Clinical Characterization and Linkage Analysis of a Family With Congenital X-Linked Nystagmus and Deuteranomaly

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Objectives: To identify a congenital nystagmus locus on the X chromosome and to characterize the phenotype of a 4-generation family affected with congenital nystagmus and color deficiency.

Methods: Sixty-five patients underwent an eye examination, including evaluation for the presence of nystagmus and color vision abnormalities. Affected patients and obligate carriers of the congenital nystagmus mutation were genotyped with short tandem repeat polymorphisms located on the X chromosome, and these data were subjected to linkage analysis.

Results: Fourteen patients were affected with a horizontal, conjugate, congenital nystagmus. All examined patients had a visual acuity of 20/60 or better. There were no associated ocular or systemic findings except that 18 of the family members had deficient red-green color vision, which was classified as deuteranomaly (the most common form of anomalous trichromacy). Five patients exhibited nystagmus and deuteranomaly. Significant linkage was demonstrated between the nystagmus phenotype and 11 markers from Xq. The maximum lod score was 4.84 (θ = 0) and was obtained with marker DXS8041. Analysis of recombinants defined the disease interval to lie between markers ATA59C05 and DXS1192 (a 5.4-centimorgan region). The proximity of this locus to the red-green opsin gene cluster (11 centimorgans more telomeric) explains the frequent coexistence of nystagmus and color vision deficiency in this family.

Conclusions: We have identified the genetic locus of the X-linked congenital nystagmus gene in this family. The critical interval in this report is less than half the size of the previously described nystagmus locus. These findings will aid in identifying the gene responsible for this condition.

PATIENTS, MATERIALS, AND METHODS

Informed consent was obtained from all patients in the study. The presence of nystagmus was confirmed by slitlamp examination. Criteria for the diagnosis of congenital nystagmus included a history of nystagmus within the first 3 months of life and evidence of bilateral, conjugate, ocular oscillations by slitlamp examination. Sixty-five family members were examined by us (M.L.M., J.B., and R.V.K.). The medical records of 3 additional family members (2 of whom were deceased) were also examined. Screening examination included determination of visual acuity, slitlamp biomicroscopy, questioning about subjective photophobia or nystagopia, and direct ophthalmoscopy. Color vision was evaluated using either Ishihara plates (Kanehara & Co Ltd, Tokyo, Japan) or standard pseudospectral plates (Igaku-Shoin, Tokyo). Selected patients also underwent Farnsworth-Munsell 100-hue testing, Nagel anomaloscope testing, or both to further characterize the color vision defect. To determine whether any retinal disease was associated with the nystagmus, dark adaptometry and electroretinography were performed on selected patients. Dark adaptation was evaluated with a Goldmann-Weekers dark adaptometer. Patients underwent 30 minutes of dark adaptation before electroretinography under full-field Ganzfeld dome stimulation (LKC Technology, Gaithersburg, Md) according to the International Society for Clinical Electrophysiology of Vision protocol.

Blood samples were obtained from affected family members and their parents, siblings, and spouses. Blood, 7 to 10 mL, was obtained from each patient in EDTA-containing glass tubes. DNA was prepared from the blood using a nonorganic method. Oligonucleotide primers flanking short tandem repeat polymorphisms located on the X chromosome were obtained from Research Genetics, Huntsville, Ala. Patients were then screened for variations in these X chromosome polymorphisms as previously described. Clinically normal women who were obligate carriers of the nystagmus mutation based on the existence of affected children were considered affected. Clinically normal female children of affected men were considered to have an “unknown” phenotype for the linkage analysis, unless they were obligate carriers.

Pairwise linkage analysis was performed with the MLINK and LODSCORE programs as implemented in the FASTLINK (version 2.3) version of the LINKAGE program package (available via the Internet at http://linkage.cpmc.columbia.edu/software/linkage). Allele frequencies were assumed to be equal for each marker. The true population allele frequencies for each marker could not be reliably estimated from the small number of spouses in the families. To show that the assumption of the equal allele frequencies would not affect our linkage results, we recalculated the lod scores using allele frequencies for the “affected” allele of 2 of the most tightly linked markers (DXS1047 and DXS8041), ranging from 0.01 to 0.50. The maximum lod score remained greater than 4 for each of these markers for all allele frequencies in this range. The genetic maps used for analysis of recombinants were obtained from the Marshfield Center for Medical Genetics, Marshfield, Wis, via the Internet at http://www.marshmed.org/genetics.

Figure 1. Pedigree. The left half of each symbol represents the clinical presence or absence of nystagmus, while the right half represents the status of the individual’s color vision. Solid symbols indicate individuals found to be clinically affected; open symbols, unaffected individuals; shaded symbols, individuals whose status could not be ascertained; open symbols with a black circle in the center, obligate carriers who are clinically unaffected; and slashed symbols, deceased individuals. All patients marked with a backward slash were examined by 1 or more of us (M.L.M., J.B., and R.V.K.). All patients marked with a forward slash were included in the linkage analysis. Individual IV-6 is represented as an obligate carrier because of her status as a molecularly confirmed identical twin of an obligate carrier. The affection status of the deceased individuals was obtained historically.

explore the relationship of the congenital motor nystagmus gene locus and the red and green opsin genes on Xq28 as an explanation for the coexistence of the 2 phenotypes in some members of this family.

RESULTS

CLINICAL FINDINGS

The pedigree structure and clinical features of each family member are summarized in Figure 1. Thirteen patients were found to have conjugate horizontal nystagmus. The waveform was pendular, jerk, or a combination of both waveforms. Examination of the medical records of 3 additional patients (II-5, III-6, and IV-13) revealed that they also had horizontal nystagmus. The visual acuity ranged from 20/20 to 20/60, and the decrease in visual acuity was proportional to the amplitude of the nystagmus. In most patients, the nystagmus had a null position in which the amplitude of the nystagmus was dampened. One family member (V-23) had undergone a Kestenbaum procedure at age 7 years for an abnormal head position associated with
the nystagmus. No other neurologic disorders were present in any of the affected patients. No patients complained of photophobia. Direct ophthalmoscopy in all patients, and indirect ophthalmoscopy in selected patients, detected only 1 isolated fundus abnormality in the entire family, a chorioid hemangioma in patient V-8.

There were no subjective complaints of nystalopia. Dark adaptometry and electroretinography were performed on 8 patients to evaluate their retinal function. In particular, we attempted to rule out conditions such as cone-rod dystrophy or congenital stationary night blindness, which could be associated with the type of nystagmus seen in this family. In 4 patients with nystagmus (V-1, V-3, III-3, and V-32) and 2 patients who were obligate carriers of the nystagmus gene (IV-10 and IV-12), the results of dark adaptation testing were normal, and the patients reached threshold after 15 minutes. Electroretinographic results were normal in these patients as well.

Of the 54 patients screened with pseudoisochromatic plates, 18 had color vision defects by color plate screening. Eleven of these patients (III-3, III-8, IV-6, IV-7, IV-9, IV-10, IV-11, IV-12, V-17, and V-40) were further characterized with the Nagel anomaloscope. All 11 patients were found to be deuteranomalous trichromats, with an average anomalocromatic value of 0.5 (mean, 24.42; range, 0-35; normal, 45). The matching range was narrow and extended from 0 to 30 on the red-green mixture scale. To further characterize the color defect, 7 patients (III-3, IV-10, IV-12, IV-16, V-32, V-39, and V-40) underwent Farnsworth-Munsell 100-hue testing under standardized conditions. A bipolar axis was evident with the highest error scores centered on caps 58 and 15, which is also characteristic of a deutan defect.

**LINKAGE ANALYSIS**

Forty-five family members (11 clinically affected, 9 obligate carriers, 13 clinically unaffected men, 10 unaffected women, and 2 informative spouses) were genotyped using short tandem repeat polymorphisms on the X chromosome. Two-point linkage analysis revealed significant linkage (lod score > 3) to 11 markers known to lie at Xq24. The maximum lod score of 4.84 (θ = 0) was obtained with marker DXS8041. The analysis of recombination events in affected individuals is also shown in Figure 2. These recombination events suggest that the X-linked nystagmus gene is located between markers ATA59C05 and DXS1192. This interval lies approximately 11 centimorgans centromeric to the red-green opsin gene cluster.

Several recombination events between the nystagmus-causing gene and the red-green opsin cluster resulted in interesting patterns of inheritance. One woman (II-3) who is a carrier for the color vision abnormality and nystagmus had 1 son affected with deuteranomaly alone and a second who inherited both conditions. Haplotype analysis revealed a recombination event that caused 1 brother to “lose” the abnormal nystagmus gene. Similarly, siblings V-1 and V-3 are affected with only nystagmus. Individual V-1 manifests only nystagmus because she has 1 normal copy of the red-green opsin cluster that she inherited from her father. In contrast, her brother (V-3) manifests only nystagmus for a different reason. In this individual, a recombination event occurred between the nystagmus locus and the red-green opsin cluster, causing him to inherit only the nystagmus gene.

**COMMENT**

The inheritance pattern of the congenital nystagmus in this family is consistent with an X-linked gene that is completely penetrant in the hemizygous state and occasionally penetrant in the heterozygous state. X-linked recessive congenital nystagmus has been reported previously.16 The presence of affected women has been previously observed, and this phenomenon could in principle be ex-

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Figure 2. Two-point linkage data and analysis of recombinant individuals. Forty-five mapped genetic markers from the long arm of the X chromosome are listed along the left side of the figure in the order they were obtained from Marshfield Center for Medical Genetics, Marshfield, Wis, via the Internet at http://www.marshmed.org/genetics. The most centromeric marker is at the top. Linkage analysis with 11 markers resulted in significant lod scores (> 3), and those scores are noted to the right of each marker. (Maximum lod scores for each marker occurred at θ = 0) were obtained with marker DXS8041. The analysis of recombination events in affected individuals is also shown in Figure 2. These recombination events suggest that the X-linked nystagmus gene is located between markers ATA59C05 and DXS1192. This interval lies approximately 11 centimorgans centromeric to the red-green opsin gene cluster. The pedigree revealed a recombination event that caused 1 brother to “lose” the abnormal nystagmus gene. Similarly, siblings V-1 and V-3 are affected with only nystagmus. Individual V-1 manifests only nystagmus because she has 1 normal copy of the red-green opsin cluster that she inherited from her father. In contrast, her brother (V-3) manifests only nystagmus for a different reason. In this individual, a recombination event occurred between the nystagmus locus and the red-green opsin cluster, causing him to inherit only the nystagmus gene.
linked congenital nystagmus have been recently reported. Identification of this gene will enable clinicians to accurately diagnose this form of congenital nystagmus with a simple genetic test. This will also enable the ophthalmologist to give accurate genetic counseling and to provide reassurance that affected individuals will not have notable visual impairment.

In summary, we describe an X-linked pedigree of congenital nystagmus that is linked to a 5.4-centimorgan interval on Xq24-q28. The location of the red-green opsin gene cluster at Xq28 explains the frequent coexistence of congenital nystagmus and deuteranomaly in this family.

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