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Fine mapping of the X-linked recessive congenital idiopathic nystagmus locus at Xq24-q26.3

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Purpose: To refine the interval for X-linked congenital idiopathic nystagmus at Xq24-q26.3 and to evaluate a novel candidate gene (Muscleblind-like 3 gene [*MBNL3*]).
Methods: A single pedigree with congenital idiopathic nystagmus (CIN) inherited as an X-linked recessive trait underwent detailed clinical examination including nystagmology and electrophysiological investigation in selected subjects. Following detailed phenotyping, genotyping was performed using 52 microsatellite markers spaced at an average of 5 cM along the X chromosome. Subsequent two-point and multipoint linkage analysis were performed and a candidate gene was screened for mutations by conventional sequencing.
Results: Linkage mapping located the disease gene to a 15.5cM interval at Xq24-q26.3, between markers DXS1212 and DXS1062 with a maximum two-point LOD score of 4.24 with both markers DXS8044 and DXS994 (θ=0). Multipoint analysis indicated a LOD score of 4.54 and a critical gene interval of 8.0 cM. No mutations were found in the *MBNL3* gene in this pedigree.
Conclusions: We describe a family with an unusual inheritance pattern most consistent with X-linked recessive inheritance with X inactivation causing manifesting females. We refine the linkage interval for X-linked recessive congenital idiopathic nystagmus and exclude *MBNL3* as the causative gene in this family.

Nystagmus is a disorder of oculo-motor control and can occur as an isolated inherited trait, Congenital Idiopathic Nystagmus (CIN), or secondary to other visual or neurological disease. In all cases the underlying patho-physiology is poorly understood. CIN is genetically heterogeneous and has been described as an autosomal dominant [1], autosomal recessive [2], and X-linked dominant [2] or recessive trait [3]. Although X-linked regions have been identified at Xp11.4-p11.3 [3] and Xq26-q27 [2] no genes have been identified. These studies used CIN families for whom the inheritance pattern was likely X-linked dominant with variable penetrance.

In 2005, the first X-linked recessive CIN locus was reported and mapped to a 37.9 cM region at Xq23-q27, between markers DXS8055 and DXS1205 [4]. This locus included the region identified previously for X-linked dominant CIN and thus raised the question of whether the X-linked recessive and X-linked dominant forms may be caused by allelic variation within the same gene. Alternatively, the disease may result from mutation in different genes within this interval. As part of our ongoing research to identify new loci for CIN we have ascertained a three generation family with X-linked CIN. This paper describes successful genetic linkage of this family and refinement of the X-linked recessive congenital idiopathic nystagmus locus at Xq24-q26.3.

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METHODS

Clinical methods: The study had the approval of the local and regional ethics committee and conformed to the tenets of the Declaration of Helsinki.

Twenty nine individuals (seven affected males, two affected females, 11 obligate female carriers and nine unaffected members) in a single CIN pedigree (Figure 1) underwent detailed clinical examination including: LogMAR visual acuity, refraction, color vision, intra-ocular pressure (IOP) recording, anterior and posterior ocular segment examination, orthoptic assessment, and 24 patients had recordings of their nystagmus waveform using a Skalar IRIS IR Light Eye Tracker equipment (Cambridge Research Systems Ltd. Rochester UK). Seven patients also had extensive central and peripheral neurological examinations carried out.

International Society for Clinical Electrophysiology of Vision (ISCEV) standardized electroretinograms (ERGs) and visual evoked potential (VEP) recordings were recorded in 2 affected males and 2 obligate female carriers.

Informed consent was obtained from all subjects for genetic studies and genomic DNA was isolated from ORAGENE saliva sample kits (DNA Genotek Inc. Ontario, Canada).

Genotyping and linkage analysis: Forty eight fluorescently labeled microsatellite markers from the ABI

Prism Linkage Mapping Set v 2.5-HD5 (Applied Biosystems, Foster City, CA) were used for genotyping (panels 28, 83, 84, 85, and 86) with the polymerase chain reaction (PCR) according to manufacturer's instructions. Four further fluorescently labeled microsatellite markers were designed for fine mapping and run using identical conditions (Operon Biotechnologies GmbH, Cologne, Germany). Amplified products were then analysed on an ABI Prism 3100 Genetic Analyser (Applied Biosystems), and the Genotyper v 3.7 NT software was used to identify alleles.

Two-point and multipoint linkage analyses were performed using the VITESSE program [5]. The PEDCHECK program [6] was used to examine the pedigree for genotyping errors and mendelian inconsistencies.

Both marker locations and inter-marker Centimorgan distances (cM) are taken from the Rutgers combined linkage-physical map of the human genome [7] based on the NCBI build 35 (National Center for Biotechnology Information, Bethesda, MD).

The inheritance pattern was assumed to be X-linked recessive and the disease gene frequency was taken at 0.001 with penetrance of 0.1 in female heterozygotes and 1 in female homozygotes and male hemizygotes. Other penetrance values between 0 and 0.25 for female heterozygotes were also tested but the results were consistent throughout.

Sequencing: Sequencing of the Muscleblind-like 3 gene (*MBNL3*, OMIM 300413) was performed for one affected and one obligate female carrier. Following standard PCR, each fragment was sequenced using the Big Dye Terminator Cycle Sequencing kit and a 3100 sequencer (Applied Biosystems) using the manufacturer's protocol (PCR primers available on request).

RESULTS & DISCUSSION

Nystagmus was transmitted as an apparent X-linked recessive trait in our family with eight affected males, 11 obligate female carriers, two affected females, and no male-male transmission. We propose that the most likely explanation for the occurrence of manifesting females is skewed X inactivation as described in other ocular diseases [8]. Thus, for obligate female carriers to manifest we suggest that the pattern of X inactivation must be skewed such that cells in the tissue responsible for nystagmus have only the mutant gene active and the wild type gene "switched off". However, it is also possible, although less likely, that the pedigree is X-linked dominant with variable penetrance.

Variable penetrance, either due to skewed X inactivation, or other causes is common in genetic disease and may explain why apparent X-linked dominant and X-linked recessive pedigrees link to the same region. It may be the case that all these pedigrees have X-linked dominant inheritance with variable penetrance or that they all have X-linked recessive inheritance with skewed X inactivation but have simply been interpreted differently. However, to further investigate X inactivation in this pedigree (or indeed other pedigrees) it would be necessary to sample the cells/tissue responsible for nystagmus, ascertain whether the abnormal phenotype causes cell selection and investigate tolerance to abnormal gene product levels. Clearly, this is not possible until the nystagmus gene is identified and therefore cannot aid gene discovery.

Flash and pattern ERG and occipital pattern VEP recordings in 2 affected males and 2 obligate female carriers were normal, thus excluding masquerading eye conditions. Computerized Tomography brain scans were performed on 2 affected individuals at diagnosis with no abnormal findings. Oph-

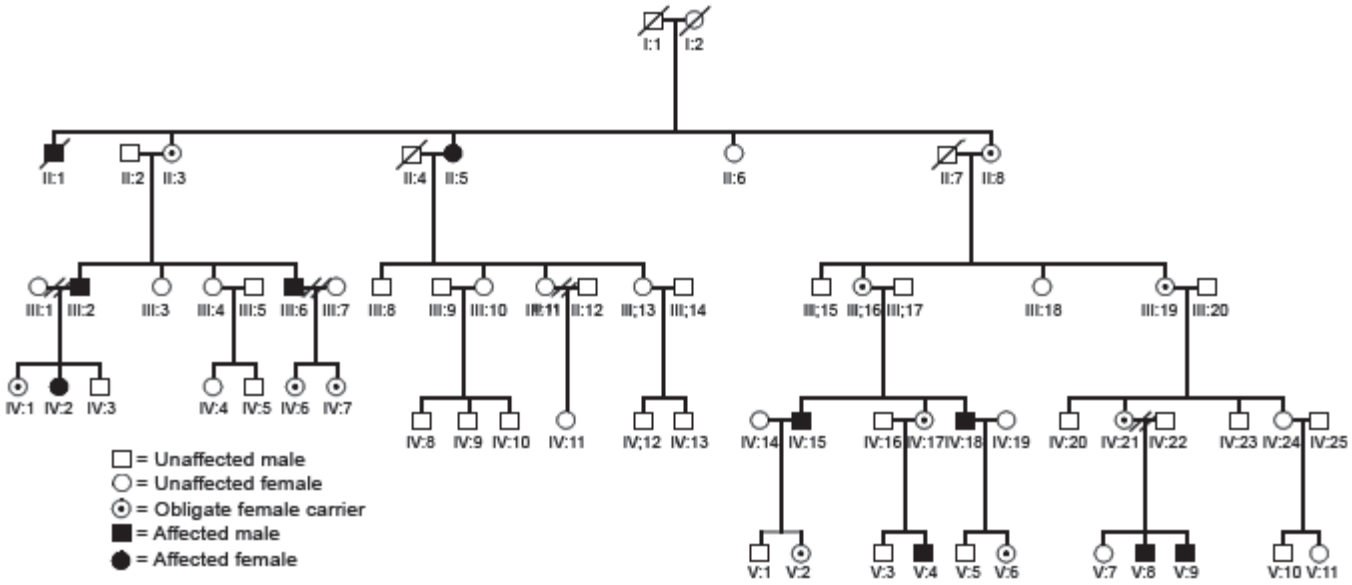

Figure 1. An X-linked CIN Pedigree. This family has 8 affected males (1 deceased), 2 affected females, and 11 obligate female carriers. Patients genotyped and informative for linkage were: II3, II:5,, III:2, III:6, III:15 III:16, III:17, III:19, III:20, IV:15, IV:17, IV:18, IV:20, IV:21, IV:23, V:3, V:4, V:8, and V:9.

thalmic and neurological examinations were also normal except for nystagmus in affected patients. All affected individuals exhibited nystagmus waveforms that were either jerk with increasing velocity slow phases, pendular, or a combination of both. No cases of manifest latent/latent nystagmus were detected (Table 1).

The highest two-point LOD score (Z) of 4.24 at θ=0 was found for both markers DXS8044 and DXS994 (Table 2).

Table 1.

Pedigree number	Clinical status	Corrected LogMAR visual acuity (R:L)	Ophthalmic examination	Orthoptic findings	Nystagmology	Flash and pattern ERG and occipital pattern VEP findings
II:2	N	0.16:0.1	NAD	N	-	-
II:3	N (OFC)	0.22:0.18	hypermetropia	N	NAD	-
II:5	A	0.32:0.16	hypermetropic astigmatism	Y (RET)	H,P,IVSP,RC	-
III:2	A	0.24:0.26	myopic astigmatism	N	H,J,IVSP,RC	-
III:6	A	0.34:0.38	hypermetropic astigmatism	Y (RET)	H,J,IVSP-	
III:8	U	0.42:0.40	congenital cataracts	Y (LET)	H,mixed J and P, mixed IVSP and LVSF,RC	-
III:10	N	0.06:0.0	NAD	N	NAD	-
III:13	N	0.06:0.04	NAD	N	NAD	-
III:15	N	0.26:0.24	cataract	N	NAD	-
III:16	N (OFC)	0.04:0.14	early cataract	Y (RET)	NAD	NAD
III:19	N (OFC)	0.22:0.28	early cataract	Y (LET)	NAD	-
III:20	N	0.16:0.12	NAD	N	NAD	-
IV:1	N (OFC)	-0.1:-0.12	myopic astigmatism	N	NAD	-
IV:2	A	0.32:0.36	myopic astigmatism	N	H,mixed J and P, mixed IVSP and LVSF,RC	-
IV3	N	0.0:0.02	NAD	N	NAD	-
IV6	N (OFC)	0.02:0.0	NAD	N	NAD	-
IV7	N (OFC)	0.00:-0.12	NAD	N	NAD	-
IV15	A	0.38:0.42	NAD	N	H,J,IVSP,RC	-
IV17	N (OFC)	0.06:0.02	NAD	N	NAD	NAD
IV18	A	0.46:0.4	NAD	Y (LET)	H,J,mixed IVSP and LVSF,RC	NAD
IV:20	N	-0.12:0.0	NAD	N	NAD	-
IV:21	N (OFC)	0.02:0.02	NAD	Y (LET)	NAD	-
IV:23	N	0.0:0.04	NAD	N	NAD	-
V:2	N (OFC)	0.04:0.08	NAD	N	NAD	-
V:3	N	0.02:0.04	NAD	N	NAD	-
V:4	A	0.5:0.46	NAD	N	H,J,mixed IVSP and LVSF,RC	NAD
V:6	N (OFC)	0.04:0.02	NAD	N	NAD	-
V:8	A	0.32:0.44	NAD	Y (RET)	H,J,IVSP,RC	-
V:9	A	0.12:0.1	NAD	N	H,J,IVSP	-

Summary of clinical findings. Clinical data collected from 29 individuals from an X-linked Congenital Idiopathic Nystagmus pedigree.

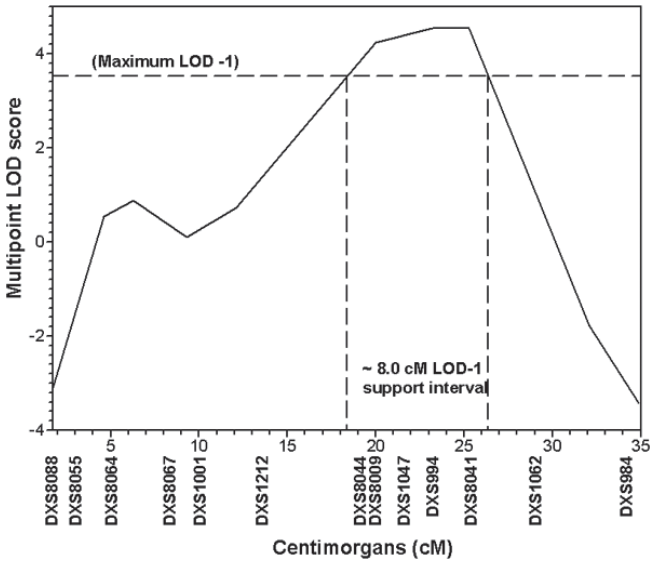

Figure 2. Multipoint LOD scores. Multipoint LOD scores with LOD-1 support interval narrow the X-Linked recessive nystagmus locus to an 8.4 cM region between markers DXS1001 and DXS1062.

TABLE 2.

Marker order	LOD score at θ= 0.0	0.1	0.2	0.3	0.4	0.5
DXS8088	-inf	1.38	1.37	1.03	0.54	0.00
DXS8055	-inf	1.64	1.57	1.17	0.59	0.00
DXS8064	-0.00	-0.00	-0.00	-0.00	-0.00	0.00
DXS8067	-inf	1.08	1.02	0.79	0.45	0.00
DXS1001	-inf	2.08	1.76	1.25	0.61	0.00
DXS1212	-inf	1.08	1.02	0.79	0.45	0.00
DXS8009	1.19	1.01	0.81	0.58	0.31	0.00
DXS8044	4.24	3.55	2.77	1.91	0.94	0.00
DXS1047	3.00	2.54	2.03	1.45	0.79	0.00
DXS994	4.24	3.55	2.78	1.91	0.94	0.00
DXS8041	3.03	2.53	1.96	1.32	0.63	0.00
DXS1062	-inf	0.66	0.85	0.73	0.44	0.00
DXS984	-inf	1.68	1.66	1.31	0.76	0.00

Two-point LOD scores. Thirteen markers from the linked region (from a total of 52) are shown and illustrate significant linkage of nystagmus to markers DXS8044-DXS8041.

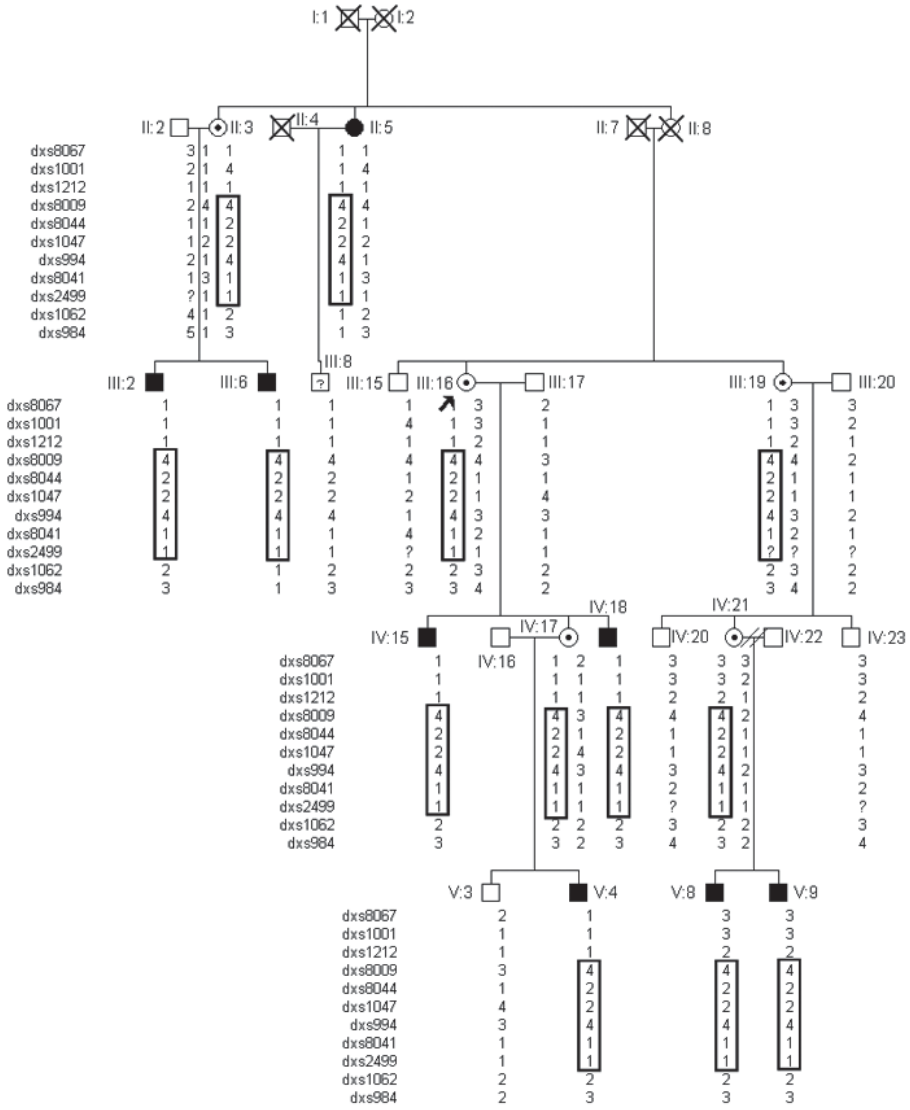

Figure 3. Haplotype analysis. Markers are listed top to bottom centromere-DXS8067-DXS1001-DXS1212-DXS8009-DXS8044-DXS1047-DXS994- DXS8041-DXS2499-DXS1062-DXS984-telomere. The haplotype co-segregating with the nystagmus phenotype is boxed. A question mark indicates that the genotype is not determined and for clarity, identifiers from Figure 1 were used. Marker DXS2499 was uninformative in this pedigree.

Multipoint analysis provided a maximum LOD score of 4.54 at marker DXS994 and a critical LOD-1 support interval of about 8.0 cM between markers DXS1212 and DXS1062 (using the Rutgers combined linkage-physical map; Figure 2). These results were confirmed by haplotype analysis (Figure 3).

Centromeric and telomeric boundaries for the shared haplotype are defined by; a recombination between markers DXS1001 and DXS1212 for individual IV:21 and a recombination between markers DXS1062 and DXS2499 for individual III:6.

These results significantly narrow the only previous X-linked recessive CIN interval identified by Guo et al. [4] (about 37.9 cM to about 8.0 cM) in a single family thus reducing the risk of error due to locus heterogeneity (Figure 4).

This interval still has a region of overlap (about 3.4 cM interval between DXS8033 and DXS1062) with the interval for X-linked dominant CIN refined by Zhang et al. [9] to a 4.4 cM region between markers DXS8033 and DXS1211. Therefore, these results do not rebuke the hypothesis that X-linked recessive CIN and X-linked dominant CIN may be caused by alternative variants of in the same gene. However, comparison of overlapping linkage intervals from multiple studies should be interpreted with caution especially when studies employ different diagnostic criteria, different methods of phenotypic assignment, different methods of critical interval calculation, and different families in a disease known to be heterogeneous. It is also important to note that previous linkage studies have employed various genetic maps to assign marker positions and cM distances. Many of these locations and even marker orders have changed over time due to updated mapping information. Therefore, using overlapping linkage intervals from different studies to reduce the nystagmus gene interval could be extremely error prone.

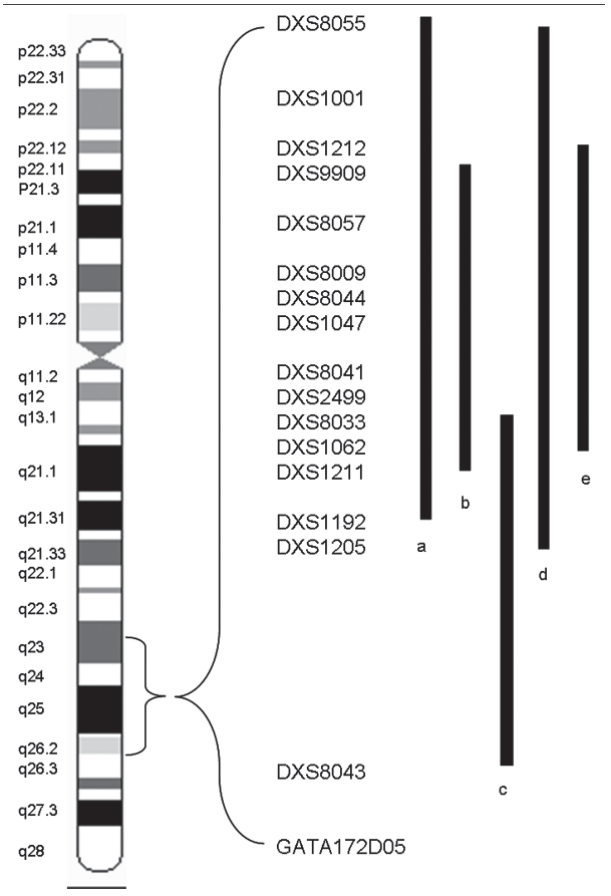

Figure 4. Diagram of the X chromosome showing the q24-q26 region for X-linked CIN. In the image, a, b, and c represent the critical regions for X-linked dominant CIN reported by: a; [2], b; [10], and c; [9]. Also, d and e represent the critical regions for X-linked recessive CIN reported by: d; [4] and e; this study.

It is perhaps worth noting that if multiple pedigrees are combined for linkage studies of nystagmus (and a standardized map, method of diagnosis, phenotyping and interval calculation are employed) then perhaps the higher number of informative meioses would allow SNP markers at a high density to provide the maximum information required to narrow the linkage interval.

Several candidate genes, including *CDR1* [2], *SOX3* [2], *SLC25A14* [10], *SLC9A6* [9], and *FGF13* [9], have been screened in patients with X-linked dominant CIN but no causative mutations have been detected. We identified a very strong candidate located in the center of our critical interval called Muscleblind-like protein 3 (*MBNL3*). This highly conserved gene is a member of a newly described family of tissue-specific alternative splicing regulators [11]. This family is known to regulate terminal muscle differentiation through alternative splicing control and several groups have suggested that the family participates in the differentiation of photoreceptors, neurons, adipocytes and blood cell types. More specifically *MBNL3* has been shown to inhibit muscle differentiation [12]. Northern blot analysis and cell culture experiments have established expression of MBNL genes in several human tissue types including brain and muscle cell types [12,13]. Furthermore, MBNL proteins have been shown to sequester foci of expanded-repeat transcripts and are thought to therefore play a role in the molecular pathology of a group of neuromuscular diseases including the Myotonic Dystrophies (DM1; OMIM 160900 and DM2; OMIM 602668) [13-15]. Knock-out mice models for MBNL genes also display a relevant phenotype including overt myotonia and ocular anomalies [16].

Direct sequencing for two affected individuals of all known transcripts of this gene (VEGAGENE transcripts OTTHUMT00000058318-OTTHUMT00000058327) revealed no sequence variations in any exon or the 200 bp flanking sequence at both the 5 and 3 prime ends.

We demonstrate that X-linked CIN families can have a small number of manifesting females and suggest that this may be due to X-linked recessive inheritance with X inactivation. We have confirmed the presence of a locus for X-linked CIN on Xq and significantly narrowed the previously described critical gene interval. We have also excluded the MBLN3 gene as the causative gene in our pedigree.

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REFERENCES

1. Kerrison JB, Arnould VJ, Barmada MM, Koenekoop RK, Schmeckpeper BJ, Maumenee IH. A gene for autosomal dominant congenital nystagmus localizes to 6p12. Genomics 1996; 33:523-6.
2. Kerrison JB, Vagefi MR, Barmada MM, Maumenee IH. Congenital motor nystagmus linked to Xq26-q27. Am J Hum Genet 1999; 64:600-7.
3. Cabot A, Rozet JM, Gerber S, Perrault I, Ducroq D, Smahi A, Souied E, Munnich A, Kaplan J. A gene for X-linked idiopathic congenital nystagmus (NYS1) maps to chromosome Xp11.4-p11.3. Am J Hum Genet 1999; 64:1141-6.
4. Guo X, Li S, Jia X, Xiao X, Wang P, Zhang Q. Linkage analysis of two families with X-linked recessive congenital motor nystagmus. J Hum Genet 2006; 51:76-80.
5. O'Connell JR, Weeks DE. The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. Nat Genet 1995; 11:402-8.
6. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998; 63:259-66.
7. Kong X, Murphy K, Raj T, He C, White PS, Matise TC. A combined linkage-physical map of the human genome. Am J Hum Genet 2004; 75:1143-8. Erratum in: Am J Hum Genet. 2005; 76:373.
8. McMullan TF, Collins AR, Tyers AG, Robinson DO. A novel X-linked dominant condition: X-linked congenital isolated ptosis. Am J Hum Genet 2000; 66:1455-60.
9. Zhang B, Xia K, Ding M, Liang D, Liu Z, Pan Q, Hu Z, Wu LQ, Cai F, Xia J. Confirmation and refinement of a genetic locus of congenital motor nystagmus in Xq26.3-q27.1 in a Chinese family. Hum Genet 2005; 116:128-31.
10. Kerrison JB, Giorda R, Lenart TD, Drack AV, Maumenee IH. Clinical and genetic analysis of a family with X-linked congenital nystagmus (NYS1). Ophthalmic Genet 2001; 22:241-8.
11. Pascual M, Vicente M, Monferrer L, Artero R. The Muscleblind family of proteins: an emerging class of regulators of developmentally programmed alternative splicing. Differentiation 2006; 74:65-80.
12. Squillace RM, Chenault DM, Wang EH. Inhibition of muscle differentiation by the novel muscleblind-related protein CHCR. Dev Biol 2002; 250:218-30.
13. Fardaei M, Rogers MT, Thorpe HM, Larkin K, Hamshere MG, Harper PS, Brook JD. Three proteins, MBNL, MBLL and MBXL, co-localize in vivo with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells. Hum Mol Genet 2002; 11:805-14.
14. Fardaei M, Larkin K, Brook JD, Hamshere MG. In vivo co-localisation of MBNL protein with DMPK expanded-repeat transcripts. Nucleic Acids Res 2001; 29:2766-71.
15. Mankodi A, Urbinati CR, Yuan QP, Moxley RT, Sansone V, Krym M, Henderson D, Schalling M, Swanson MS, Thornton CA. Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2. Hum Mol Genet 2001; 10:2165-70.
16. Kanadia RN, Urbinati CR, Crusselle VJ, Luo D, Lee YJ, Harrison JK, Oh SP, Swanson MS. Developmental expression of mouse muscleblind genes Mbnl1, Mbnl2 and Mbnl3. Gene Expr Patterns 2003; 3:459-62.

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