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MAPPING THE DISEASE GENE IN TWO CONGENITAL MOTOR NYSTAGMUS FAMILIES

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Backgrounds. Congenital motor nystagmus CMN is a subtype of Congenital nystagm and usually diagnosed after sensory defect nystagmus is excluded. Although four sites have been located on X chromosome but we think there is still another disease gene on X chromosome. So in our study, we will narrow down the candidate region and identify the candidate gene for X-linked CMN of two Congenital motor nystagmus families.

Methods. Two families with CMN were investigated. Genotyping and linkage analysis were conducted in these two Chinese families.

Results. These two families were affected by X-linked CMN with incomplete penetrance. Two-point linkage analysis revealed significant maximum logarithm of odds (LOD) scores of 8.55 (DXS1047, sita = 0) and 3.91 (DXS1211 and DXS1205, sita = 0) at the family nys-01 and the family nys-02 respectively. Haplotype construction and multipoint linkage analysis also confirmed the locus and refined the locus to a 7.1 cM interval between the markers DXS8044 and DXS8041 in chromosome Xq25-q26.3.

Conclusion. We have mapped the nystagemus gene to an interval of 7.1 cM, at the location of Xq15-q26.3, such interval shares no overlap with previous Xq26-q27 locus.

Key words: congenital motor nystagmus, X-linked, linkage analysis, X-chromosome randomly-inactivation, gene map.

Congenital nystagmus (CN) is a hereditary disease characterized by bilateral ocular oscillations that begin in the first 6 month of life [1]. Congential nystagmus always accompanied with other diseases such as leukotrichia, the monochromasia, cataracts and optic atrophy et al. Congenital motor nystagmus CMN is a subtype of CN and usually diagnosed after sensory defect nystagmus is excluded [2]. CMN is a genetic heterogeneity eye disease to date, various inheritance patterns for CMN have been reported including autosomal dominant, autosomal recessive and X-linked [3]. Among these types, X-linked dominant inheritance with incomplete penetrance is the most probable mode of inheritance. So far, four sites have been located, Xp11.4-p11.3 [4], Xq26-q27 (NYS1, OMIM 310700, X-linked dominant and recessive inheritance) [3; 4], 6p12 (NYS2, OMIM 164100) [5; 6] and 7p11.2 (NYS3, OMIM 608345) [7].The virulence gene of Xq26-q27 is *FRMD7* [8], but there maybe another virulence genes in this site [9].

Methods

Subjects

Two families with CMN from Shangdong and Henan province were investigated they were respectively named nys-01 and nys-02. There are four generation of family nys-01 including 15 patients 22 normal individuals and 15 spouse (Fig. 1). There are four generation of family nys-02 9 patients 9 normal individuals and 8 spouse included (Fig. 2). 69 individuals of the two families gave consent informed to the study protocol which was approved by the Ethics committee of Tianjin Eye Hospital (Tianjin, China). 53 members of them were invited for a detailed clinical examination including direct-flashlight test, visual loss test, examination with slit-lamp microscope and examination of the fundus. Criteria for the diagnosis of CMN included onset of nystagmus before the age of 6 month 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 [4]. Electroretinography, which is useful in the evaluation of the patient with nystagmus, was performed in two random selected individuals.

Linkage analysis

As we investigated there is no male-to-male transmission but frequent male-to-female transmission; about half unaffected female were born to affected men, and some unaffected women passed the disease to the next generation. These indicated the disease gene of CMN is in X chromosome and its inheritance mode is X-linked dominant inheritance with incomplete penetrance. So we scanned the X chromosome only.

Blood samples were collected with informed consent from 69 pedigree members of the two families. Genomic DNA of all 23 affecteds family members were

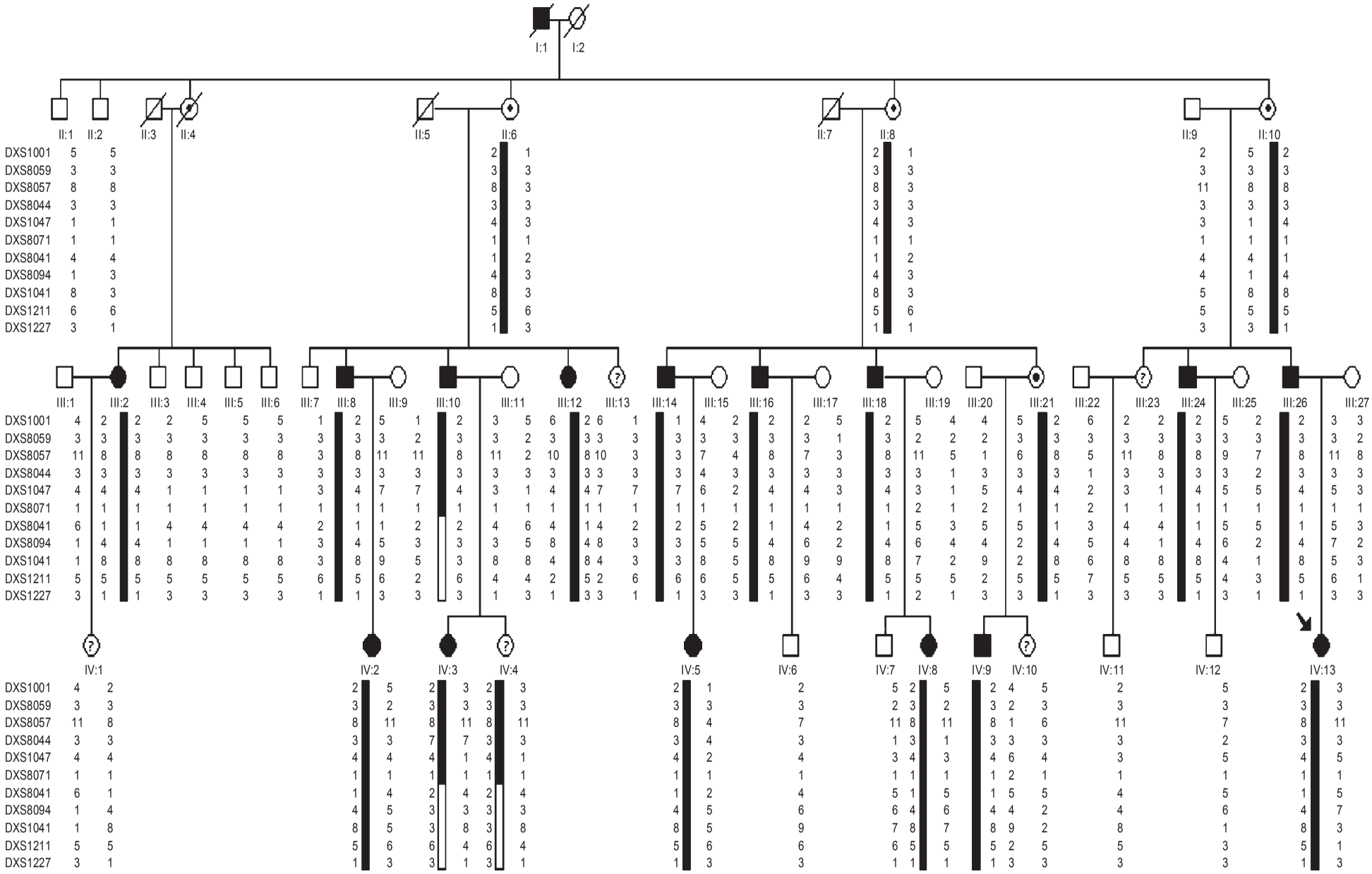

Fig. 1. Haplotype analysis of Family nys-01. Markers are listed from top to bottom:
centromere-DXS1001-DXS8059-DXS8057-DXS8044-DXS1047-DXS8071-DXS8041-DXS8094-DXS1041-DXS1211-DXS1227-telomere

isolated from peripheral blood samples by standard procedures. All specimens were quantified by spectrophotometry and diluted to 50 ng/мL for polymerase chain reaction (PCR) amplificaton. At the same time we take family CEPH-02 from CEPH for control group. Genome-wide screening was performed with 382 markers spaced about 10 cM interval (ABI PRISM Linkage Mapping Set, Version 2.5, Applied Biosystems, USA). Fine mapping was accomplished using fluorescein-labeled primers from the Decode linkage map (Kong et al., 2002). Multiplex PCR was performed in standard techniques with primers and Ampli Taq Gold DNA polymerase from Perkin Elmer. The reaction products 1 мL, Liz Size Standard-500 0.2 мL and Hi-Di formanamide 9 мL,were electrophoresed and visualized on 3130 Genetic Analyzer. Alleles were analyzed by GENESCAN Analysis version 3.7 and GENOTYPER version 3.7 software. Two-point LOD scores were calculated by the MLINK program of the LINKAGE package (version 5.1). We assumed the disease is an autosomal dominant trait with 99% penetrance. Marker allele frequencies were set at 1/*n*, where *n* is the number of alleles observed. We assumed gene frequencies of 0.0001 and no sex difference in recombination rates. Multi-point linkage analysis was used to estimate the optimal position. For multi-point linkage calculation, the genetic distance between loci was calculated by the Gene Browser (http://www.genome.ucsc.edu). The haplotype was constructed using the Cyrillic program to define the borders of the cosegregating region.

Results

Clinical findings

After clinical diagnose 23 members of the two families were affirmed have been affected

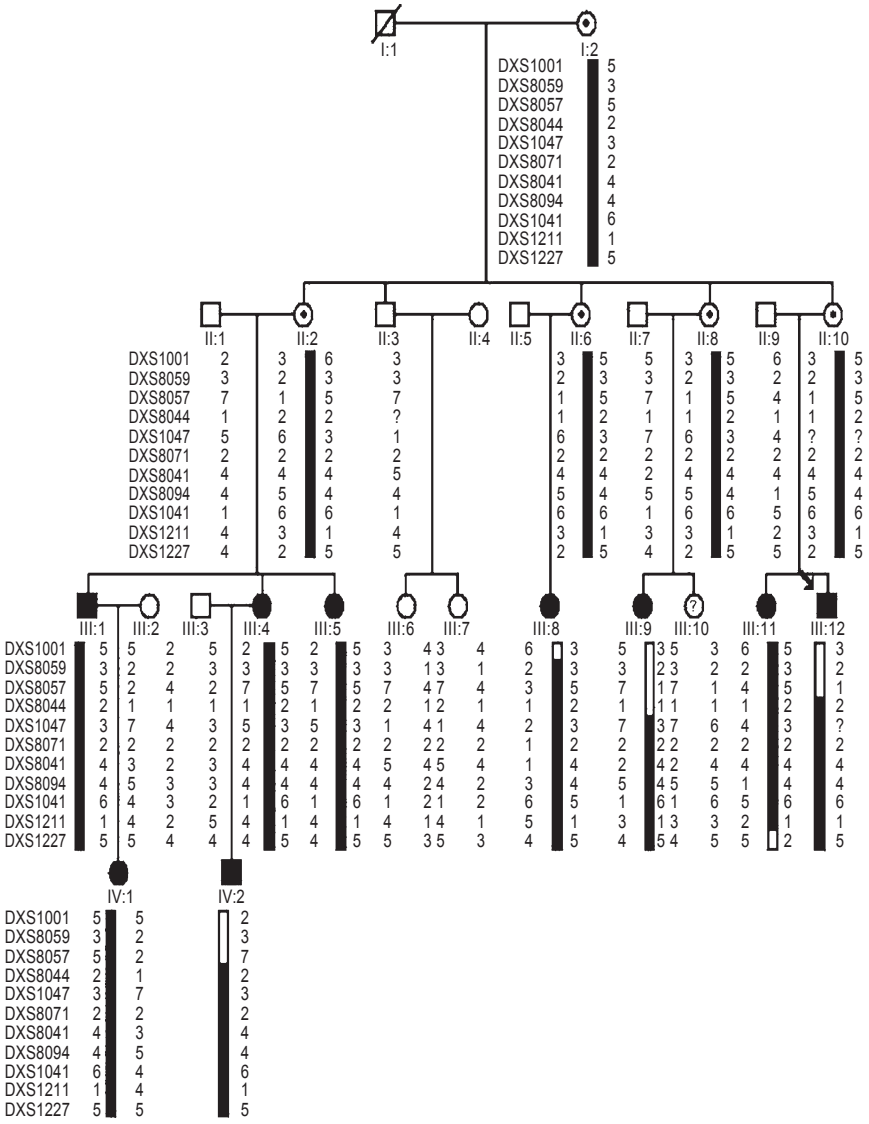

Fig. 2. Haplotype analysis of Family nys-02. Markers are listed from top to bottom:centromere-DXS1001-DXS8059-DXS8057-DXS8044-DXS1047-DXS8071-DXS8041-DXS8094-DXS1041-DXS1211-DXS1227-telomere

CMN. All of these patients have different visual loss 0.2–1.2 no other oculopathy accompanied except CMN and there was no history of other ocular or systemic abnormalities of their spouse.

Genetyping and Linkage Analysis

69 members of the two families 23 clinically affected 46 unaffected were genetyped. Linkage study was performed using 16 microsatellite markers at about 10 cM intervals on the X chromosome. Microsatellite marker DX1047 in both families, generated significant positive LOD scores with LOD2 and = 0. Later, linkage study was performed using 20 microsatillite markers around DXS1047,in nys-01 family, some microsatillites such as DX8059, DX8071 and so on,also revealed positive high LOD scores (Table 1). While, we also got high LOD scores between DXS1047 and DXS1205 in family nys-02 (Table 2). Haplotypes analysis of family nys-01 shows that there are special haplotypes between DXS8055 and DXS1211 in all affecteds and carries, recombination occurred in individual 10, 20 and 22 (see fig. 1). Therefore we assigned a locus for CMN to a 13 cM interval between DXS1001 and DXS8041 in family nys-01. Haplotypes were also constructed for the

Table 1

Two-point LOD Score with Polymorphic DNA Markers on the X Chromosome (Family nys-01)

Order	Position, Mb	LOD score at 0 =					Z_{max}	θ_{max}
		0.0	0.1	0.2	0.3	0.4		
DXS8055	114.5	-0.44	2.72	2.38	1.69	0.84	2.72	0.1
DXS8053	115.4	2.85	3.90	3.14	2.14	1.00	3.90	0.1
DXS8059	122.0	2.59	1.76	1.68	1.29	0.72	2.59	0.0
DXS8078	126.3	3.77	3.15	2.45	1.67	0.84	3.77	0.0
DXS1047	128.8	8.55	7.17	5.63	3.87	1.86	8.55	0.0
DXS8071	131.2	3.65	3.04	2.37	1.61	0.81	3.65	0.0
DXS8041	133.4	1.11	6.42	5.17	3.59	1.73	6.42	0.1
DXS8074	133.8	3.72	3.01	2.25	1.46	0.67	3.72	0.0
DXS8033	133.9	-3.66	4.00	3.16	2.09	0.93	4.00	0.1
DXS8094	136.0	-3.85	6.47	5.23	3.64	1.76	6.47	0.1
DXS1041	136.3	-6.44	4.21	3.36	2.25	1.02	4.21	0.1
DXS1211	138.0	-6.73	3.97	3.17	2.11	0.95	3.97	0.1

Table 2

Two-point LOD Score with Polymorphic DNA Markers on the X Chromosome (Family nys-02)

Order	Position, Mb	LOD score at 0 =					Z_{max}	θ_{max}
		0.0	0.1	0.2	0.3	0.4		
DXS8059	121.9	-4.11	-0.12	0.22	0.29	0.20	0.29	0.3
DXS8098	122.6	-3.69	0.81	0.85	0.58	0.22	0.85	0.2
DXS8057	123.3	-9.79	0.41	0.75	0.65	0.33	0.75	0.2
DXS8009	125.8	-3.07	2.03	1.67	1.10	0.44	2.03	0.1
DXS8044	126.3	-4.89	0.32	0.42	0.36	0.22	0.42	0.2
DXS1047	128.8	3.61	3.02	2.35	1.60	0.78	3.61	0.0
DXS1041	136.2	1.81	1.49	1.13	0.73	0.31	1.81	0.0
DXS1211	138.0	3.91	3.27	2.56	1.75	0.86	3.91	0.0
DXS1205	139.9	3.91	3.27	2.56	1.75	0.86	3.91	0.0
DXS1227	140.5	-3.99	1.34	1.23	0.95	0.54	1.34	0.0

analyzed markers on family nys-02 (see Fig. 2). On individual 18 and 22, recombination events were found. Then according our multipoint linkage analysis on the two families Fig. 3 for nys-01, Fig. 4 for nys-02, the disease gene were located between DXS8044 and DXS1227. Finally, according with the genetypes of nys-01 and nys-02, we located the disease gene of CMN to a 7.1 cM interval between DXS8041 and DXS8044 on Xq25-26.3 (Fig. 5).

So we located the CMN virulence genes to a 7.1 cM interval between Xq25 and Xq26.3 and supposed there were two independent CMN virulence gene.

Discussion

This study suggested that the most common mode of inheritance for CMN is X-linked dominant with incomplete penetrance and the morbidity of the female family members is 60%.This study also validated a classical hypothesis — Lyon hypothesis. As we all known, female with two X chromosomes just have equal X chromosome common gene products with male this means that both males and females rely on the information from only a single X chromosome. Therefore, it is only one X chromosome that provides genetic information in both males and females. This phenomenon was interpreted as a means of dosage compensation for X-linked genes. On 1960 Ohno and his colleagues showed that female mice consisted of a single condensed X-chromosome. On 1961 geneticists Mary Lyon proposed the condensed X chromosome is inactivated that's famous Lyon Hypothesis. (1) In the day 15–16 of embryonic development one of the two copies of the X chromosome present in female mammals is inactivated while the whole cell number is about 5000. (2) The choice of which X chromosome will be inactivated is random it may be from father or mother. (3) Once an X chromosome is inactivated it will remain inactive throughout the lifetime of the cell and its descendants in the organism. But in the next meiotic mitosos term the inactivated X chromosome will be resurrection and on the next new random inacvation trip again. This hypothesis can explain why some female carriers such as 14, 28, 42 didn't affected CMN it may because the disease gene just right on the inactivated X chromosome.

On 1999 Three families with CMN inherited in an X-linked, irregularly dominant pattern were investigated with linkage analysis by Kerrison. He located NYS1 gene between GATA172DO5 and DXS1192 on Xq26-q27 [4] later this gene were located to 15.8 cM interval between ATA59C05 and DXS1192 [10]. On 2011 he narrowed the CMN locus down to a region between ATA9909 and DXS1211 [11]. However, these results is still overlaps 8.7 cM than our defined region in this study. It's interesting there is a report find another inheritance pattern — X linked dominant pattern with 100% penetrance, in a Chinese family, and they refines a locus for X-linked dominant CMN to a 4.4 cM re-

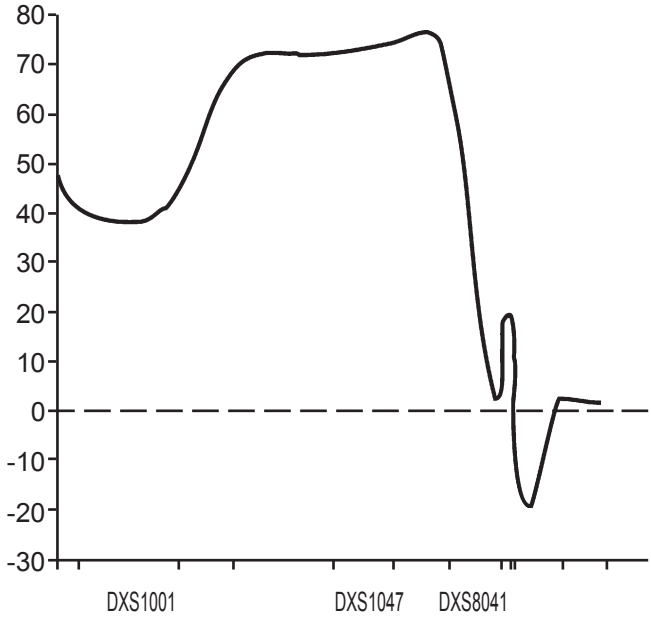

Fig. 3. Multipoint LOD score with support interval localizes the gene in a region between DXS1001 and DXS8041 in family nys-01. The markers and intervals: DXS8055-0.9cM-DXS8053-4.2cM -DXS1001-2.3cM -DXS8059-4.2cM –DXS 8078- 2.53cM -DXS1047-2.3cM -DX8071-2.26cM -DXS8041-0.38cM -DXS8074-0.1cM -DXS8033-2.1cM -DXS8094-0.03cM -DXS1041-1.77cM -DXS1211

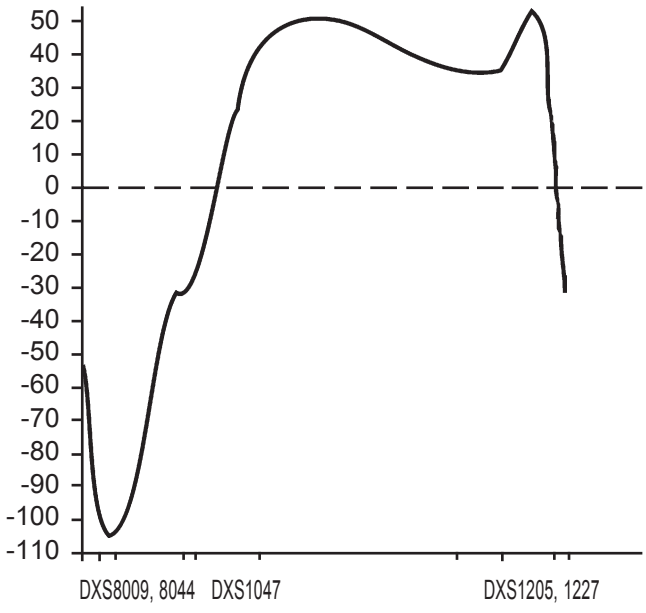

Fig. 4. Multipoint LOD score with support interval localizes the gene in a region between DXS8044 and DXS1227 in family nys-02. The markers and intervals: DXS8059-0.65cM-DXS8098-0.66cM-DXS8057-2.6cM-DXS8009-0.43cM-DXS8044-2.47cM-DXS1047-7.46cM-DXS1041-1.76cM-DXS1211-1.95cM-DXS1205-0.54cM-DXS1227

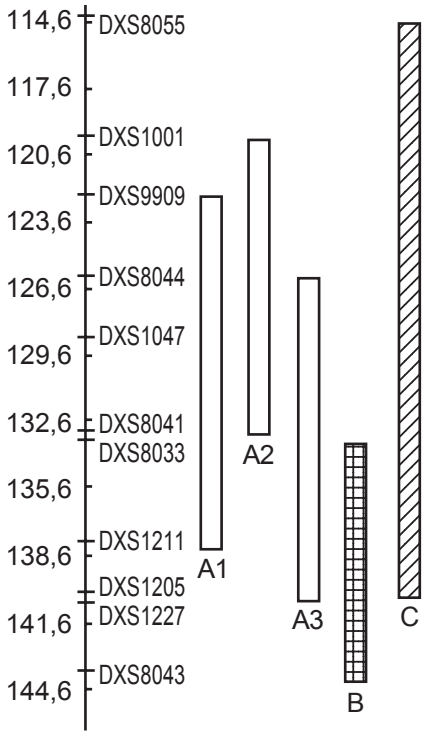

Fig. 5. Xq-linked nystagmus with different inheritance pattern Series A shows the X-linked dominant pattern with incomplete penetrance (A1, assigned by Kerrison et al., 2001; A2 and A3 refined in family nys-01 and nys-02, respectively). B shows the X-linked dominant pattern with 100% penetrance. C shows the X-linked recessive pattern

gion at Xq26.3-q27 [9], and in another study performed in two X-linked recessive pattern families by, the CMN disease gene were located to Xq23-27 [2].

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