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Deletion Mapping of Åland Island Eye Disease to Xp21 between DXS67 (B24) and Duchenne Muscular Dystrophy

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Summary

Åland Island Eye Disease (AIED) is an X-linked form of ocular hypopigmentation—also known as Forsius-Eriksson, or type 2, ocular albinism—in which affected males demonstrate subnormal visual acuity, protanomalous red-green colorblindness, axial myopia, astigmatism, hypoplasia of the fovea, and hypopigmentation of the fundus. A patient has previously been described who, in addition to AIED, manifested a contiguous gene syndrome which included congenital adrenal hypoplasia (AHC), glycerol kinase deficiency (GKD), and Duchenne muscular dystrophy (DMD). In the present paper report we report the molecular genetic analysis of his deletion. Initially, multiplex polymerase-chain-reaction amplification was used to screen for a DMD-locus deletion which was then further characterized, using DMD cDNA and genomic probes, via Southern blot analysis. The deletion includes the region encompassed by probes C7 (DXS28) and DMD cDNA 8. Probes B24 (DXS67) and DMD cDNA 5b-7 show normal hybridization patterns and appear to flank the deletion, while the DMD cDNA 8 detects a junction fragment. Molecular genetic techniques have mapped the deletion in this patient to the subbands Xp21.3-21.2, between DXS67 and DMD.

Introduction

Complex glycerol kinase deficiency (CGKD) is a contiguous gene syndrome characterized by glycerol kinase deficiency (GKD) in association with congenital adrenal hypoplasia (AHC) and/or dystrophic myopathy (McCabe et al. 1977; Guggenheim et al. 1979; Wieringa et al. 1985; McCabe 1989). Individuals affected by the CGKD contiguous gene syndrome have been described with cytogenetically detectable deletions at Xp21 (Hammond et al. 1985; Patil et al. 1985; Wieringa et al. 1985; Bartley et al. 1986; Marlhens et al. 1987; Chelly et al. 1988; McCabe 1989). Molecular analyses have been applied to confirm deletions (Francke et al. 1987; Chelly et al. 1988; Matsumoto et al. 1988) and to detect them when cytogenetic resolution fails (Dunger et al. 1986; McCabe et al. 1989; Towbin et al. 1989). The sequence of these gene loci is believed to be Xpter–AHC–GK–DMD–cen (Chelly et al. 1988; Davies et al. 1988; Matsumoto et al. 1988; McCabe 1989).

Recently, a 6-year-old adopted boy was described (Pillers et al. 1988, 1990) who may be instrumental in expanding the definition of the CGKD contiguous gene syndrome. In addition to Duchenne muscular dystrophy (DMD), GKD, and AHC, the patient also manifests Åland Island eye disease (AIED). AIED is an X-linked form of ocular hypopigmentation which was reported by Forsius and Eriksson (1964) in 1964. Initially described in an inbred family living on the Åland Islands of Finland, this disorder has also been referred to as type 2, or Forsius-Eriksson, ocular albinism (OA2). It is characterized in males by subnormal visual acuity, protanomalous (red-green) colorblindness, axial myopia, astigmatism, hypopigmentation of the fundus, and hypoplasia of the fovea (MIM 30060; McKusick 1988). Ophthalmological examination, including a characteristic electroretinogram (ERG), and a skin biopsy showing normal melanocytes are fundamental to the diagnosis (Weleber et al. 1989).

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Previous cytogenetic analysis revealed a deletion at Xp21 in patient JR (Pillers et al. 1990). Molecular genetic studies were performed to better define the deletion. Multiplex PCR amplification was used to screen the region to be probed (Chamberlain et al. 1988), with the extent of the Oregon-JR deletion determined using DMD cDNA probes (Koenig et al. 1987), genomic probes, and Southern blot analysis.

Material and Methods

Material

Restriction enzymes were purchased from Boehringer-Mannheim Biochemicals (Indianapolis; *Bam*HI, *Eco*RI, and *Hin*dIII) or Bethesda Research Laboratories (Gaithersburg, MD; *Msp*I and *Pst*I). Agarose grade Seakem-ME was purchased from FMC Bioproducts (Rockland, ME). Zetabind nylon membrane was obtained from Bio-Rad Laboratories (Richmond, CA). Kodak X-AR film (Rochester, NY) was used for autoradiography. Random primer labeling kits were bought from Boehringer-Mannheim Biochemicals.

DNA Isolation

High-molecular-weight genomic DNA was isolated from lymphoblastoid cells according to methods described elsewhere (Kan et al. 1977; Poncz et al. 1982). After overnight proteinase K digestion, DNA was recovered by an Applied Biosystems DNA extractor (Model 340A). The purified DNA was resuspended in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and was quantitated spectrophotometrically at OD_{260}. The ratio OD_{260}/OD_{280} was used to confirm purity.

Multiplex PCR Screening

Multiplex polymerase chain reaction (PCR) was performed according to a method described elsewhere (Chamberlain et al. 1988, 1990) by using a modified reaction mixture which amplified nine separate regions of the DMD locus. Reactions utilized 500 ng of patient DNA and were performed on a Perkin Elmer–Cetus automated thermal cycler for 23 cycles. One-seventh of the final product was electrophoresed through a 1.5% agarose gel containing 0.5 μg ethidium bromide/ml.

Southern Blotting

Genomic DNA was digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Msp*I, or *Pst*I. Ten-microgram aliquots of digested DNA were electrophoresed on a horizontal 1% agarose gel. DNA was transferred to a nylon membrane (Zetabind) according to the method of Southern (1975), adapted to use 0.4 N NaOH as the transfer solution (Reed and Mann 1985). Blots were prehybridized at 65°C for 1 h with Church buffer (Church and Gilbert 1984) and then were hybridized for 18 h. The blots were washed two times for 30 min each at 65°C in 40 mM Na_2HPO_4, pH 7.2/1% SDS (Church and Gilbert 1984) and were autoradiographed for 1–7 d at −70°C.

DNA Probes

The following DMD cDNA subclones were used as probes: 1-2a, 3, 5b-7, 8, 9-10EHc, 11Hc, 12Hc, 12b-14HcE, and 14. The subclones approximate the cDNA nucleotide regions defined by Koenig et al. (1987) and have been described in detail elsewhere (McCabe et al. 1989; Towbin et al. 1989). The following Xp21 genomic probes, ordered Xcen to pter, were also used: OTC, cX5.4/cX5.7 (DXS148), 754 (DXS84), pERT 87-15 (DXS164), P20 (DXS269), J66 (DXS268), C7 (DXS28), B24 (DXS67), 99-6 (DXS41), and D2 (DXS43). All probes were labeled to high specific activity (10^9 dpm/μg) with ^{32}P-dCTP by using random primers (Feinberg and Vogelstein 1983).

Results

Multiplex PCR Screening

Multiplex PCR amplification using the nine-plex method of Chamberlain et al. (1988, 1990) was performed to screen for a deletion involving the dystrophin locus. Seven of the nine primer sets generated the expected size amplification products from the patient's genomic DNA (fig. 1*A*). However, two of the nine fragments (i.e., fragments f and h; figure 1*A*) produced from normal control DNA were absent from the patient DNA reaction products. These results demonstrated that the patient's DNA contained a partial intragenic deletion encompassing a portion of the 3′ end of the DMD gene (fig. 1*B*).

Southern Blot Analysis: Centromeric Breakpoint

The centromeric breakpoint of the deletion was determined using Southern blots of *Hin*dIII-digested DNA probed with DMD cDNAs (fig. 2). The banding pattern obtained using probe 5b-7 was identical to that of a control individual (fig. 2*A*), as were results of studies using genomic probes for the DMD region, i.e., DXS164, DXS268, and DXS269. None of the expected bands were observed when DMD cDNA probe 8 was used, although a single 6.6-kb fragment was detected,

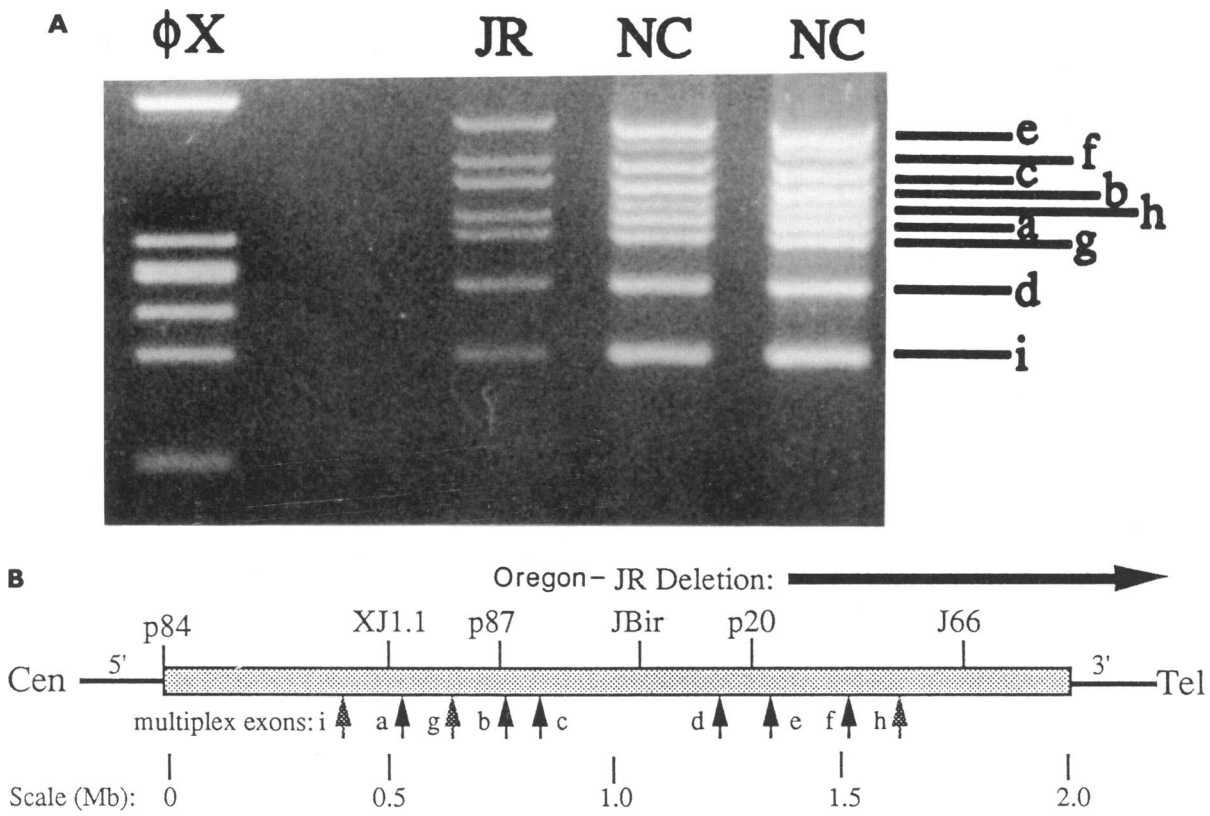

Figure 1 *A*, Agarose gel electrophoresis of multiplex PCR products. Note the absence of bands f and h in patient JR as compared with control. *B*, Molecular map of dystrophin locus. Intragenic genomic probe sites are indicated above, and exon primer sites for the multiplex procedure are shown below. The deletion Oregon-JR begins centromeric to primer f and extends telomeric beyond the dystrophin locus.

indicating the presence of a deletion junction fragment (fig. 2*B*). This was confirmed using restriction enzymes *Pst*I, *Msp*I, *Eco*RI, and *Bam*HI, each of which also detected a junction fragment (fig. 3). Data indicating the position of the centromeric breakpoint are summarized in figure 4.

Southern Blot Analysis: Telomeric Breakpoint

The telomeric breakpoint of the deletion was determined using genomic and DMD cDNA probes known to detect regions telomeric to DMD cDNA probe 8 (fig. 4). The remainder of the dystrophin gene extending 3′ from cDNA 8 was deleted. Genomic probe C7 (DXS28) was deleted, whereas genomic probe B24 (DXS67) showed a normal hybridization pattern (data not shown).

Discussion

A patient has been described who manifests AIED in the presence of an Xp21 deletion and phenotypic features of DMD, GKD, AHC, and mental retardation (Pillers et al. 1988, 1990). Multiplex PCR analysis of this patient's genomic DNA showed absence of the two amplification products normally generated by primer sets f and h. This demonstrates that the centromeric, or 5′, breakpoint of the deletion must lie within the region flanked by primer sets e and f. Only two exon-containing *Hin*dIII genomic fragments are located in this part of the dystrophin gene: a 1.5-kb fragment (detected with cDNA probe 5b-7) and a 10-kb fragment which was not detected with cDNA probe 8 in this patient (Koenig et al. 1987; Chamberlain et al. 1988; Baumbach et al. 1989). The observation that cDNA probe 8 detects only a single 6.6-kb fragment with the patient DNA demonstrates that at least a portion of the exon normally present on the 10-kb *Hin*dIII fragment must be intact. We propose that the 6.6-kb hybridizing fragment is derived from a deletion junction fragment resulting from a deletion with one breakpoint

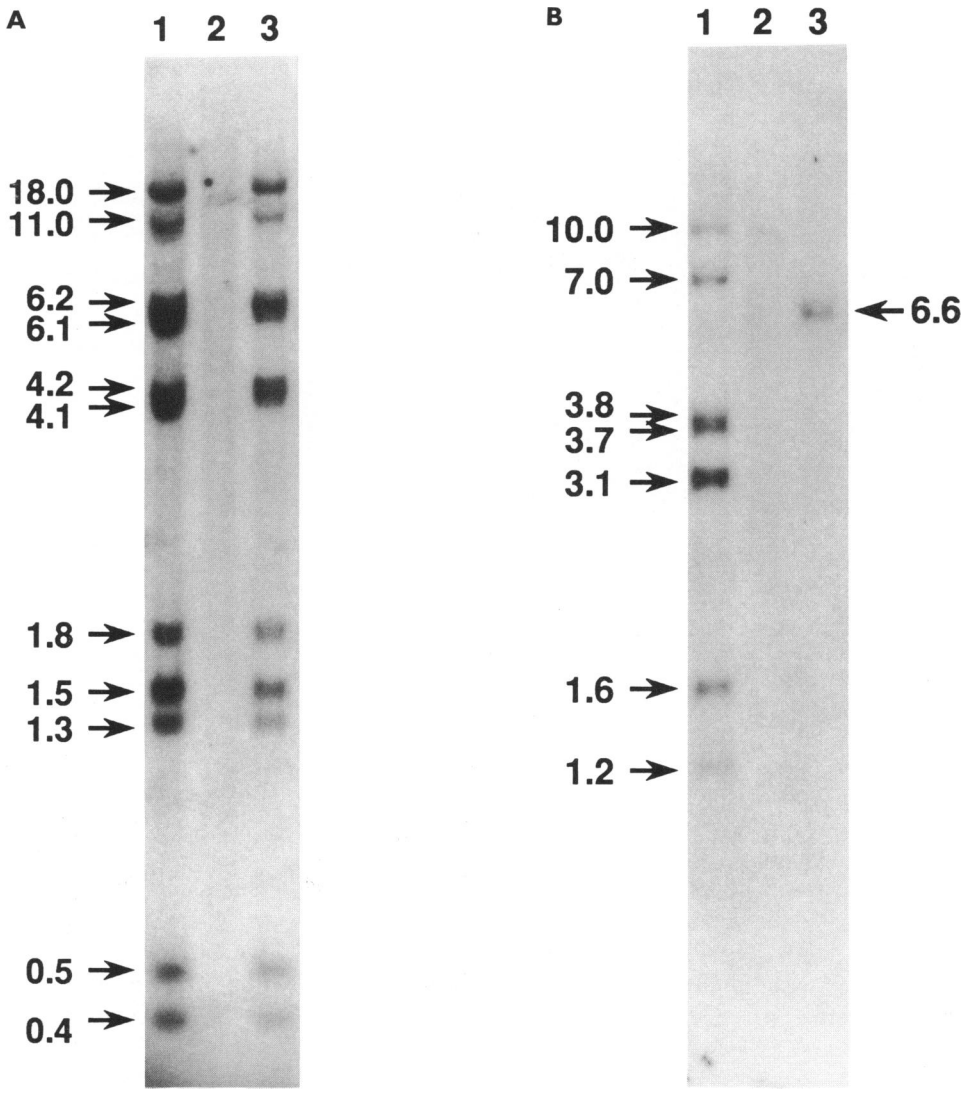

Figure 2 Autoradiography of *Hin*dIII-digested DNA on Southern blots using dystrophin cDNA probes. Lane 1, Normal control. Lane 2, Control known to be deleted in this region. Lane 3, Patient JR. *A,* Results for probe cDNA 5b-7, showing normal banding pattern. *B,* Results for probe cDNA 8, demonstrating that anticipated bands are absent, consistent with PCR multiplex screening. A single 6.6-kb band in lane 3 suggests the presence of a junction fragment in the DNA from patient JR.

within the 10-kb *Hin*dIII fragment. Southern analysis with 3′ DMD cDNA and genomic probes demonstrated that the deletion extends at least through the genomic region corresponding to C7. However, genomic probe B24 (DXS67) displays a normal hybridization pattern, indicating that the telomeric deletion breakpoint is located between C7 and B24 (fig. 4). We have thus been able to resolve the ambiguity regarding the subband observed to be deleted at the cytogenetic level. These molecular analyses map the Oregon-JR deletion to Xp21.3-21.2.

AIED (i.e., OA2) and Nettleship-Falls ocular albinism

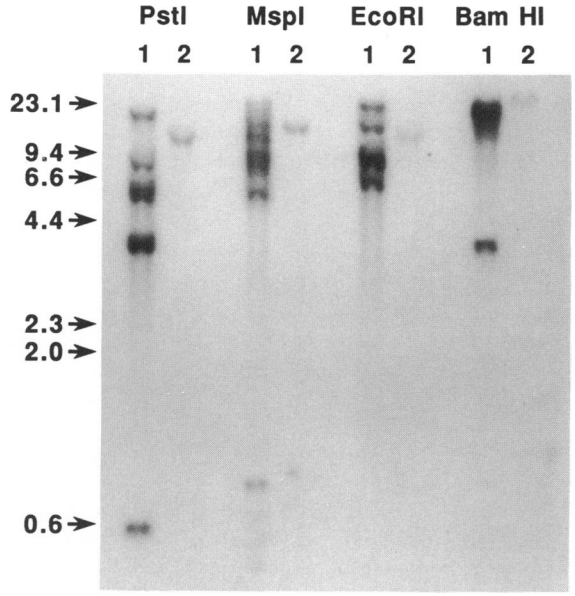

Figure 3 Autoradiography of Southern blots prepared from DNA digested with *Pst*I, *Msp*I, *Eco*RI, and *Bam*HI. Samples of DNA from a normal control (10 μg; lane 1) and patient JR (20 μg; lane 2) were electrophoresed, blotted, probed with cDNA 8, and autoradiographed. A single band consistent with a junction fragment was seen in each digest of the DNA from patient JR.

(OA1) have been considered to be allelic because of similar recombination fractions with the Xg blood group (OA1, .15 [Pearce et al. 1971]; OA2, .12 [Race and Sanger 1968, p. 549]). Placement of "ocular albinism" at Xp22 has been based on studies of Nettleship-Falls patients. The assignment of AIED to this locus was inferred. The recent findings of Schnur et al. (1989) show that their patients with ichthyosis and OA1 had deletions limited to Xp22. In contrast, our data indicate that the Oregon-JR deletion, associated with AIED, is localized to Xp21.3-21.2. This would support arguments that the two disorders relate to defects at distinct gene loci (Weleber et al. 1989).

As the clinical diagnosis of AIED is technically difficult (Weleber et al. 1989), it is unclear whether other CGKD patients also have this eye defect. With the molecular genetic delineation of this patient, it is a distinct possibility that other patients with CGKD will have AIED. Ophthalmologic examination, including electroretinography, of patients with defined deletions must be performed before AIED can be mapped relative to the other loci in this region.

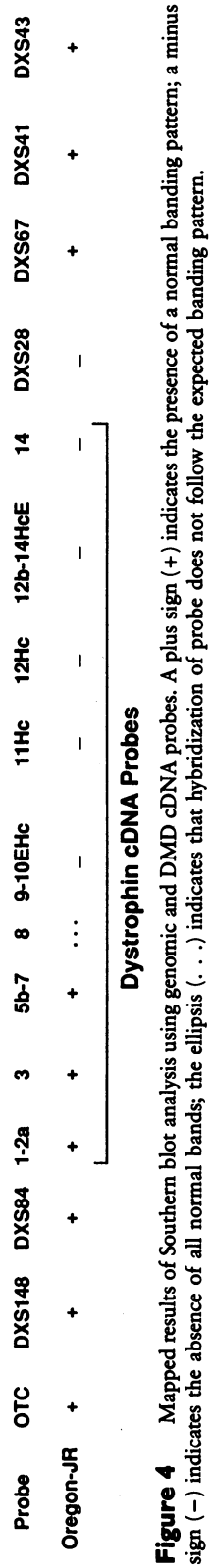

Figure 4 Mapped results of Southern blot analysis using genomic and DMD cDNA probes. A plus sign (+) indicates the presence of a normal banding pattern; a minus sign (–) indicates the absence of all normal bands; the ellipsis (. . .) indicates that hybridization of probe does not follow the expected banding pattern.

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