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Report

A Novel X-Linked Dominant Condition: X-Linked Congenital Isolated Ptosis

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We present a large family with a previously undescribed condition: X-linked dominant congenital bilateral isolated ptosis. Linkage analysis defined a critical region between Xq24 and Xq27.1, with a maximum single-point LOD score of 2.88 at DXS1047 and DXS984. Male and female family members are equally affected, providing an example of an X-linked, truly dominant condition.

The word "ptosis," an abbreviation for the term "blepharoptosis," is used to describe the drooping of the upper eyelid. Ptosis may be congenital or acquired, and it may be unilateral or bilateral. It can occur in isolation or in syndromic form, and it is a feature of many conditions. Here, we discuss dominantly inherited congenital isolated ptosis. Work by Engle et al. (1997) on a pedigree with this condition, first described by Cohen (1972), has demonstrated linkage to markers in the region of 1p32-1p34.1 (MIM 178300) with a maximum two-point LOD score of 8.8. We describe the analysis of an independent large pedigree with dominant congenital isolated ptosis and linkage to the X chromosome.

A family whose members had congenital ptosis was noted by one of us (A.G.T.), who came across the family as part of his oculoplastic practice. We made a detailed assessment of the phenotype of members of this family willing to participate in the study. The pedigree is that of a white family from the southwest of England. Members of the pedigree exhibit dominantly inherited congenital isolated bilateral ptosis, as shown in figure 1. In particular, there was no evidence of blepharophimosis, epicanthus inversus, or prevailing ocular motility disorder. The ptosis was strikingly symmetrical and equal in both male and female family members (fig. 1*A*,*B*). The affected family members were born with bilateral ptosis, which resulted in an abnormally low lid position that almost impinges on the visual axis in the primary position of gaze (superior margin–reflex distance of ~0 mm). Patients had minimal levator function bilaterally (<5 mm), associated with an absent upper-lid skin crease. They had pronounced frontalis overaction and a characteristic chin-up head posture. Best corrected visual acuity showed no evidence of amblyopia in any patient studied. Ocular motility was normal in most affected individuals, but there was reduced upgaze in some affected family members—individuals I:2, II:8, II:21, III:8, and III:26 (fig. 2). The patients had a good Bell's response. No patients reported progression or variability of their ptosis; for this reason, myasthenia gravis is unlikely, and edrophonium testing and electromyographic studies were not performed.

The portion of the pedigree available for study spanned four generations; 15 affected and 17 unaffected individuals were available for analysis, including 19 individuals that are potentially informative for the X chromosome (fig. 2). All procedures were undertaken with the patients' informed consent and with ethical approval from the Salisbury Research Ethics Committee.

We obtained blood and extracted DNA from family members for linkage analysis. We performed PCR amplification under standard conditions. Primers were obtained (MWG-BIOTECH AG) according to sequences obtained from the Medical Research Council linkage-mapping set (Reed et al. 1994) supplemented by markers from the Genome Database. One primer of each pair was labeled with a fluorochrome at the 5′ end. Hotstar *Taq* reagents were used (Qiagen) in the following reactions, per sample: 1 μl DNA (100 μg/ml); 0.1 μl (0.5

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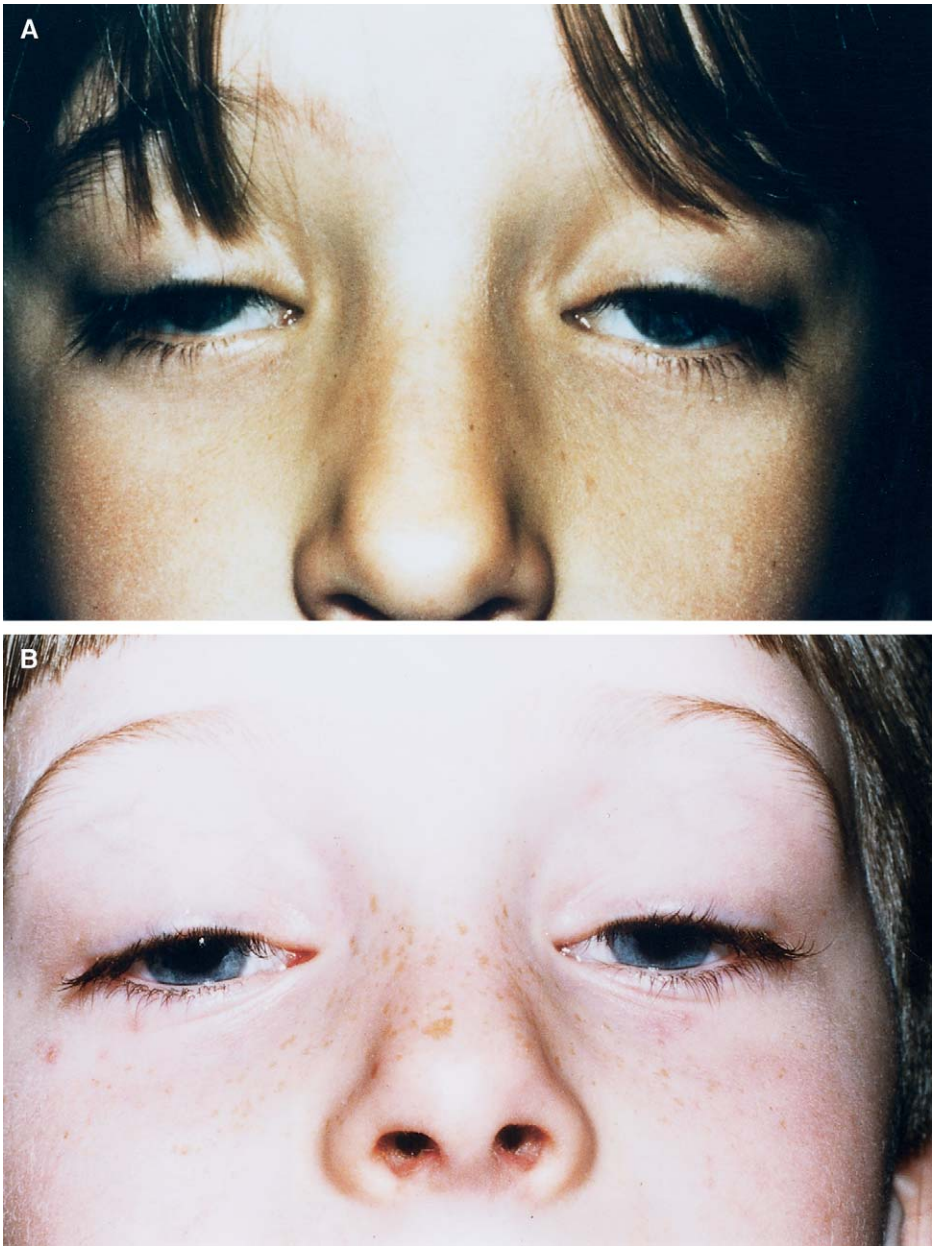

Figure 1 *A,* Female member of the pedigree showing phenotype. *B,* Male member of the pedigree showing phenotype.

U) Hotstar *Taq* polymerase; 2 μl 10 × PCR buffer; 2 μl 100 mM dNTP; 13.9 μl dH_20; and 1 μl each primer (33 ng/μl). Typical thermocycling parameters were as follows: 15 min at 94°C, followed by 32 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, followed by 2 min at 72°C and 60 min at 60°C, and a 4°C soak.

We used the following primer pairs to test for linkage to the chromosome 1 locus: D1S2733, D1S2677, D1S2797, D1S2134, D1S2824, D1S1616, and D1S2748. PCR reaction products were separated by polyacrylamide gel electrophoresis on an ABI 377 analyzer. GENESCAN and GENOTYPER software (ABI) were used to genotype the individuals. For chromosome 1, we typed seven markers and performed single-point analysis by MLINK, assuming penetrances to be 90%. We estimated allele frequencies for the seven markers from the pedigree.

The results of linkage analysis for chromosome 1 (table 1) show no single-point LOD scores to support linkage of the condition to markers in the region of interest on chromosome 1, as defined by Engle et al. (1997). LOD scores of −3.22 and −1.80 for markers D1S2733

and D1S2748, which are at either end of their critical region, exclude linkage to these markers in the pedigree that we studied. We obtained a LOD value of −3.09 for D1S2677, the marker with the highest LOD score in the pedigree that they describe. Single-point tests were repeated with equal allele frequencies. This was found to have little effect on the LOD scores. The largest changes were for D1S2677, where the LOD at recombination fraction (θ) 0 changed from −3.09 to −3.21, and at D1S2824, where the LOD at $\theta = 0$ changed from −1.81 to −1.62.

The mode of inheritance in our pedigree was such that no male-to-male transmission was observed in the wider pedigree (fig. 3), including individuals unavailable for study; all six sons of affected men were unaffected. However, the four daughters of affected men were affected. This is consistent with an X-linked dominant mode of inheritance. We calculated a LOD score for X linkage by means of the pedigree alone without haplotype data. Since women who transmit ptosis are not informative (their X chromosomes cannot be distinguished without markers), only the men who transmit ptosis are useful in this respect. There are a total of six normal sons and four affected daughters from affected fathers in the wider pedigree, including individuals unavailable for study (fig. 3). There are thus 10 meioses (not including the index case) that are consistent with disease linkage to the X chromosome corresponding to a LOD of $10\log_{10}2 = 3.01$.

Table 1

Single-Point LOD Scores for Chromosome 1 Markers

	LOD AT θ				
PRIMER	0	.1	.2	.3	.4
D1S2733	−3.22	−1.16	−.50	−.18	−.04
D1S2677	−3.09	−1.97	−1.08	−.49	−.14
D1S2797	−5.15	−2.49	−1.33	−.62	−.20
D1S2134	−5.51	−2.75	−1.80	−1.02	−.40
D1S2824	−1.81	−1.67	−1.20	−.61	−.21
D1S1616	−1.54	−1.52	−1.18	−.63	−.23
D1S2748	−1.80	−1.54	−1.38	−.95	−.38

We therefore searched for linkage to polymorphic markers on the X chromosome by means of the methods described earlier. We used the following markers: HUMAR, DXS8092, DXS990, DXS8063, DXS1220,

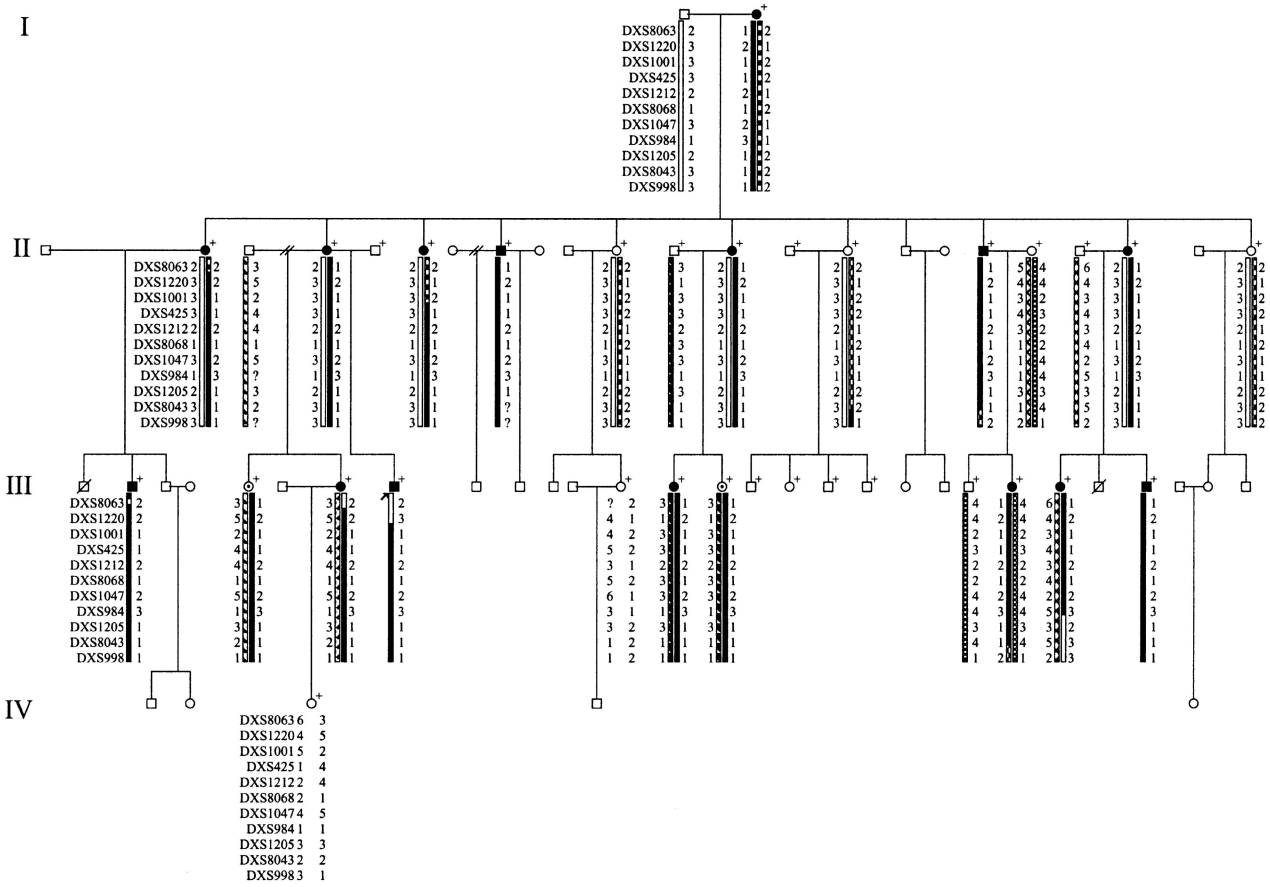

Figure 2 Ptosis pedigree with haplotypes. Plus signs (+) denote samples obtained for analysis; blackened symbols denote affected status.

DXS1001, DXS425, DXS1212, DXS8068, DXS1047, DXS994, DXS8094, DXS8050, DXS1062, DXS1192, DXS984, DXS1205, DXS1227, DXS8043, DXS998, and DXS548.

The map for multipoint analysis was obtained by the Map+ program (Collins et al. 1996), which we used to combine CEPH V8.2–derived pairwise LOD scores with two-point LODs derived from the pedigree. The interference parameter in the Rao et al. (1977) map function was estimated at 0.366, and typing-error frequency (Shields et al. 1991) was .0012.

We performed multipoint analysis (table 2) by the LINKMAP option by Vitesse, version 2 (O'Connell and Weeks 1995), and single-point analysis by MLINK. Penetrances were assumed to be .1, .9, and .9 for the X-chromosome genotypes dd, dD, and DD in women (where D is the disease allele) and 0.1 and 0.9 for the corresponding d and D genotypes in men, because there were 2 women, among 19 informative transmissions, who possessed the at-risk haplotype but did not have ptosis. We estimated allele frequencies for the 21 markers from the pedigree.

X-chromosome linkage from haplotype analysis showed evidence for linkage to markers mapped to Xq24–Xq27.1. A highest single-point LOD score of 2.88 with no recombination was established at DXS1047 and DXS984 (table 2). The assumption of 100% penetrance reduced the maximum LOD to 2.71 at $\theta = 0$ with DXS1212 and DXS8068. Employing equal allele frequencies gave a single-point LOD of 2.88, implying, as before, insensitivity to allele frequencies. Recombinations showed the critical region to lie between DXS1001 and DXS1205 (fig. 2). Under these conditions, a LOD of 2 that uses markers meets the conventional criteria for X linkage (Terwilliger and Ott 1994). However, this is conservative since the LODs from both segregation and marker linkage are independent.

Table 2

X-Chromosome LOD Scores

Primer	Single-Point LOD at θ					Multipoint LOD
	.0	.1	.2	.3	.4	
HUMAR	−4.70	−2.20	−1.18	−.53	−.16	−4.70
DXS8092	−3.50	−2.09	−1.21	−.60	−.21	−3.50
DXS990	−2.19	−1.30	−.72	−.35	−.12	−2.79
DXS8063	−1.63	−.46	.01	.14	.08	−1.61
DXS1220	.97	1.35	1.28	.96	.45	.97
DXS1001	1.92	2.01	1.73	1.24	.59	1.84
DXS425	2.62	2.46	2.03	1.42	.66	2.87
DXS1212	2.70	2.23	1.72	1.13	.48	2.88
DXS8068	2.52	2.08	1.60	1.05	.44	2.88
DXS1047	2.88	2.67	2.19	1.53	.72	2.88
DXS994	.31	.53	.54	.44	.26	2.85
DXS8094	.37	.58	.59	.48	.28	2.78
DXS8050	.32	.46	.46	.38	.23	2.78
DXS1062	.27	.49	.51	.42	.25	2.75
DXS1192	.31	.53	.54	.44	.26	2.69
DXS984	2.88	2.59	2.10	1.46	.68	2.88
DXS1205	1.93	2.01	1.74	1.25	.58	1.93
DXS1227	1.67	1.80	1.57	1.12	.52	1.93
DXS8043	1.67	1.80	1.57	1.12	.52	1.88
DXS998	−2.47	−.24	.24	.30	.17	−2.03
DXS548	−2.47	−.24	.24	.30	.17	−2.03

If T represents transmission of disease from fathers and S is transmission of markers | disease transmission and X represents X linkage and $\overline{X}$ autosomal linkage, then

$$\frac{P(T \mid X)}{P(T \mid \overline{X})} \times \frac{P(S \mid T,X)}{P(S \mid T,\overline{X})} = \frac{P(S,T \mid X)}{P(S,T \mid \overline{X})} = 2^{10} \times 10^{z} .$$

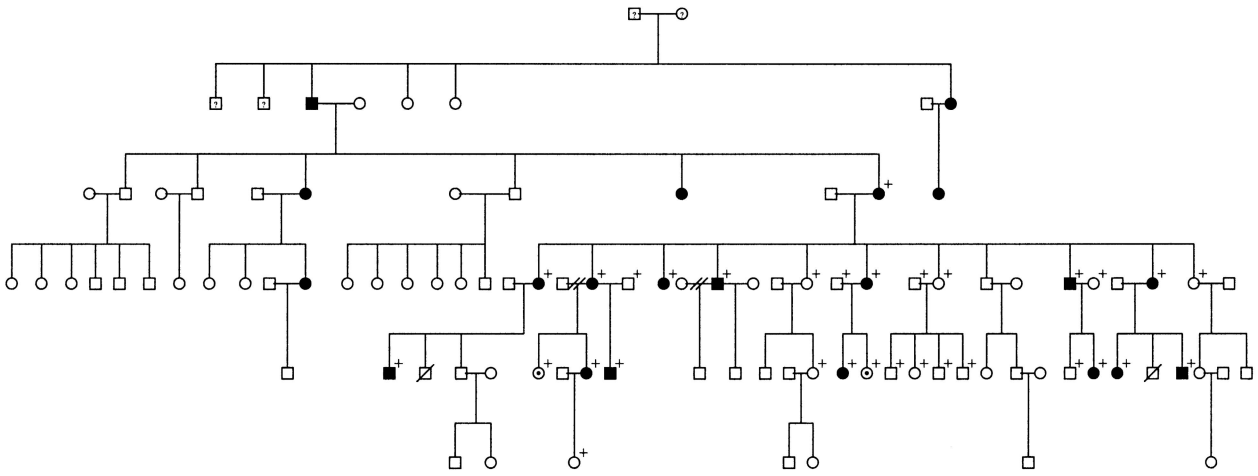

Figure 3 The wider pedigree

The LOD is therefore 3.01 + 2.88 = 5.89. This provides overwhelming evidence for X linkage. Two women, patients III:5 and III:15, carried the at-risk haplotype but were unaffected, with no expression of the ptosis phenotype.

The X-inactivation status of women was investigated by PCR amplification of a polymorphic site at the human androgen receptor (HUMAR) locus before and after digestion with methylation-sensitive restriction enzymes *Hpa*II and *Cfo*I as described by Pegoraro et al. (1994). PCR products were separated on an ABI 377 sequencer and quantified by Genescan and Genotyper software (ABI), and the degree of X inactivation was determined by comparison of the ratios of the two alleles before and after restriction and expression of this comparison as a percentage. We assessed which disease allele was active by genotyping at a mean average of 8.2 cM between HUMAR and the disease-interval haplotype. This allowed identification of any recombination between the inactive allele at HUMAR and the disease interval; it also allowed us to assess the inactivation status of the at-risk haplotype.

We analyzed 12 women with the at-risk haplotype in the pedigree for X-inactivation status at the human androgen receptor locus in DNA from blood. The blood we analyzed included the blood of the two women (patients III:5 and III:15) who did not express the ptosis phenotype. In 8 of 10 affected women, the chromosome with the mutation was active more often than the normal chromosome, although in only three patients was the skew >80%. The remaining two affected women had a slight skew against activity of the chromosome with the mutation. Of the two nonexpressing women with the at-risk haplotype, one, patient III:5, had a unilateral pattern of X inactivation, with the chromosome carrying the mutation exclusively inactive. This probably explains her normal phenotype in the presence of the at-risk haplotype. The other, patient III:15, exhibited a random pattern of X-chromosome inactivation, which does not explain why the at-risk haplotype does not confer ptosis.

Blood for cytogenetic analysis was obtained from affected individuals II:21 and III:26. Blood lymphocytes were cultured with conventional techniques. No chromosome abnormalities were identified at the 600-band level in either individual.

The genetic cause of ptosis in the pedigree studied is clearly distinct from the only other documented isolated ptosis pedigree subjected to linkage analysis, as described by Engle et al. (1997), who found linkage to 1p32-p34.1. We excluded linkage to chromosome 1 after testing seven polymorphic markers in the region of interest (table 1) and demonstrated linkage to Xq24–Xq27.1. Therefore, this defines a new condition: X-linked dominant congenital isolated ptosis. As can be seen from figure 1, the phenotype is identical in both men and women, making this an X-linked, truly dominant condition. This is very unusual, as most X-linked dominant conditions manifest less severely in women—for example, X-linked Charcot-Marie-Tooth (MIM 302800) and hypophosphatemia (vitamin D–resistant rickets [MIM 307800]). More extreme X-linked dominant diseases, such as X-linked dominant chondroplasia punctata (MIM 302960), are lethal in men because of their hemizygous state. The second X chromosome has an ameliorating effect in women.

That women with Turner syndrome do not have congenital isolated ptosis and that ptosis is not common to all men suggests that the disease is not due to haploinsufficiency but, rather, to a dominant negative effect in carriers of the mutant allele. The locus falls outside the pseudoautosomal regions, and, therefore, the mutant allele is likely to be randomly inactivated, although some nonpseudoautosomal genes escape X-chromosome inactivation. X-chromosome inactivation studies in women carrying the at-risk haplotype show that there is no consistent skewing to favor the expression of the mutant allele in blood. There is, therefore, no evidence that the mutant allele affects X-chromosome inactivation. It may be that the mutant allele overrides the function of the normal allele at a critical time in neuromuscular development, with permanent effects—for example, by occupation of a significant proportion of receptors for neurotrophic factors, with a resulting failure to switch the relevant cell-signaling pathways on or off.

In conclusion, we have defined X-linked dominant congenital bilateral isolated ptosis, an X-linked, truly dominant condition that has not previously been reported. We are not aware of any other X-linked dominant condition with equal expression in men and women. Linkage for the condition to Xq24–Xq27.1 has been demonstrated with a single-point LOD score of 2.88 and a cumulative LOD score of 5.89 when combined with a LOD of 3.01 from analyses of the mode of inheritance from the pedigree alone.

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Electronic-Database Information

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

The Genome Database, http://www.hgmp.mrc.ac.uk/gdb (for markers)

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim (for 1p32-1p34.1 [MIM 178300], X-linked Charcot-Marie-Tooth [MIM 302800], hypophosphatemia [MIM 307800], and X-linked dominant chondroplasia punctata [MIM 302960])

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