

## Report

# Terminal Osseous Dysplasia with Pigmentary Defects Maps to Human Chromosome Xq27.3-Xqter

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We have identified a four-generation family with 10 affected females manifesting one or more of the following features: osseous dysplasia involving the metacarpals, metatarsals, and phalanges leading to brachydactyly, campodactyly, and other digital deformities; pigmentary defects on the face and scalp; and multiple frenula. There were no affected males. We performed X-inactivation studies on seven affected females, using a methylation assay at the androgen receptor locus; all seven demonstrated preferential inactivation of their maternal chromosomes carrying the mutation, and two unaffected females showed a random pattern. These findings indicate that this disorder is linked to the X chromosome. To map the gene for this disorder, we analyzed DNA from nine affected females and five unaffected individuals, using 40 polymorphic markers evenly distributed throughout the X chromosome. Two-point and multipoint linkage analyses using informative markers excluded most of the X chromosome and demonstrated linkage to a region on the long arm between DXS548 and Xqter. A maximum LOD score of 3.16 at recombination fraction 0 was obtained for five markers mapping to Xq27.3-Xq28. The mapping data should facilitate the identification of the molecular basis of this disorder.

We identified a novel limb-malformation syndrome in a four-generation family (fig. 1). The syndrome is characterized by abnormal and delayed ossification of bones in the hands and feet, leading to brachydactyly, campodactyly, and clinodactyly of the digits, severe limb deformities, and joint contractures. In addition, the affected individuals have pigmentary skin lesions on the face and scalp, dysmorphic features such as hypertelorism, and multiple frenula. The spectrum of phenotypic manifestations in this pedigree is reminiscent of those described by Horii et al. (1998) and Bloem et al. (1974) in a few sporadic cases.

Because only females in the family are apparently affected and the number of male offspring from affected women is reduced, we hypothesized that this is an X-linked dominant, male-lethal disease. (Unaffected women had a male:female offspring ratio of 1.25:1. The af-

ected women, however, had a ratio of 1:3.4.) We obtained blood samples from 15 family members and isolated genomic DNAs either from lymphoblast cell lines or peripheral blood leukocytes (PBLs), using procedures described elsewhere (Ellison et al. 1992).

We performed X-inactivation studies with PBL DNA from seven affected and two unaffected females, using the previously described methylation assay at the androgen-receptor (AR) locus (Allen et al. 1992). Results were analyzed as described elsewhere (Pegoraro et al. 1994) and are shown in figure 2.

All seven affected females examined had clearly skewed X-inactivation patterns, ranging from 82:18% (IV.5) to 93:7% (IV.3) (fig. 2). Furthermore, all of the affected females studied showed preferential inactivation of their maternal X chromosomes, which carried the mutated gene. These results are consistent with an X-linked dominant, male-lethal inheritance pattern. Two unaffected individuals (II.2 and IV.2) have random X-inactivation, as revealed by approximately equal intensity of the two amplified alleles from *HpaII*-digested DNA. To map the gene, we genotyped all 15 samples with 40 highly polymorphic markers that cover the entire X chromosome at intervals of 10–20 cM. PCR was

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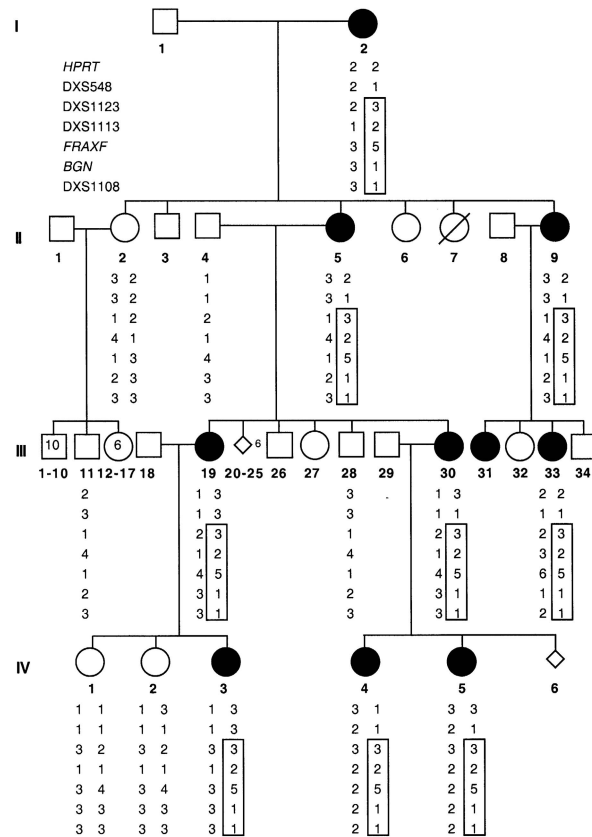
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performed by use of published primer sequences and conditions (Genome Database). Haplotype analysis demonstrated that all affected females, but no unaffected individuals, shared an identical haplotype on the distal part of Xq demarcated proximally by DXS548 (fig. 1). To confirm the linkage of the disease locus to this region, we performed two-point linkage analysis (Schaffer et al. 1994). A maximum LOD score of 3.16 with recombination fraction ( $\theta$ ) 0 was obtained for each of the five markers that had alleles cosegregating with the disease locus (table 1). The rest of the X chromosome was excluded on the basis of LOD scores  $< -2$ . Taken together, these results provide strong evidence for the existence of a gene responsible for terminal osseous dysplasia and pigmentary defects in the region flanked by marker DXS548 and Xqter.

Although all affected females preferentially inactivated their maternal X chromosomes, there is no direct correlation between the severity of clinical phenotype and the degree of the skewing of X-inactivation. Within the range of skewing (93:7%–82:18%), there is phenotypic variability, most likely because of differing inactivation patterns between different tissues.

The candidate region spans Xq28, one of the most gene-rich regions in the human genome. Approximately 60 cDNAs (National Center for Biotechnology Information Human Gene Map), 37 characterized genes (Integrated X Chromosome Database, version 2.2), and a number of human disorders have been mapped to this 8.7-Mb region. Christian syndrome (CHRS, MIM 309620) causes limb abnormalities, along with mental retardation, short stature, ridging of the metopic suture, and vertebral anomalies (fusions, hemivertebrae, and scoliosis); patients with otopalatodigital syndrome type 1 (OPD1, MIM 311300) are short in stature and mentally retarded, and they have cleft palate and a variety of limb anomalies. Despite the overlap in limb abnormalities with the syndrome in our pedigree, most of the features of CHRS and OPD1 are not present, making it



**Figure 1** Family pedigree with haplotype analysis of seven representative markers in Xq26-Xqter region. The represented markers are shown, left, in generation I. The identical haplotype shared by affected females is boxed.

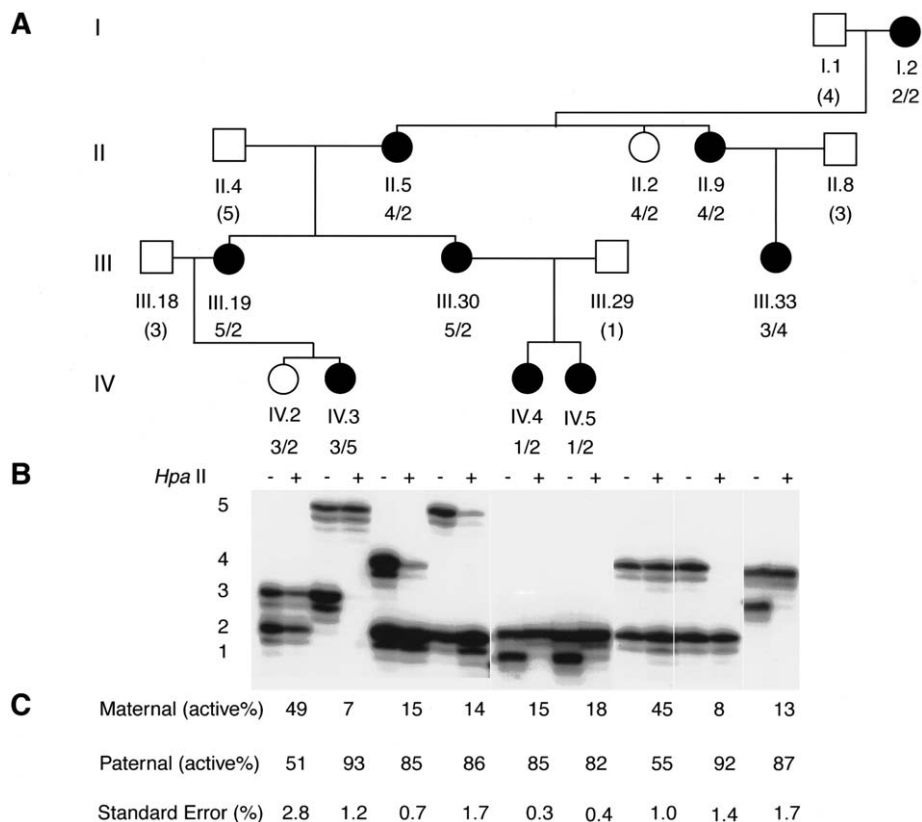
unlikely that these diseases are allelic with the syndrome we describe here.

Some of the clinical features seen in this family are comparable to features seen in tattered (*Td*), bare

**Table 1**  
LOD Scores of Two-Point Linkage Analysis for Selected Markers

MARKER	LOD SCORE AT $\theta =$						
	.00	.01	.05	.10	.20	.30	.40
HPRT	-10.795	-4.415	-2.280	-1.370	-.539	-.161	-.003
DXS548	-4.135	.228	.791	.915	.836	.604	.308
DXS1123	3.164	3.113	2.906	2.635	2.051	1.410	.722
DXS1113	3.164	3.113	2.906	2.635	2.051	1.410	.722
FRAXF	3.164	3.113	2.906	2.635	2.051	1.410	.722
BGN	3.164	3.113	2.906	2.635	2.051	1.410	.722
DXS1108	3.164	3.113	2.905	2.633	2.047	1.406	.718

NOTE.—Markers are listed in order from Xpter to Xqter. Penetrance of 90% and disease-allele frequency of .0001 were used for calculation.



**Figure 2** Analysis of X-inactivation patterns using the methylation assay at the androgen receptor. *A*, Partial pedigree of the family, showing the members analyzed by the AR assay. Individuals are numbered according to the full pedigree in figure 1. The genotypes at the AR locus are shown beneath the symbol for each individual, with inferred genotypes in parentheses. *B*, Autoradiographs of labeled PCR products. PBL DNA from each individual was either used directly as a template (– lanes) or digested with *HpaII* prior to PCR amplification (+ lanes). For each individual, the two alleles were present in the undigested samples. In the *HpaII*-digested samples of the affected females, the alleles from the maternal X chromosomes were overrepresented, indicating preferential inactivation of these X chromosomes. *C*, Percentage of paternal and maternal active alleles. Ratios of active X chromosomes are determined with use of a correction factor to account for preferential amplification of the smaller allele (Pegoraro et al. 1994). The numbers shown here represent the means calculated from at least two separate experiments, with standard errors shown below.

patches (*Bpa*), and striated (*Str*) mice. *Td* is an X-linked, semidominant mouse mutation associated with prenatal male lethality. Heterozygous females have patches of scarred skin at age 4–5 d. Because no hairs grow in the scarred areas, adults have tattered coats. The tattered coat may resemble the pigmented lesions and alopecia seen in the family described here, but it seems to be a generalized phenomenon that is not limited to the face and scalp as in our patients. Craniofacial defects and twisted toes have also been observed in some *Td* mice. Recently, the defect in *Td* mice has been identified as a mutation in a sterol isomerase encoded by the mouse emopamil-binding protein (*Ebp*) gene (Derry et al. 1999). Alterations in human *EBP* have been found in *CDPX2* patients, whose phenotype bears similarity to that of the *Td* mouse (MIM 302960). The phenotypes

of *Bpa* and *Str* mice are very similar to that of *Td* mice and are caused by mutations in the NAD(P)H steroid dehydrogenase-like gene (*Nsdhl*), which also encodes an enzyme involved in sterol biosynthesis (Liu et al. 1999). *EBP* maps to Xp11.23-p11.22 and *NSDHL* is located in Xq28. *NSDHL* is thus a candidate gene, and it will be interesting to examine sterol metabolism in our patients.

Although hundreds of human disorders involving defects in limb development have been described, only ~25 have been mapped (Bamshad et al. 1999). We report the molecular studies and genetic mapping of a novel X-linked dominant, male-lethal disorder. We demonstrated a skewed pattern of X-inactivation in all affected females examined and mapped the gene responsible for this disorder to an 8.7-Mb region telomeric to DXS548 on the

distal part of Xq. This will allow screening of candidate genes in this region for mutations. Identification of additional cases in the extended pedigrees of this and other families may help to further refine the critical interval.

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## Electronic-Database Information

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

Genome Database, <http://gdbwww.gdb.org/> (for PCR primers and conditions of the markers used)

Integrated X Chromosome Database, version 2.2, <http://ixdb.mpimg-berlin-dahlem.mpg.de/> (for markers and genes from DXS548 to Xqter)

National Center for Biotechnology Information Human Gene Map, <http://www.ncbi.nlm.nih.gov/SCIENCE96/> (for cDNAs in DXS548-Xqter)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for OPD1 [MIM 311300], CHRS [MIM 309620], and CDPX2 [MIM 302960])

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