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Congenital Motor Nystagmus Linked to Xq26-q27

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Summary

Congenital motor nystagmus (CMN) is a hereditary disorder characterized by bilateral ocular oscillations that begin in the first 6 mo of life. It must be distinguished from those genetic disorders—such as ocular albinism (OA), congenital stationary night blindness (CSNB), and blue-cone monochromatism (BCM)—in which nystagmus accompanies a clinically apparent defect in the visual sensory system. Although CMN is presumed to arise from a neurological abnormality of fixation, it is not known whether the molecular defect is located in the eye or in the brain. It may be inherited in an autosomal dominant, autosomal recessive, or X-linked pattern. Three families with CMN inherited in an X-linked, irregularly dominant pattern were investigated with linkage and candidate gene analysis. The penetrance among obligate female carriers was 54%. Evaluation of markers in the region of the genes for X-linked OA, CSNB, and BCM revealed no evidence of linkage, supporting the hypothesis that CMN represents a distinct entity. The gene was mapped to chromosome Xq26-q27 with the following markers: GATA172D05 (LOD score 3.164; recombination fraction [θ] = 0.156), DXS1047 (LOD score 10.296; $\theta = 0$), DXS1192 (LOD score 8.174; $\theta = 0.027$), DXS1232 (LOD score 6.015; $\theta = 0.036$), DXS984 (LOD score 6.695; $\theta = 0$), and GATA31E08 (LOD score 4.940; $\theta = 0.083$). Assessment of haplotypes and multipoint linkage analysis, which gave a maximum LOD score of 10.790 with the 1–LOD-unit support interval spanning ~7 cM, place the gene in a region between GATA172D05 and DXS1192. Evaluation of candidate genes *CDR1* and *SOX3* did not reveal mutations in affected male subjects.

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Introduction

Amblyopia and congenital nystagmus may develop in response to abnormal visual stimulation during infancy. Amblyopia manifests as poor vision, most commonly found in the deviating eye in patients who have ocular misalignment at an early age. It may be reversed with early intervention. Whereas amblyopia is typically a defect of monocular stimulation, more severe, bilateral visual defects are associated with congenital nystagmus, which is characterized by binocular spontaneous oscillations. Congenital nystagmus predominantly occurs secondary to genetic ocular diseases such as albinism, achromatopsia, and Leber congenital amaurosis. It may also develop in the setting of bilateral congenital cataracts, in which case, early cataract removal may result in improvement or prevention of the nystagmus. In a cohort of children in England who were followed from birth through the age of 5 years, nystagmus was present in 1/1,000 children (Stayte et al. 1993).

In contrast to nystagmus that arises as a result of aberrant visual stimulation, nystagmus may also occur in the absence of any apparent ocular disease. Whereas amblyopia is associated with abnormal maturation of the visual cortex, congenital nystagmus, in the setting of otherwise normal examination findings, is thought to represent abnormal development of those ocular motor areas of the brain that control fixation. As these patients may have normal visual acuity, it has been presumed that the nystagmus represents a primary defect in the parts of the brain responsible for ocular motor control—thus the term congenital "motor" nystagmus (CMN).

Several patterns of inheritance of CMN have been described, including X-linked (MIM 31700) (Nettleship 1911; Waardenburg 1963; Rosenblum and Rosenblum 1987), autosomal recessive (MIM 257400) (Nettleship 1911), and autosomal dominant (MIM 164100) (Allen 1942; Dichgans and Kornhuber 1964; Kerrison et al. 1998). It is not known which of these various patterns of genetic inheritance is most common. The patterns cannot be distinguished clinically by visual acuity or waveforms observed in eye movement recordings.

Autosomal dominant CMN has been investigated with cytogenetic and linkage analysis. Patton and co-

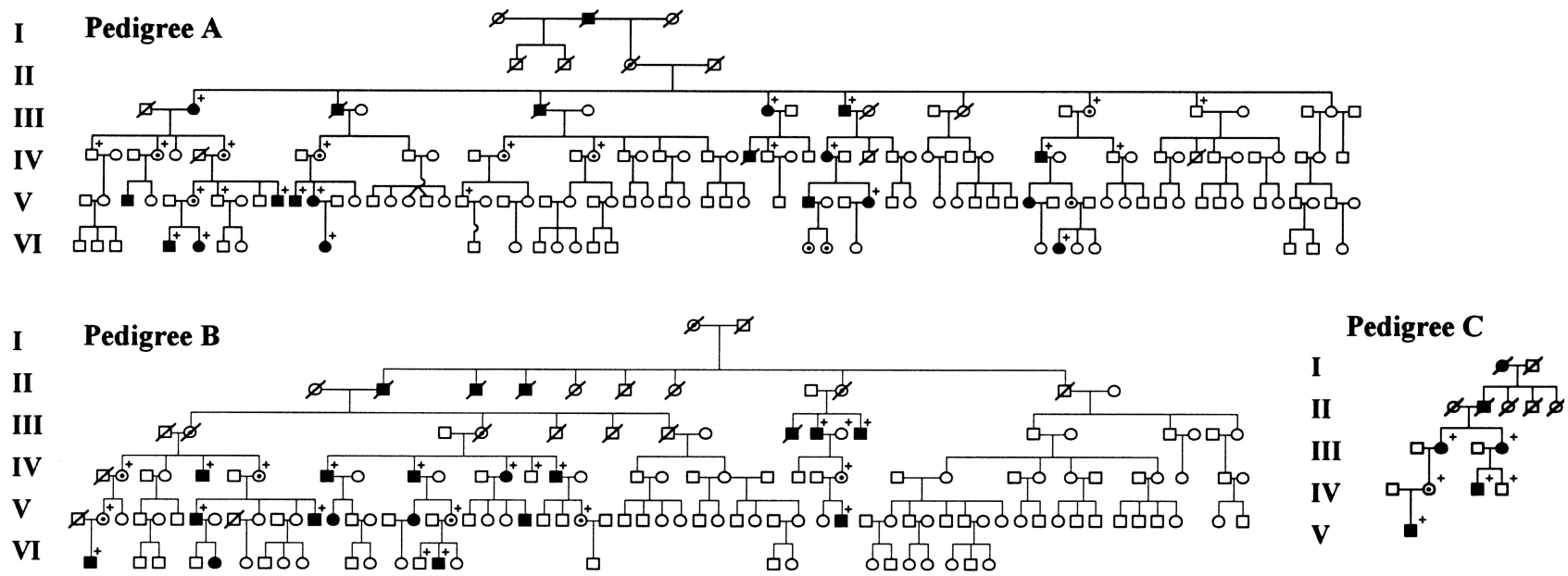

Figure 1 Three complete pedigrees with X-linked CMN. Note partial penetrance among carrier mothers. Plus (+) symbols indicate individuals who were genotyped.

Table 1

Linkage Results with Markers from Xq26-27 in Three Families with CMN

Marker and Pedigree	LOD Score at θ = 0	.01	.05	.1	.2	.3	.4	LOD_{max}	θ
GATA172D05									
A	−∞	−2.463	.034	.847	1.204	.975	.489	1.204	.196
B	−∞	.046	1.221	1.531	1.503	1.151	.611	1.579	.140
C	.602	.585	.514	.426	.253	.110	.024	.602	.000
Total	−∞	-1.707	1.890	2.918	3.062	2.320	1.181	3.164	.156
DXS1047									
A	5.322	5.240	4.904	4.464	3.507	2.424	1.198	5.321	.000
B	4.042	3.978	3.722	3.386	2.663	1.861	.965	4.042	.000
C	.903	.886	.814	.721	.522	.316	.128	.903	.000
Total	10.296	10.134	9.468	8.597	6.713	4.617	2.300	10.296	.000
DXS1192									
A	4.419	4.345	4.047	3.658	2.824	1.904	.898	4.418	.000
B	−∞	2.688	3.108	3.044	2.556	1.846	.969	3.116	.060
C	.903	.886	.814	.721	.522	.316	.128	.903	.000
Total	−∞	8.017	8.064	7.514	5.983	4.133	2.043	8.174	.027
DXS1232									
A	3.369	3.330	3.143	2.864	2.207	1.473	.720	3.369	.000
B	−∞	1.837	2.310	2.316	1.976	1.435	.754	2.340	.074
C	.602	.589	.535	.465	.318	.170	.049	.602	.000
Total	−∞	5.757	5.989	5.645	4.502	3.078	1.523	6.015	.036
DXS984									
A	3.737	3.670	3.393	3.034	2.264	1.419	.526	3.737	.000
B	2.628	2.601	2.472	2.274	1.800	1.257	.658	2.627	.000
C	.301	.297	.279	.255	.204	.146	.079	.301	.000
Total	6.696	6.597	6.172	5.588	4.289	2.836	1.271	6.695	.000
DXS8013									
A	1.360	1.348	1.287	1.191	.954	.675	.359	1.360	.000
B	.700	.694	.660	.603	.464	.309	.152	.694	.000
C	…[a]	…	…	…	…	…	…	…	…
Total	2.060	2.042	1.947	1.795	1.418	.984	.511	2.060	.000
GATA31E08									
A	−∞	3.484	3.814	3.633	2.896	1.943	.897	3.815	.046
B	−∞	−.547	.663	1.021	1.097	.867	.478	1.119	.163
C	.301	.292	.258	.215	.134	.064	.017	.030	.000
Total	−∞	3.281	4.785	4.917	4.168	2.908	1.412	.083	4.940

[a] Ellipses (…) indicate that the marker was uninformative.

workers (1993) described a mother and child with isolated CMN and a balanced 7:15 translocation. Kerrison and coworkers (1996, 1998) described a large pedigree with autosomal dominant CMN in which six markers were linked at recombination fraction (θ) = 0 in the 6p12 region. This is the first report of linkage analysis in X-linked CMN.

Several X-linked disorders may be associated with nystagmus, in particular Nettleship-Falls ocular albinism (OA; Xp22.3), complete congenital stationary night blindness (CSNB; Xp11.3), and blue-cone monochromatism (BCM; Xq27). Three families with X-linked CMN were investigated with linkage analysis and candidate gene analysis to determine whether a mutation in a gene for one of the above-mentioned disorders results in isolated nystagmus or whether a separate locus is responsible.

Families and Methods

Pedigree Assessment

The database of the Johns Hopkins Center for Hereditary Eye Diseases was screened for families with hereditary CMN. Criteria for the diagnosis of CMN in probands included onset of nystagmus before the age of 6 mo and ocular examination findings that were normal except for visual acuity and nystagmus: normal color vision, pupillary light reflexes, intraocular pressure, anterior segment, optic nerves, and retina. Electroretinography, which is useful in the evaluation of the patient with nystagmus, particularly with preverbal children and in the absence of a well-documented family history, was performed in selected individuals (Pearce et al. 1973; Ruttum et al. 1992). Affection status for additional fam-

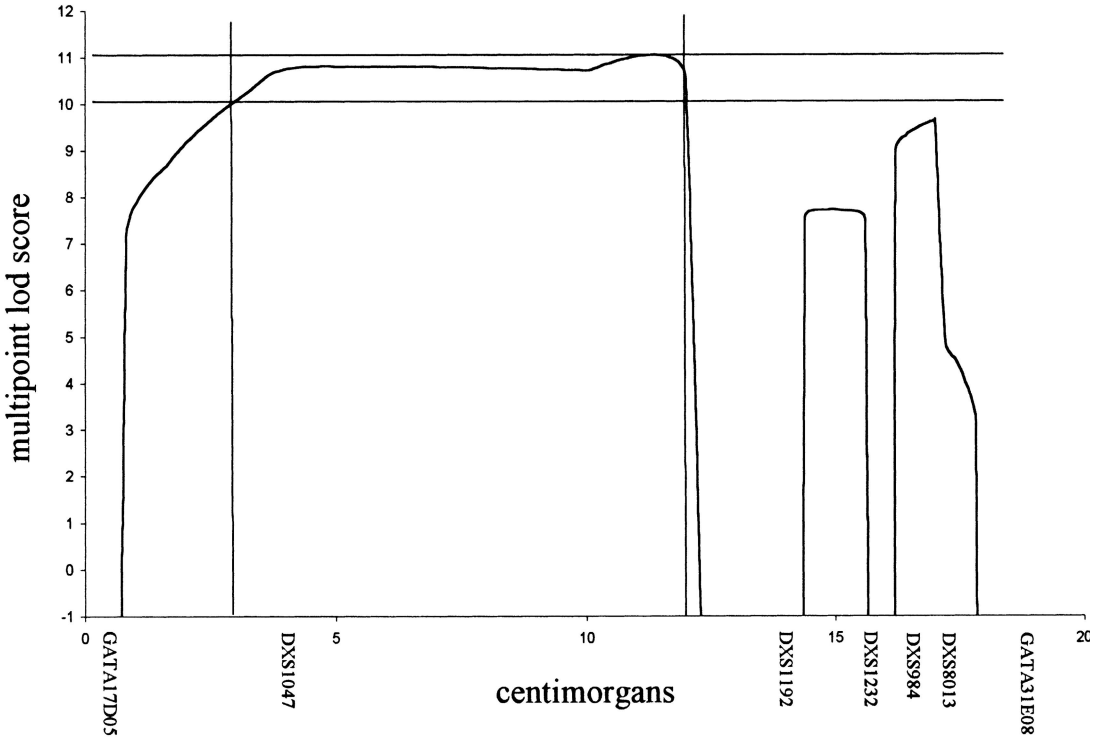

Figure 2 Multipoint LOD score with 1–LOD-unit support interval localizes the gene in a region between GATA172D05 and DXS1192

ily members was based on a history of nystagmus with onset within the first 6 mo of life and detection of ocular oscillations on examination by one of us or by another eye care professional.

Linkage Analysis

Blood samples or mouth swabs were obtained with informed consent from 53 members of three families (fig. 1) (25 female subjects, 19 affected male subjects, and 9 unaffected males) in accordance with a protocol approved by the Johns Hopkins Joint Committee on Clinical Investigation. Daughters of the female subjects were not included unless they were affected or had affected offspring. DNA was extracted in accordance with standard procedures. PCR was performed, in accordance with standard techniques, with primers obtained from Research Genetics (MapPairs) and with *Taq* polymerase (Perkin-Elmer-Cetus). PCR products were pooled and separated by gel electrophoresis with a fluorescence-based 373 DNA sequencer (Applied Biosystems). Assessment of DNA fragment size was performed with GENESCAN and GENOTYPER (Applied Biosystems). Linkage analysis was performed with the MLINK and ILINK programs of the FASTLINK package (Lathrop and Lalouel 1984; Lathrop et al. 1984, 1986; Cottingham et al. 1993; Schaffer et al. 1994). We assumed a gene frequency of .0001 and no sex difference in recombination rates. We estimated allele frequencies from family members. Multipoint analysis was performed with the VITESSE algorithm (O'Connell and Weeks 1995). Marker order and intervals for X chromosomal markers were obtained from Généthon (Dib et al. 1996) and the Genetic Location Data Base (Collins et al. 1996).

Candidate Gene Analysis

CDR1, consisting of a single exon, was PCR amplified in a single 1.3-kb fragment with the following primers: forward 5′-GGAAGACCTGGAGATGTTGGAAGACGAGCAGA-3′; reverse 5′-AATGTTTCAATGTCAGGAGTTCCGATGGCACC-3′. After gel purification, each sample was prepared for sequencing with the following nested primers: 5′-TTCGGAAGCTATGGATTTGA-3′; 5′-TTGTTGCGAGCTTAGTTGGA-3′; and 5′-GTAGATTTTCAGGAAGACCCA-3′. Sequencing was performed with the Thermo-Sequenase radiolabeled terminator cycle sequencing kit (Amersham Life Technologies).

The initial third and HMG domain of *SOX3,* consisting of a single exon, were PCR amplified and sequenced. The primers for the initial third of the gene, amplified in two fragments, were as follows: forward 5′-GGGGCTCGGTAATGATTGG-3′ and reverse 5′-CTACGGGGTTCTTGAGTTCAGTCT-3′; forward 5′-GGGGCTCGGTAATGATTGG-3′ and reverse 5′-CTA-

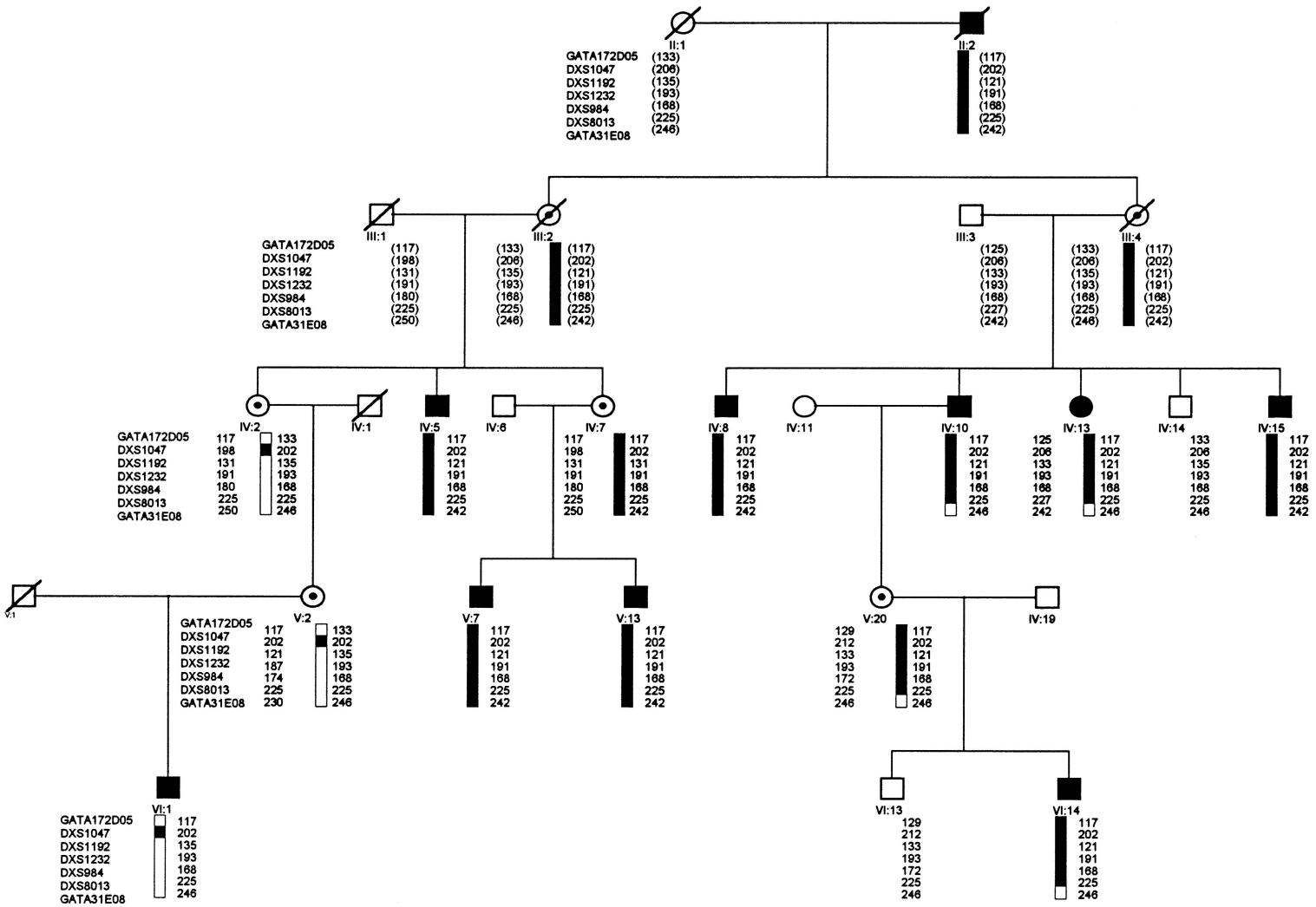

Figure 3 Haplotypes of left portion of pedigree B. Dark bars represent chromosome bearing disease gene; unshaded bars depict crossovers. Inferred haplotypes are in parentheses.

CGGGGTTCTTGAGTTCAGTCT-3′. S3F1 and S3F3 were used to amplify the HMG domain (Stevanovic et al. 1993).

Results

Descriptions of Families

Of 19 pedigrees with CMN, 4 had a pattern of autosomal dominant inheritance, with ~50% of offspring affected in the presence of father-to-son transmission. One family had a pattern of autosomal recessive inheritance with a history of consanguinity. Ten families had an X-linked pattern of inheritance with no father-to-son transmission, and four had an indeterminate pattern of inheritance. Of the X-linked pedigrees, two were X-linked recessive with only males affected, and eight were X-linked dominant with incomplete penetrance among female carriers. Three of the latter eight families consented to participate in, and are the subject of, the present study (fig 1).

No affected men in these three families had affected sons, consistent with X-linked inheritance. Among the three families, the penetrance among obligate female carriers (daughters of affected men) was 54% (pedigree A, two of six; pedigree B, three of five; pedigree C, two of two). Nystagmus had its onset before 6 mo of age in all affected individuals. Visual acuity among patients ranged from 20/20 to 20/100, with a median of 20/40. Apart from their reduced visual acuity and nystagmus, affected individuals had normal color vision, pupillary light reflexes, intraocular pressure, anterior segment, optic nerves, and retina. A normal electroretinogram (ERG) was obtained from the two individuals tested (pedigree B, V-40; pedigree C, III-4).

Linkage Analysis

Evaluation of markers within or in the vicinity of genes for OA (*OA1, DXS1043*), CSNB (*DXS6810, DXS1003*), and BCM (*DXS1108*) revealed no evidence of linkage in any of the three families. This confirmed that a separate locus is responsible for CMN.

The remainder of the X chromosome was screened with markers spaced at ~8-cM intervals. Linkage was initially established for all three pedigrees with marker DXS1047, with a combined LOD score of 10.296 without recombination. Evaluation of adjacent markers demonstrated evidence of linkage (table 1). Linkage without recombination was also established ~13 cM distal to DXS1047 with markers DXS984 (LOD score 6.695; $\theta = 0$) and DXS8013 (LOD score 2.060; $\theta = 0$).

Multipoint linkage analysis was performed by use of the following six markers and intervals: GATA172D05–4 cM–DXS1047–10 cM–DXS1192–2 cM–DXS1232–1-cM–DXS984–1 cM–DXS8013–1 cM–GATA31E08. A maximum multipoint LOD score of 10.790 was obtained around DXS1047 with a 1–LOD-unit support interval spanning ~7 cM in the region between GATA172D05 and DXS1192 (fig. 2). This interval excluded DXS984 and DXS8013, which were also linked without recombination by two-point analysis.

Affected individuals in all three pedigrees had a different allele size for the most closely linked marker, DXS1047.

Haplotype Analysis

Evaluation of haplotypes supports the conclusion that the gene for CMN is most closely linked to marker DXS1047. In pedigree B (fig. 3), a crossover occurred on either side of DXS1047 in individual IV-2, and it was passed to her carrier daughter (V-2) and affected grandson (VI-1). These recombinations were confirmed by repeated genotyping. Markers DXS984 and DXS8013 are noninformative in these individuals. Although a small inversion or recombinations encompassing DXS984 and DXS8013 may have occurred, these events are unlikely. For pedigree A, recombination occurred between GATA172DO5 and DXS1047 in three individuals and between GATA31E08 and DXS1232 in one individual. For pedigree C, no recombinations occurred in affected males or female carriers over the entire haplotype. X-linked inheritance or linkage to this region cannot be rigorously established in pedigree C alone, given its limited size.

Candidate Gene Analysis

Two genes closely linked to DXS984 were evaluated as candidates: *CDR1* and *SOX3*. A 1.3-kb fragment, which included the single exon of the *CDR1* gene, was successfully amplified with flanking primers and sequenced with nested primers. No mutations were found in two patients (IV-7 and V-10) from pedigree A, one unaffected male from pedigree A (V-9), and one unrelated control.

The initial third and HMG-box region of *SOX3* were PCR amplified and sequenced in three individuals from pedigree A (IV-7, V-9, and V-10), two individuals from pedigree B (VI-1 and VI-14), and one unrelated control. No mutations were observed.

Discussion

This study suggests that the most common mode of inheritance for CMN is X-linked dominant with incomplete penetrance. As these families were ascertained from a referral population of patients, they may not be representative of the true prevalence in the general population. The penetrance among obligate female carriers of ~54% distinguishes it from other X-linked disor-

ders—such as OA, CSNB, and BCM—that manifest nystagmus. Female carriers of BCM, which is considered X-linked recessive, have not been reported to manifest nystagmus. Although OA and CSNB are considered to be X-linked recessive, female carriers may rarely exhibit nystagmus (Pearce et al. 1973; Ruttum et al. 1992). Reasons for incomplete penetrance among female carriers include patterns of skewed X inactivation, interactions with other genes, and nongenetic, developmental influences on oculomotor development. In some instances, unaffected relatives, who do not manifest nystagmus, may nevertheless have subclinical eye movement abnormalities demonstrated by sensitive recording techniques (Dell'Osso et al. 1993).

The identification of a separate locus for X-linked CMN establishes that it is a distinct genetic entity. In the three pedigrees studied, CMN is most closely linked to marker DXS1047. Multipoint and haplotype analyses place the gene in an interval of ~7 cM between markers GATA172DO5 and DXS1192. The observation that affected individuals in all three families have a different allele size for the most closely linked marker, DXS1047, suggests that mutations within the gene may have arisen independently.

Two candidate genes were assessed for possible mutations: *CDR1* and *SOX3*. *CDR1* encodes the protein, cerebellar degeneration–related antigen, which is an immunogenic protein expressed in Purkinje cells. This protein is the autoimmune target in patients with paraneoplastic cerebellar degeneration consisting of ataxia and nystagmus (Peterson et al. 1992). The gene maps to Xq24-27 (Chen et al. 1990; Siniscalco et al. 1993) and consists of one exon with multiple tandem hexapeptide repeats coding for Glu-Asp (Dropcho et al. 1987). Its repetitive structure, which is thought to be the reason for its high immunogenicity, makes it difficult to amplify. No mutations were found.

SOX3 was also examined for mutations. This single-exon gene, which is expressed in fetal brain tissue (Stevanovic et al. 1993), is closely linked to this region (Gedeon et al. 1996; Mumm et al. 1997). No mutations were found.

X-linked CMN with incomplete penetrance among female carriers is likely the most common form of hereditary CMN. It maps to Xq26-27 and represents a distinct genetic entity apart from other X-linked disorders that feature nystagmus as a manifestation.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, ftp://ftp.genethon.fr/pub/Gmap/Nature-1995/data/data_chromX (for marker order and intervals for X chromosomal markers)

Genetic Location Data Base, http://cedar.genetics.soton.ac.uk/pub/chromX (for marker order and intervals for X chromosomal markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for X-linked CMN [MIM 31700], autosomal recessive CMN [MIM 257400], and autosomal dominant CMN [MIM 164100])

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