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DNA VARIANTS

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Dinucleotide polymorphism at the *DXS1178* locus is tightly linked to *PGK1* at Xq13

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Abstract A polymorphic CA repeat (locus name *DXS1178*) was isolated from a 1-megabase YAC (OTCC) containing the *OTC* gene, located at Xp21.1. However, amplification in human-rodent hybrid cells and segregation analysis in three CEPH families mapped the *DXS1178* locus at Xq13. The mapping ambiguity is apparently caused by the chimeric nature of the OTCC YAC clone.

Sourceldescription. A 1-megabase YAC, OTCC, was isolated by screening the Washington University yeast artificial chromosome (YAC) library (Brownstein et al. 1989) with primer sequences derived from the *OTC* gene (Fujita et al. 1993). The OTCC YAC was localized to Xp11.3-p21.1 by fluorescence in situ hybridization; however, analysis of derivative clones indicated that OTCC was a chimeric clone. A 450-kb *Mlu*I fragment adjacent to the *OTC* gene was purified, digested with *Hind*III and subcloned into pBluescript KSII plasmid (Stratagene). Hybridization of about 200 independent plasmid clones with a poly (dC-dA) probe (Pharmacia) identified a 0.8-kb subclone, OCP1B. The sequence of OCP1B (locus *DXS1178*; GenBank no. U07360) revealed a segment $(CA)_2TA(CA)_{14}TACA(CT)_8$. The primers flanking the repeat amplified a 167-bp fragment in OCP1B and revealed a dinucleotide polymorphism in 65 unrelated individuals.

Polymerase chain reaction (PCR) conditions. Sequences CS13 (5′ GTTGGAATCAGTTGGAGAGTCGTG 3′) and CS14 (5′ TGCAGTTCCAGGAGCAGCAACAG 3′), flanking the microsatellite sequence, were used to design PCR primers. Amplification was carried out in 50 µl reaction containing 50 ng of DNA, 10 ng of each primer (one of these labeled with γ-^{32}P ATP and polynucleotide kinase), 200 µ*M* each dNTP, 10 m*M* Tris-Cl (pH 8.3), 50 m*M* KCl, 1 m*M* $MgCl_2$ and 0.25 units of AmpliTaq (Perkin-Elmer Cetus). Reaction was performed for 30 cycles of 45 s at 94°C, 45 s at 65°C, and 45 s at 72°C. A 2-µl aliquot of the reaction was loaded on an 8% polyacrylamide-50% urea denaturing gel, pre-run for 30 min.

Chromosomal localization. *DXS1178* was amplified only in rodent-human hybrid cells containing the long arm of the X chromosome. Segregation analysis in Centre d'Etude du Polymorphisme Humain (CEPH) families 884, 1331, and 1333 revealed linkage of *DXS1178* to the *PGK1* locus with a maximum lod score of 6 at $q = 0$ (lod-1 support interval 0.0–0.1). The localization of *PGK1* in Xq13 agrees with the amplification in rodent-human hybrid cells. No significant score was obtained with markers located on the short arm of the X chromosome. This result confirmed that the OTCC YAC is a chimeric clone.

Inheritance. X-linked segregation was observed in two large three-generation families with X-linked retinitis pigmentosa and in CEPH families 884, 1331, and 1333. Alleles in mothers from CEPH families are: 884-2 (A3, A4);

Table 1 Allele sizes and frequencies of the PCR-amplified (CA)*n* polymorphism at the *DXS1178* locus from 46 unrelated females (92 chromosomes). Observed heterozygosity is 0.56

Allele	Size (bp)	Frequency
A1	185	0.07
A2	183	0.09
A3	181	0.43
A4	179	0.09
A5	177	0.02
A6	171	0.01
A7	169	0.25
A8	167	0.04

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1331-2 (A1, A3); 1333-2 (A3, A4); and 1335-2 (A3, A3). The location of *DXS1178* on the X chromosome was confirmed by the heterozygosity observed only in females in a sample of 46 females (Table 1) and 19 males.

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