X-linked CSNB lies between the markers DXS722 and DXS8023 (family 60), in Xp11.23. This finding is also consistent with the recombination-based mapping information that we have presented for the other incomplete-CSNB families.

To put published clinical and recombination data in the context of findings presented in this report, we identified individuals, in eight families reported by other laboratories, who carry recombinant chromosomes (fig. 3). Except for two instances, the published crossovers were not accompanied by sufficient clinical information to establish clearly whether the individual families were segregating the complete or the incomplete form of X-linked CSNB.

Of the two families for which sufficient clinical information was available, one family could be classified as having complete X-linked CSNB (Gal et al. 1989), and the other could be classified as having incomplete X-linked CSNB (Bergen et al. 1994) (see fig. 3). In the complete-CSNB family, two recombinant chromosomes localized a CSNB gene distal to TIMP1 (Gal et al. 1989; Li et al. 1991), whereas, in the incomplete-CSNB family, one recombinant chromosome localized a CSNB gene proximal to MAOB (Bergen et al. 1994). This mapping information is consistent with the two-locus hypothesis that we have presented here.

The clinical findings reported for the remaining six families precluded classification on the basis of complete or incomplete CSNB. In the initial report on the mapping of X-linked CSNB, Gal et al. (1989) observed crossovers that, in their family 2, placed the CSNB gene distal to DXS255 and that, in their family 3, placed the CSNB gene distal to the region between OTC and DXS255. The mapping information for these two families overlaps with the proposed minimal regions for both the CSNB1 and the CSNB2 genes. In two other families (families 3 and 4; Aldred et al. 1992), crossovers localized the CSNB gene to the region proximal to DMD, again overlapping with proposed minimal regions for both the CSNB1 and the CSNB2 genes. Reported crossovers in a Dutch family localized the CSNB gene to the region between OTC and DXS1003 (Berger et al. 1995); this finding is consistent with segregation of the CSNB1 gene in this family. An affected male in another Dutch CSNB family (Bergen et al. 1995) carried a double-recombinant chromosome that localized the CSNB gene to the region between DMD44 and DXS228, which again overlaps with the CSNB1 minimal region. If this proves to be a family with complete CSNB, then the minimal region for the CSNB1 gene would be refined to the interval between DXS556 and DXS228, in Xp11.4.

In the present study, the paucity of critical crossovers

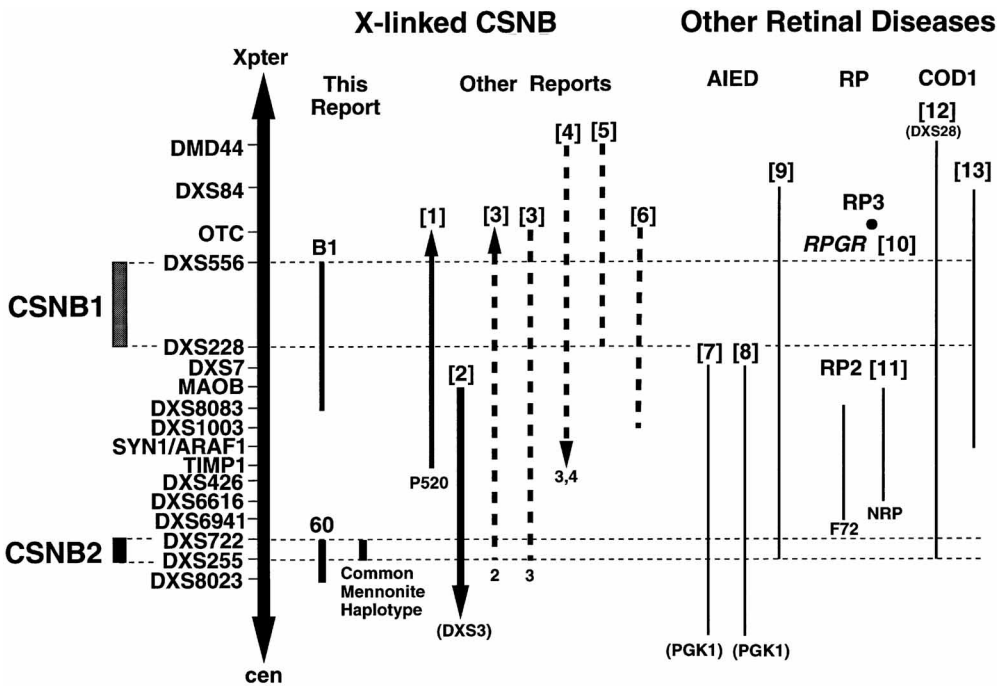

Figure 3 Recombination-based localization of genetic eye disease genes in Xp11. Part of the X chromosome is represented by the thicker line, on the left of the figure, and the polymorphic markers are indicated along its left side. In the X-linked CSNB portion of the figure, the vertical lines delineate minimal regions, determined on the basis of crossover information for complete-CSNB families (*thinner line*), for incomplete-CSNB families (*thicker line*), and for several CSNB families that could not be classified as having the complete or the incomplete form of CSNB (*broken horizontal lines*). Immediately to the right of X chromosome line, minimal regions for the CSNB1 and CSNB2 loci are shown; these were determined on the basis of findings, for two families, that gave the finest localization of the complete (family B1) and the incomplete (family 60) X-linked CSNB genes. The region spanned by the common Mennonite haplotype is also indicated. Eight recombinant chromosomes from previously reported CSNB families are also shown; one family (P520) is known to have complete CSNB, one is known to have incomplete CSNB, and five others are of unknown status (*broken vertical lines*). An arrow indicates where the distal or proximal limit of the CSNB gene in that family was not established, and the lines are drawn to the most distal or proximal marker tested. The current minimal regions for the CSNB1 and CSNB2 loci are represented on the extreme left side of the figure. Previously published recombination-based mapping information on other X-linked retinal disorders—AIED, RP2, RP3, and COD1—appears in the right portion of the diagram. Numbers in square brackets, above the vertical lines that represent the minimal regions, refer to specific family studies: [1] = Li et al. (1991); [2] = Bergen et al. (1994); [3] = Gal et al. (1989); [4] = Aldred et al. (1992); [5] = Bergen et al. (1995); [6] = Berger et al. (1995); [7] = Alitalo et al. (1991); [8] = Schwartz and Rosenberg (1991); [9] = Glass et al. (1993); [10] = Meindl et al. (1996); [11] = Thiselton et al. (1996); [12] = Bergen et al. (1993); and [13] = Hong et al. (1994).

in the smaller incomplete-CSNB families prompted us to attempt to refine the location of the CSNB2 gene, by determination of the ancestral relationship among these families, by means of disease-associated–haplotype analysis. Results of this analysis suggest that most of the chromosomes in our set of incomplete-CSNB families were derived from a single founder chromosome (table 1). Derivatives of this haplotype were found in 17 of the 21 incomplete-CSNB families, and the portion of the retained haplotype varied, from the entire region tested (DXS8026–DXS991), a distance of 30 cM, to the region between DXS722 and DXS255, a distance estimated to be <1 cM (NIH/CEPH Collaborative Mapping Group 1992; Buetow et al. 1994; Gyapay et al. 1994; Matise et al. 1994; GDB).

A review of the genealogies of all the incomplete-CSNB families that share the common haplotype suggests that they all have Mennonite ancestry. Between 1874 and 1880, almost 7,000 Mennonites immigrated to Manitoba from Russia (Epp 1974). Some descendents of these immigrants are found in Alberta, where members of many of the families reported here still reside. Investigation has so far failed to find a common ancestor among any of these families; this raises the possibility that more than one member of the initial immigrant population had incomplete CSNB. Consequently, it is likely that some descendants of the Anabaptist movement and members of the Mennonite community, in Russia and in other parts of the world, are also affected with incomplete CSNB.

Of the 21 families with incomplete CSNB, 4 do not share the common haplotype, nor do they share a com-

mon haplotype with each other. This would suggest that there are as many as five independent mutations that cause incomplete X-linked CSNB in our set of families: the mutation associated with the common Mennonite haplotype and four other mutations, one each associated with families 100, 140, 230, and M10. Family M10 is of Japanese origin, but detailed ancestry data for families 100, 140, and 230 are not available.

Both the genetic and the clinical information presented in figure 3 are consistent with two loci for X-linked CSNB: one (CSNB1) for the complete form, in Xp11.4-p11.3, and one (CSNB2) for the incomplete form, in Xp11.23. Earlier reports suggested, on the basis of two families reported to contain both complete-CSNB and incomplete-CSNB patients (Khouri et al. 1988; Pearce et al. 1990), that X-linked CSNB is a single disease entity, with highly variable clinical expression (Pearce et al. 1990). This raises important points regarding the clinical assessment of CSNB patients. In particular, if testing criteria include only measurement of adaptation to darkness, and if test results for the first individual in each family show absence of rod adaptation and indicate that the patient has CSNB, no distinction between the incomplete and the complete forms of X-linked CSNB is possible. Ideally, this distinction requires the results of a scotopic ERG, generated with a dim blue flash. Alternatively, it may be possible, by examination of several patients in the same family by means of measurement of adaptation to darkness, to observe, in all patients, either complete loss of rod function, which is associated with a diagnosis of complete CSNB, or variability in rod function, which is associated with incomplete CSNB.

The two types of X-linked CSNB also appear to be nonallelic, since the fundamental defect appears to be different, based on different ERG responses. In patients with complete CSNB, the rod b wave is not detectable by means of scotopic dim blue–flash stimulus, and the photopic "on" response (b wave) is significantly reduced; these features suggest a defect, within the middle retinal layer, that may prevent the rod signal from reaching the bipolar cells (Carr et al. 1966; Miyake et al. 1987, 1994; Young 1991). In contrast, patients with incomplete CSNB show variably but significantly reduced b waves, in response to scotopic dim blue–flash stimulus, and significantly reduced "off" and "on" responses to photopic testing (Miyake et al. 1987, 1994). Therefore, the pathophysiology that underlies incomplete CSNB includes both rod and cone disturbances; these disturbances may involve structures that are responsible for building the b wave (Tremblay et al. 1994).

It has been suggested elsewhere that the condition known as "Åland Island eye disease" (AIED) is clinically indistinguishable from incomplete CSNB (Krill 1977; Musarella et al. 1989; Weleber et al. 1989). Studies of three AIED families (Alitalo et al. 1991; Schwartz and Rosenberg 1991; Glass et al. 1993) defined recombinant chromosomes that together localized the AIED gene between DXS7 and DXS255, a region that overlaps with the minimal region for the CSNB2 gene (fig. 3). This lends further support to the notion that these conditions are most likely allelic.

Other retinal disorders also map to Xp11. Combined mapping information for two families with X-linked cone dystrophy (COD1) localized a gene for COD1, between DXS84 and SYN1/ARAF1 (Bergen et al. 1993; Hong et al. 1994). Two genes (RP2 and RP3) that are responsible for X-linked retinitis pigmentosa (RP) also map to Xp21.1-p11. Night blindness is a well-known early-onset symptom of RP, a set of degenerative retinal diseases that are characterized by progressive concentric field loss, pigmentary retinopathy, and reduced ERG amplitudes. Autosomal forms of CSNB and RP have been shown to be caused by different mutations in the rhodopsin gene, on chromosome 3q (Dryja et al. 1993; Rao et al. 1994), and in the gene that encodes the β subunit of the rod cGMP phosphodiesterase, on chromosome 4p (Gal et al. 1994). In light of these findings, it has been speculated that the genes responsible for X-linked CSNB may be allelic with the RP2 or RP3 genes. RP2 was recently sublocalized to the region between DXS8083 and DXS6616, on the basis of recombinant chromosomes from two families with this condition (Thiselton et al. 1996) (fig. 3). Our mapping information localizes the gene for incomplete CSNB proximal to DXS6616—namely, at DXS722; this finding shows that these two genes are not allelic. A gene for RP3 (RPGR) was recently cloned, and it maps just distal to OTC, in Xp21.1 (Meindl et al. 1996; Roepman et al. 1996) (fig. 3). Our mapping information localizes the complete-CSNB gene as proximal to OTC; this finding shows that complete CSNB and RP3 are also not allelic. Although neither RP2 and incomplete CSNB nor RP3 and complete CSNB are allelic, a mutation in the RPGR gene appears to be responsible for CSNB in at least one family (Herrmann et al. 1996). As in the autosomal forms of RP and CSNB, it is possible that a few families with X-linked CSNB have disease-causing mutations in RP2 or RP3. Further analysis is required to determine whether this is indeed the case.

Finally, we would like to address the issue of nomenclature for X-linked CSNB. Musarella et al. (1989) originally used "CSNB1" to designate the locus that segregates in their group of complete-CSNB families. The work that we have presented, together with published information, defines a locus for the complete form of X-linked CSNB, in Xp11.4, between the markers DXS556 and DXS228. We propose that this locus should continue to be known as "CSNB1." The use of the abbreviation "CSNB1," in previous publications (Bech-Hansen et al. 1990, 1991, 1992; Bech-Hansen and Pearce

1993) from the laboratory of N.T.B.-H., referred, in each case, to the X-linked form of CSNB rather than to the complete-CSNB or incomplete-CSNB clinical status of the family reported.

Furthermore, "CSNB2" has been used to designate the locus that segregates in families with incomplete X-linked CSNB (Musarella et al. 1992; Bergen et al. 1994). The data that we have presented here define a locus for the incomplete form of X-linked CSNB, in Xp11.23, between the markers DXS722 and DXS255. We propose that this locus should continue to be referred to as "CSNB2." The region that contains the CSNB2 gene is 1.2 Mb in size (Boycott et al. 1996; Schindelhauer et al. 1996), and this region can now be targeted for transcript identification by one or more of the many techniques available to positional cloners. Identification of the CSNB1 and CSNB2 genes will ultimately resolve the uncertainty that surrounds the clinical and genetic heterogeneity of X-linked CSNB; it will also provide information about the basic defects in this X-linked retinal condition, which, for years, has intrigued and puzzled investigators.

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