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A Gene for X-Linked Idiopathic Congenital Nystagmus (NYS1) Maps to Chromosome Xp11.4-p11.3

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Summary

Congenital nystagmus (CN) is a common oculomotor disorder (frequency of 1/1,500 live births) characterized by bilateral uncontrollable ocular oscillations, with onset typically at birth or within the first few months of life. This condition is regarded as idiopathic, after exclusion of nervous and ocular diseases. X-linked, autosomal dominant, and autosomal recessive modes of inheritance have been reported, but X-linked inheritance is probably the most common. In this article, we report the mapping of a gene for X-linked dominant CN (NYS1) to the short arm of chromosome X, by showing close linkage of NYS1 to polymorphic markers on chromosome Xp11.4-p11.3 (maximum LOD score of 3.20, over locus DXS993). Because no candidate gene, by virtue of its function, has been found in this region of chromosome Xp, further studies are required, to reduce the genetic interval encompassing the NYS1 gene. It is hoped that the complete gene characterization will address the complex pathophysiology of CN.

Introduction

Congenital nystagmus (CN) is a relatively common oculomotor disorder that has an estimated frequency of 1/1,500 live births, based on a population study of school children in Sweden (Forssman and Ringner 1971). It is an involuntary, bilateral, often symmetric, invariably conjugated, and predominantly horizontal ocular oscillation that is present at birth or that develops within the first few months of life. The rapid to-and-fro oscillations of the eyes produce corresponding oscillations of images on the retina, so that both visual acuity and contrast sensitivity are expected to be severely impaired. In patients with idiopathic CN (ICN), however, visual acuity usually is only moderately impaired, a feature that contrasts with the variable degree of visual loss observed in nystagmus due to primary ophthalmological disorders. Indeed, CN also can occur in association with various manifestations, such as strabismus and head or posture nystagmus.

CN has been divided into sensory and motor types (Kestenbaum 1961, p. 356; Forssman 1964; Cogan 1967; Harcourt 1970; Wybar 1972; Gelbart and Hoyt 1988). The sensory type is ascribed to patent visual-pathway diseases, including strabismus, aniridia, cataracts, and coloboma, or to recognizable conditions (e.g., Leber congenital amaurosis, optic nerve hypoplasia or atrophy, ocular or oculocutaneous albinism, retinoschisis, achromatopsia, and X-linked congenital stationary night blindness). For the motor type, all known diseases are excluded, and ICN usually is observed in children with no eye or CNS anomaly.

Various patterns of inheritance have been described for ICN, including X-linked dominant and X-linked recessive (MIM 310700), autosomal dominant (MIM 164100), and autosomal recessive (MIM 257400) inheritances. X-linked inheritance with incomplete penetrance and variable expressivity is probably the most common. In 1996, a gene for autosomal dominant CN was mapped to chromosome 6p12 (Kerrison et al. 1996), but no chromosomal mapping for X-linked nystagmus has been reported thus far. In this article, we report the first mapping of a gene for X-linked ICN, to chromosome Xp11.4-p11.3.

Patients and Methods

Patients

One large French family—including 12 individuals with ICN (7 female and 5 male, of whom only 3 were available), over four generations, and 10 healthy relatives—was recruited (fig. 1). Inclusion criteria were nys-

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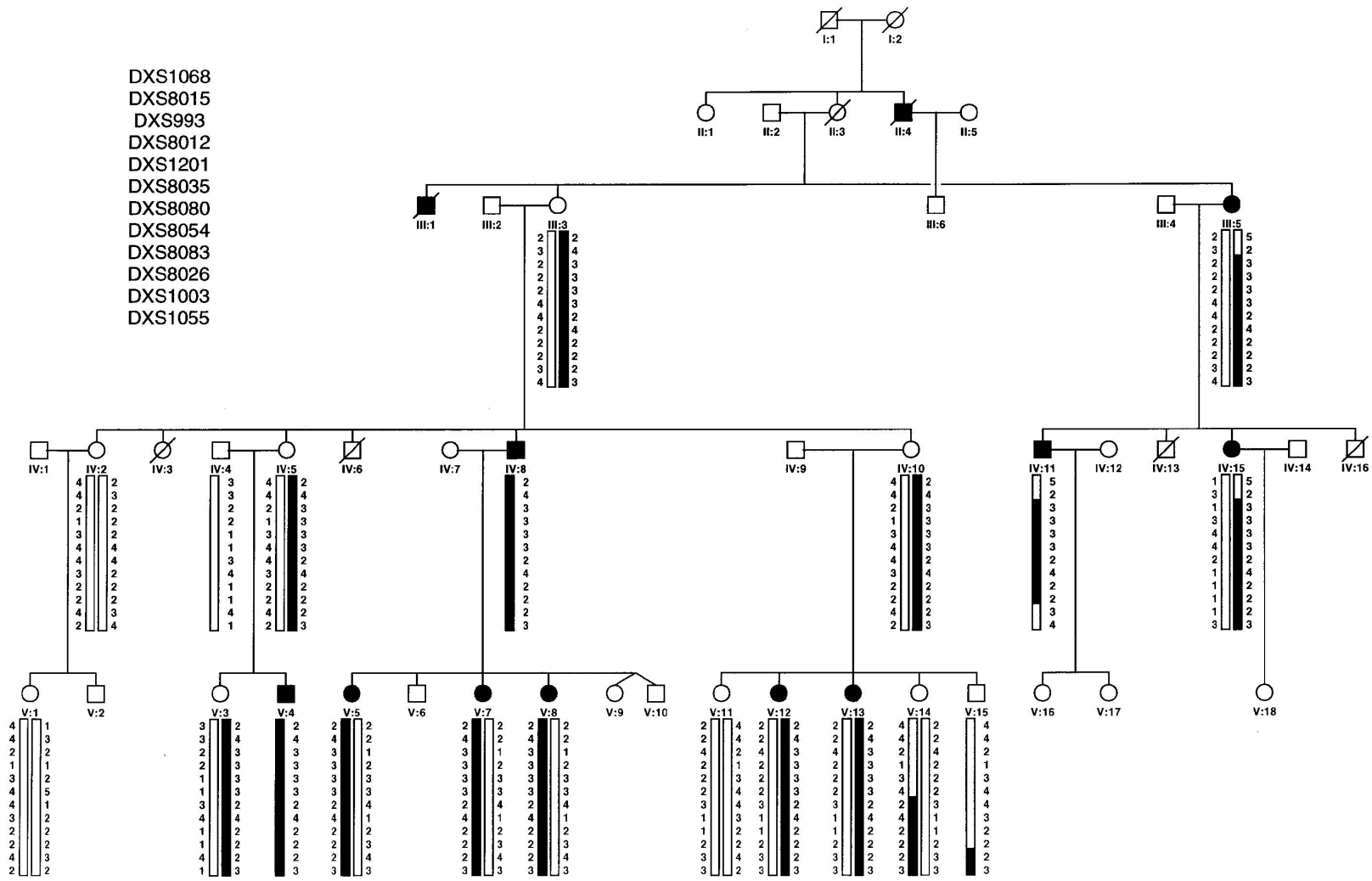

Figure 1 Haplotype analyses of X-linked dominant ICN. Blackened regions of haplotypes indicate linkage between the markers and the disease.

Table 1

Pairwise Linkage Between NYS1 and 11 Polymorphic DNA Markers of the Xp Chromosome

		LOD Score at θ =						
Locus	Marker	.00	.05	.10	.20	.30	Z_{max}	θ_{max}
DXS1068	AFM200ye7	$-\infty$	1.02	1.16	1.10	.86	1.16	.10
DXS8015	AFMa130yb1	$-\infty$	.81	.96	.93	.72	.97	.15
DXS8012	AFMa124xd9	3.13	2.89	2.64	2.09	1.48	3.13	.00
DXS993	AFM203wf4	3.13	2.89	2.64	2.09	1.48	3.13	.00
DXS1201	AFM256ze5	1.86	1.71	1.55	1.2	.84	1.86	.00
DXS8035	AFMa246ze9	3.13	2.89	2.64	2.09	1.48	3.13	.00
DXS8080	AFMc012zc1	2.74	2.54	2.33	1.86	1.33	2.74	.00
DXS8054	AFMb291yc9	2.74	2.54	2.33	1.86	1.33	2.74	.00
DXS8083	AFMc024xc5	.20	.17	.14	.08	.04	.20	.00
DXS1003	AFM276xf5	$-\infty$	$-.32$	.12	.36	.32	.36	.20
DXS1055	AFM168ya3	$-\infty$	$-.02$	.42	.66	.59	.66	.20

tagmus recorded at birth or within the 1st year of life and exclusion of recognizable abnormalities of the ocular or neural visual pathway, by detailed clinical studies including visual acuity, fundus aspect, visual field, and color vision testing, electroretinography, and visual-evoked potentials. X-linked dominant inheritance with incomplete penetrance is the most probable mode of inheritance, as indicated by no male-to-male transmission but frequent male-to-female transmission; several apparently unaffected girls (V-9, V-16, and V-17) were born to affected men, and several apparently unaffected women (III-3, IV-5, and IV-10) passed the disease to the next generation. The penetrance of the disease apparently did not change with age (fig. 1).

DNA Analysis

Blood samples (20 ml) were obtained, with informed consent, from the family members, and DNA was prepared from lymphocyte pellets by use of SDS lysis, proteinase K digestion, phenol/chloroform extraction, ethanol precipitation, and Tris-EDTA resuspension. Hypervariable microsatellites developed by Dib et al. (1996) were used for genotyping by PCR. Genomic DNA (200 ng) was amplified by use of 0.5 U *Taq* polymerase (Life Technologies), in the recommended buffer (1.5 mM $MgCl_2$, 20 μM each deoxynucleotide, and 20 μM primers in a final volume of 20 μl). Amplification conditions were 95°C for 10 min, followed by 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 10 min. PCR products were incubated at 94°C for 5 min before loading (3 μl) on a 6%-acrylamide, 7.5 M–urea gel. Electrophoresis was performed at 1,400 V, 65 W at 50°C for 3 h. The gels were blotted onto nylon membranes (Appligene), labeled by chemiluminescence according to the manufacter's instructions (enhanced-chemiluminescence direct nucleic acid labeling and detection systems; Amersham Life Science), and exposed to x-ray films for 10 min.

Linkage Analysis

Markers containing short tracts of $(CA)_n$ repeats were chosen from the Généthon linkage map, on the basis of their informativity, and had an average recombination fraction (θ) of $\leqslant$.02. All nucleotide sequences are available in the Genome Database. NYS1 was tested under the assumption that the disease is transmitted as an X-linked dominant trait with penetrances of 1.00 and .60 in males and females, respectively (fig. 1).

Two-point LOD scores for linkage between NYS1 and each microsatellite DNA marker were calculated by use of the MLINK option of the LINKAGE program, version 5.1 (Lathrop et al. 1985). Multipoint LOD-score analysis (LINKMAP program; Lathrop et al. 1985) was used to estimate the position of NYS1 with respect to the intervals on the following genetic map (genetic distance between adjacent loci in parentheses): pter–DXS1068–(2.2 cM)–DXS8015–(7.7 cM)–DXS993–(0.0 cM)–DXS8012–(2.1 cM)–DXS1201–(0.0 cM)–DXS8035–(2.0 cM)–DXS8080–(1.4 cM)–DXS8054–(1.4 cM)–DXS8083–(2.1 cM)–DXS8026–(1.9 cM)–DXS1003–(0.9 cM)–DXS1055–cen (Dib et al. 1996). In this procedure, the map of the marker loci is fixed, and the position of the disease locus varies throughout the map.

X-Inactivation Assay

Genomic DNA (500 ng) was incubated overnight with or without the restriction enzyme *Hpa*II, in the buffer supplied by the manufacturer and in a total volume of

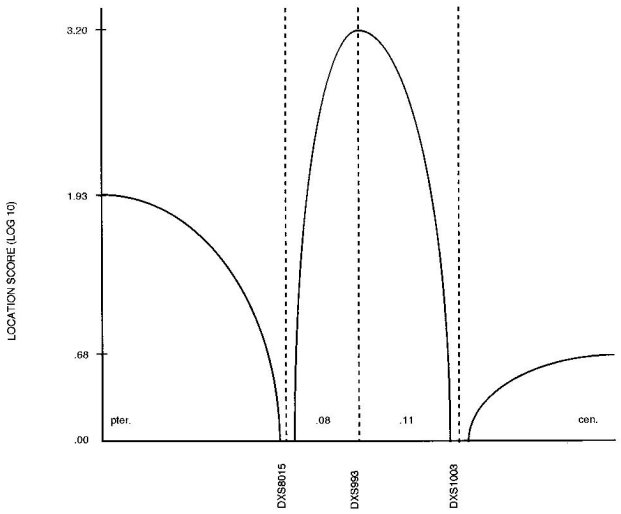

Figure 2 Support for location of an X-linked ICN gene, with respect to three chromosome Xp markers. Likelihood estimates are given in $\log_{10}$. Distances between marker loci, in centimorgans, are shown along the *X*-axis. The maximum location score for NYS1 is between DXS8015 and DXS1003, over the locus DXS993.

Table 2

Summary of Key Recombination Events Involving NYS1 and Xp Probes

Individual	Phenotype	Sex of Parent Studied	Recombination Event				
			DXS1058	DXS8015	DXS993	DXS1003	DXS1055
III-5	AF	F	+	+	−	−	−
IV-11	AM	F	+	+	−	+	+
V-15	HM	F	−	−	−	+	+

Note.—A plus sign (+) indicates presence of a recombination event, and a minus sign (−) indicates absence of a recombination event. AM = affected male, AF = affected female, HM = healthy male, and F = female.

20 μl. One in two dilutions with water were made, and 1.5 μl was used in a PCR reaction to amplify the polymorphic glutamine repeat of the androgen-receptor locus, by use of primers HARA1 and HARA2, at an annealing temperature of 60°C (Allen et al. 1992). Products were denatured in formamide-loading dye, and the single strands were separated on 1-mm–thick 8%-polyacrylamide denaturing gels containing 7-M urea and 1 × Tris borate–EDTA. The gels were run for 3 h at a constant 40 mA and then were silver stained as described by Jouet et al. (1994). A sample from a male control was used to assess the completion of *Hpa*II digestion of DNA.

Results

We generated an exclusion map of the X chromosome, starting from the telomeric end of the short arm, and found evidence for linkage of the disease gene (NYS1) to the DXS993 locus on chromosome Xp11.4 (table 1). Additional markers in the region were typed and also gave positive pairwise LOD scores (table 1). The maximum pairwise LOD score (Z_{max}), with no recombination event, was found at loci DXS993, DXS8012, and DXS8035 ($Z_{max} = 3.13$ at $\theta = .00$, for all three markers; table 1). Multipoint linkage analyses were performed to estimate the best position of NYS1. Figure 2 shows that the maximum likelihood estimate for the location of NYS1 was over the DXS993 locus ($Z_{max} = 3.20$). The odds against alternative orders were >1,000:1 in all cases.

Haplotype analyses supplemented the multipoint analysis by providing direct evidence for recombination events (fig. 1 and table 2). With respect to the marker loci, all haplotypes were resolved with a maximum of one recombination event. The positioning of recombination events by this method was consistent with the placement of NYS1 between DXS8015 and DXS1003.

In the X-inactivation studies, individuals IV-2 and V-1, who do not carry the affected haplotype, displayed a random X-inactivation pattern (fig. 3). Conversely, individuals III-3, III-5, IV-5, IV-10, IV-15, and V-3, who do carry the affected haplotype, showed a skewed X-inactivation pattern of either the affected haplotype (III-3, III-5, IV-10, and IV-5) or the unaffected haplotype (IV-5 and V-3) (fig. 3). Finally, individuals V-11, V-12, and V-13 were not informative.

Discussion

We report here the mapping of a gene causing CN to the short arm of chromosome X, owing to close linkage

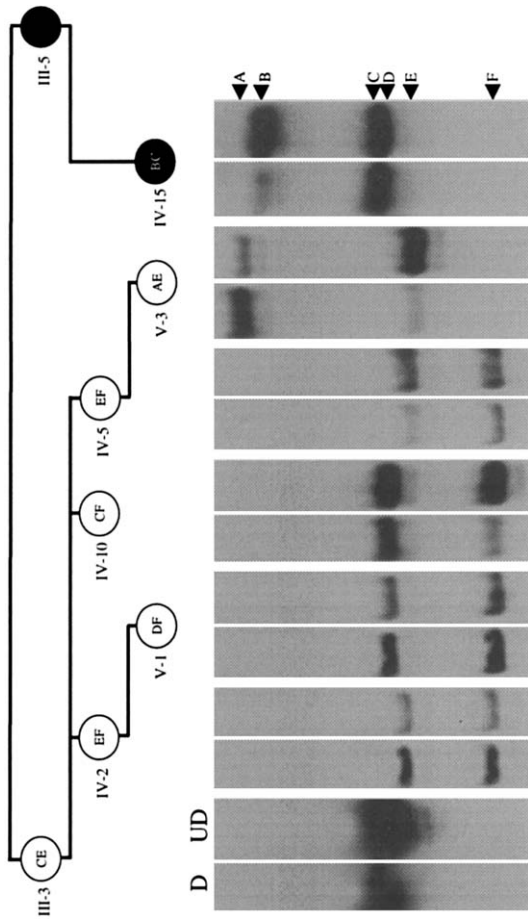

Figure 3 X-inactivation patterns of the informative females in the family studied. The different alleles at the X androgen-receptor locus are shown (*arrowheads labeled A–F*). D = DNA digested by *Hpa*II, and UD = undigested DNA.

to eight highly polymorphic markers on Xp11.4-11.3. This study supports the localization of the disease-causing gene in the 18.6-cM genetic interval defined by loci DXS8015 and DXS1003. It is worth noting that the two females who do not carry the affected haplotype displayed a random X-inactivation pattern, whereas all females who do carry the mutated gene displayed a skewed X-inactivation pattern of either the affected or the unaffected haplotype. Nevertheless, we did not observe any correlation between the X-inactivation pattern and phenotype, strongly suggesting that NYS1 is transmitted as an X-linked dominant trait with incomplete penetrance in females. The question of whether genetic heterogeneity exists in X-linked CN remains open to debate, especially since some reported pedigrees are consistent with strict X-linked recessive inheritance, with no expression in females (Forssman and Ringner 1971).

Interestingly, NYS1 maps to the genetic interval encompassing the genes for congenital stationary night blindness (CSNB1, Xp11.4-p11.3), retinitis pigmentosa (RP2, Xp11.3), cone dystrophy (COD1, Xp11.4), Norrie disease protein (NDP, Xp11.4), exsudative vitreoretinopathy (EVR2, Xp11.4), Aland island eye disease (AIED, Xp11.4-p11.23), and X-linked optic atrophy (OPA2, Xp11.4-p11.21) (Genatlas; see fig. 4). This observation raises the question of whether ICN is allelic to one of these oculopathies. Yet, the striking differences in clinical profile and evolution suggest that ICN is indeed a genetically distinct entity.

No candidate gene, by virtue of its function, has been found in the Xp region encompassing the NYS1 gene, but several observations support the view that a neural-defect gene underlies the disease—namely, (1) a high frequency of afferent visual-pathway dysfunctions, (2) CN damping by use of contact lenses and cutaneous stimulation of the trigeminal nerve, (3) the presence of a null zone, (4) neurological origin of acquired nystagmus in adults, and (5) requirement of a functioning (even leaky) geniculostriate system for the development of horizontal CN. However, the search for an identifiable neuroanatomic substrate is difficult, since nystagmus probably results from instability of the ocular motor system, rather than from a precise defect in a neurological or visual pathway (Dell'Osso et al. 1974).

Several hypotheses concerning the origin of CN have been ruled out, including the presence of a leaky neural integrator (Dell'Osso et al. 1993), misdirection of the retinogeniculate or subcortical visual pathway (Fielder and Evans 1988), and albino-like abnormal chiasmatic decussation of the optic axons (Apkarian and Shallo-Hoffman 1991). Indeed, recent studies by Dell'Osso et al. (1992*a*, 1992*b*, 1992*c*) showed that CN patients had intense fixation reflexes and stable pursuing eye movements with normal vestibulo-ocular reflexes (ocular-stabilization systems) and proposed that CN was caused by unstable efferent impulses from the extraocular muscles. Ultrastructural studies support the disorganization of the architecture of the extraocular muscles, with degenerative changes of the mitochondria in CN patients (Adachi et al. 1973; Pepin et al. 1980; Mencucci et al. 1995; Peng et al. 1998). Yet, it also has been suggested that the extraocular muscle changes are secondary to nystagmus (Hamed 1995). Whatever the mechanism, it is hoped that the identification of the NYS1 gene will address the complex pathophysiology of CN.

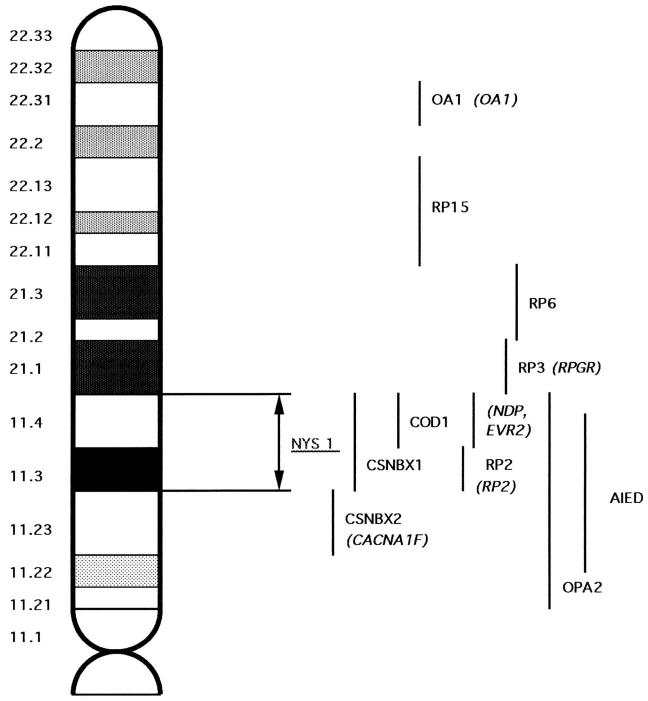

Figure 4 Ocular disease loci and genes (*italicized*) on chromosome Xp. OA1 = ocular albinism; RP2, RP3, RP6, and RP15 = X-linked retinitis pigmentosa; CSNBX1 and CSNBX2 = X-linked congenital stationary night blindness; COD1 = X-linked cone dystrophy; AIED = Aland island eye disease; OPA2 = X-linked optic atrophy; NDP = Norrie disease protein; EVR2 = exudative vitreoretinopathy; CACNA1F = calcium channel α1-subunit; and RPGR = retinitis pigmentosa GTPase regulator.

Acknowledgment

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Electronic-Database Information

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

Genatlas, Infobiogen, http://www.Infobiogen.fr (for the localization of CSNBX1 and CSNBX2, RP2, RP3, RP6, RP15, COD1, NDP, EVR2, AIED, and OPA2)

Généthon, http://www.genethon.fr (for the genetic map of the X chromosome)

Genome Database, http://gdbwww.gdb.org (for the nucleotide sequences of markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for X-linked dominant and X-linked recessive [MIM 310700], autosomal dominant [MIM 164100], and autosomal recessive [MIM 257400] CN)

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