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Linkage analysis of two families with X-linked recessive congenital motor nystagmus

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Abstract X-linked recessive congenital motor nystagmus was identified in two Chinese families living in the Guangdong province of China. Nystagmus was noticed in early childhood. Only males in the families were affected and all obligate carriers did not have nystagmus. Linkage study was performed using microsatellite markers at about 10 cM intervals on the X chromosome. The nystagmus in these two families is linked to markers in the region of chromosome Xq23–q27, including *DXS1001*, *DXS8009*, and *DXS1047*. *DXS1047* gave the highest lod score of 3.53 at $\theta=0$.

Keywords Congenital motor nystagmus · X-linked recessive · Linkage · Locus · NYS1

Introduction

Congenital nystagmus (CN) is an ocular condition characterized by bilateral involuntary ocular oscillation. CN is commonly observed in ocular diseases such as albinism, Leber congenital amaurosis, aniridia, cone or cone-rod dystrophy, macular coloboma, optic nerve dysplasia, etc. In rare cases, CN may occur without any other known ocular or systemic disease, and is referred to as idiopathic CN or congenital motor nystagmus (CMN) (Cabot et al. 1999; Kerrison et al. 1999). Patients with CMN have normal or mild-to-moderately reduced visual acuity. Their optic media, fundus appearance, and

cone-rod function are rather normal. These lead to a presumption that the primary defect of CMN is in the central nervous system, where ocular motor function is controlled.

CMN has been described as being transmitted as an autosomal dominant (OMIM 164100, *NYS2*; OMIM 608345, *NYS3*; OMIM 193003), autosomal recessive (OMIM 257400), and X-linked dominant or recessive (OMIM 310700) trait. No gene has been identified as being responsible for CMN, although linkage studies have suggested several loci on chromosome Xp11.4–p11.3, Xq26–q27, and 6p12, for X-linked dominant (Cabot et al. 1999; Kerrison et al. 1999, 2001; Zhang et al. 2005) and autosomal dominant (Kerrison et al. 1996) CMN. The genetic locus for X-linked recessive CMN has not been reported. Here, we describe two families with X-linked recessive CMN and map the disease to Xq23–q27, between *DXS8055* and *DXS1205*.

Materials and methods

Families and clinical data

Two families living in Guangdong province with X-linked recessive CMN, including nine affected and ten unaffected individuals, participated in the linkage study. Informed consent conforming to the tenets of the Declaration of Helsinki and following the Guidance of Sample Collection of Human Genetic Diseases (863-Plan) by the Ministry of Public Health of China was obtained from the participating individuals prior to the study. Ophthalmological examination (by X.G. and Q.Z.) included visual acuity, color vision, slit-lamp, and fundoscopic examinations. A subject was considered to have CMN if the following criteria were met: (1) CN noted before 1 year old; (2) clear optic medium with normal appearance of fundus; (3) normal color vision and no night blindness; and (4) exclusion of other known ocular or systemic diseases. Electroretinogram (ERG) responses were recorded in selected patients

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Fig. 1 Fundus photograph and electroretinogram (ERG) recording. An example of the normal fundus appearance and normal cone-rod response on ERG recording of the individual III6 in family B is shown

consistent with International Society for Clinical Electrophysiology of Vision standards (ISCEV 1989). Genomic DNA was prepared from venous blood.

Genotyping and linkage analysis

Genotyping for all participating family members was performed using 5'-fluorescently labeled microsatellite

markers. An X chromosome-wide scan was carried out using 28 panels of the ABI PRISM linkage Mapping Set Version 2, which included 18 markers spaced at intervals of about 10 cM. Polymerase chain reaction (PCR) was conducted at 94°C for 8 min, followed by 10 cycles of amplification at 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s; then 20 cycles at 89°C for 15 s, 55°C for 15 s, 72°C for 30 s; finally at 72°C for 10 min. After mixing with GENESCAN 400HD [ROX] standard (ABI) and

Table 1 Two-point linkage results between microsatellite markers of the X chromosome and congenital motor nystagmus (CMN) in the Chinese families

Markers ^a	Position		Lod score at $\theta =$							$Z_{\max}$	$\theta_{\max}$
	cM	Mb	0	0.01	0.05	0.1	0.2	0.3	0.4		
DXS1060	10.10	4.87	−∞	−6.71	−3.33	−1.96	−0.76	−0.23	−0.01	0.02	0.46
DXS8051	15.70	8.91	−∞	−6.50	−3.14	−1.81	−0.67	−0.19	0.00	0.02	0.45
DXS987	25.50	14.07	−∞	−3.91	−1.90	−1.11	−0.44	−0.15	−0.03	0.00	0.48
DXS1226	36.80	?	−∞	−0.81	0.40	0.75	0.84	0.63	0.31	0.85	0.17
DXS1214	46.20	30.62	−∞	−0.73	0.49	0.87	0.98	0.80	0.47	0.99	0.17
DXS1068	56.20	37.94	−∞	−3.10	−1.14	−0.41	0.12	0.26	0.20	0.26	0.31
DXS993	66.10	40.90	−∞	−5.61	−2.88	−1.77	−0.78	−0.30	−0.07	0.00	0.50
DXS991	86.90	55.40	−∞	−5.18	−2.47	−1.38	−0.46	−0.08	0.04	0.05	0.41
DXS986	95.90	79.19	−∞	−1.32	−0.05	0.39	0.63	0.58	0.36	0.64	0.22
DXS990	104.90	92.81	−∞	−4.37	−2.30	−1.45	−0.67	−0.30	−0.10	0.02	0.57
DXS1106	115.10	102.54	−∞	−3.37	−1.41	−0.68	−0.13	0.04	0.06	0.06	0.37
DXS1210	119.20	108.40	−∞	−0.56	0.06	0.25	0.33	0.27	0.15	0.33	0.19
DXS8055	126.80	114.48	−∞	0.88	1.39	1.44	1.22	0.83	0.39	1.45	0.09
DXS1001	139.40	119.62	2.33	2.29	2.13	1.93	1.50	1.04	0.55	2.33	0.00
DXS8009	148.40	125.90	2.37	2.32	2.15	1.92	1.44	0.92	0.38	2.37	0.00
DXS1047	150.30	128.80	3.53	3.48	3.25	2.95	2.32	1.63	0.86	3.53	0.00
DXS1205	163.70	139.99	−∞	−0.26	0.34	0.51	0.53	0.42	0.23	0.55	0.16
DXS1227	164.70	140.53	−∞	−2.26	−0.94	−0.45	−0.07	0.05	0.06	0.06	0.36
DXS8043	176.70	143.73	−∞	−4.81	−2.16	−1.16	−0.37	−0.11	−0.04	0.28	0.74
DXS8091	186.30	147.31	−∞	−6.37	−3.58	−2.40	−1.27	−0.67	−0.27	0.32	0.75
DXS1073	196.50	153.39	−∞	−7.60	−4.16	−2.73	−1.38	−0.67	−0.25	0.11	0.63

^aDXS8081 and DXS8064 between DXS8055 and DXS1001, DXS8074 and DXS1062 between DXS1047 and DXS1205, as well as DXS8057 between DXS1001 and DXS8009, were genotyped but were uninformative in these families

deionized formamide, PCR products were denatured at 96°C for 5 min and then immediately placed on ice for 5 min. The amplicons were separated on an ABI3100 DNA sequencer. Genotyping data were analyzed using the Gene Mapper version 3.5 software package from ABI. Two-point linkage analysis was performed by using the MLINK program of the FASTLINK implementation of the LINKAGE program package (Lathrop and Lalouel 1984; Schaffer et al. 1994). Only one set of genotyping data from the homozygote twins (individuals V1 and V2 in family B) was used to calculate lod scores. CMN in the two families was analyzed as an X-linked recessive trait with full penetrance and a disease-gene allele frequency of 0.0001. Additional markers were selected around the candidate region according to the National Center for Biotechnology Information map.

Haplotypes were generated using the program Cyrillic 2.1, and confirmed by inspection.

Results

In the two families with X-linked recessive CMN, the disease was transmitted from female carrier to affected son. All affected individuals were male and all obligate carriers had no sign of nystagmus. All affected individuals were noticed to have nystagmus in early childhood. Of the six affected individuals, visual acuity was over 0.8 in three cases and between 0.3 and 0.5 in three cases. Complaints of night blindness or photophobia were not recorded in any affected individual in the two families. In a recent examination, the optic medium and fundus of

Fig. 2 Pedigree and haplotype of the two families with congenital motor nystagmus (CMN). *Black bars* Disease allele, *open bars* normal alleles. *Black squares* Individuals affected with CMN, *circles with black dots* obligate carriers

Fig. 3 Diagram of the X chromosome showing the critical region for X-linked CMN. *A*, *B*, *C* Critical regions for X-linked dominant CMN reported by Kerrison et al. (1999, 2001) and Zhang et al. (2005), respectively; *D* linked region for X-linked recessive CMN in this study

all affected individuals were normal (Fig. 1). Normal color vision was recorded in four affected individuals (individuals III6, IV3, IV4, and IV5), and the other two were too young to read Ishihara plates for color vision evaluation. ERG recorded in individual III6 showed normal cone and rod response (Fig. 1).

Upon an initial X chromosome scan, two-point linkage analysis excluded all X chromosome markers with lod scores of minus infinity except *DXS1001* and *DXS1047*, which gave lod scores greater than 2. Fine mapping and haplotype analysis confirmed the locus on Xq23–q27 (Table 1, Fig. 2). Three microsatellite markers, *DXS1001*, *DXS8009*, and *DXS1047*, generated positive lod scores, with the highest lod score of 3.53 for *DXS1047* at $\theta=0$.

Haplotypes in this region of both families supported the linkage results (Fig. 2). Recombination at *DXS8055* for individual IV3 set the centromeric boundary, and recombination at *DXS1205* in individual IV5 with further recombination at *DXS1227* in individual IV1 (confirmed in individuals V1 and V2) set the telomeric

boundary for the linked region. Therefore, the disease gene should be located between *DXS8055* and *DXS1205*.

Discussion

In this study, X-linked recessive CMN in two Chinese families was mapped to a locus on chromosome Xq23–q27 between *DXS8055* and *DXS1205*. Exclusion of other regions in the X chromosome by lod scores of minus infinity, lod scores greater than 2 for three markers inside the linked region (Terwilliger and Ott 1994), and haplotype observation, all support the conclusion that this locus is linked to X-linked recessive CMN.

X-linked dominant CMN with incomplete penetrance among female carriers has been mapped to two regions: Xp11.4–p11.3 (Cabot et al. 1999) and Xq26–q27 (Kerrison et al. 1999, 2001; Zhang et al. 2005). The X-linked recessive CMN in the two Chinese families examined

here mapped to Xq23–q27, which harbors the X-linked dominant CMN locus (Fig. 3). The present result provides evidence that X-linked recessive CMN and X-linked dominant CMN may be caused by the same gene with different mutations. Different mutations in the same gene causing a similar phenotype but with a different pattern of inheritance have been identified in several genes related to ocular diseases, e.g., *RP1* (Khaliq et al. 2005; Riazuddin et al. 2005; Sullivan et al. 1999), *RHO* (Dryja et al. 1990; Rosenfeld et al. 1992), *LRP5* (Jiao et al. 2004; Toomes et al. 2004), etc. On the other hand, linkage to the same region does not rule out the possibility that the disease may result from mutation of different genes (Toomes et al. 2004). These possibilities can be easily resolved upon cloning of the causative gene.

Several candidate genes, including *CDRI*, *SOX3*, *SLC25A14*, *SLC9A6*, and *FGF13*, have been screened in patients with X-linked dominant CMN but no causative mutations were detected (Kerrison et al. 1999, 2001; Zhang et al. 2005). Other potential candidate genes inside the linked interval include, but are not limited to, *GRIA3*, *MBNL3*, and *FHL1*.

Three other X-linked disorders, including Nettleship-Falls ocular albinism (OMIM 300500), *CSNB1* (OMIM 310500), and blue-cone monochromacy (OMIM 303700), have nystagmus in addition to their specific defects. Linkage to loci for these three disorders in the two Chinese families studied here is excluded by linkage analysis, and the clinical manifestation is different.

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