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Familial Inheritance of a DXS164 Deletion Mutation from a Heterozygous Female

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SUMMARY

Restriction-fragment-length-polymorphism analysis was used to examine a female who is segregating for Duchenne muscular dystrophy (DMD) and a deletion of the DXS164 region of the X chromosome. The segregating female has no prior family history of DMD, and she has two copies of the DXS164 region in her peripheral blood lymphocytes. The following two hypotheses are proposed to explain the coincidence of the DMD phenotype and deletion of the DXS164 region in her offspring: (1) she may be a gonadal mosaic for cells with two normal X chromosomes and cells with one normal X chromosome and an X chromosome with a deletion of the DXS164 region; and (2) she may carry a familial X;autosome translocation in which the DXS164 region is deleted from one X chromosome and translocated to an autosome. The segregation of DMD and the DXS164 deletion in this family illustrates the importance of extended pedigree analysis when DXS164 deletions are used to identify female carriers of the DMD gene.

INTRODUCTION

The DXS164 region of the X chromosome is closely linked to the locus for Duchenne muscular dystrophy (DMD) (Monaco et al. 1985) with $\theta = 0.06$ centimorgans (confidence limits 0.02–0.12) (Fischbeck et al. 1986). Part or all

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of the DXS164 region is deleted in 8.3% of familial and 5.8% of isolated cases of DMD, and the DXS164 deletions have never been observed in phenotypically normal males in these families (Kunkel et al. 1986).

To investigate the relationship between deletions in the DXS164 region and mutations at the DMD locus, we used restriction-fragment-length polymorphisms (RFLPs) to examine a family in which a DXS164 deletion and the DMD gene are segregating. In this family, the proband and two of his sisters inherited a DXS164 deletion and the DMD gene from their mother, who carries two copies of the DXS164 region in her peripheral blood lymphocytes.

MATERIAL AND METHODS

Family Data

The family was ascertained in 1975 through the Duke Neuromuscular Research Clinic. The extended pedigree as of 1977 is family BE of Roses et al. (1977). Blood was obtained for DNA isolation, establishment of lymphoblastoid cell lines, and genotyping for paternity testing. All family members in this communication were resampled and retyped.

Analysis of DNA Polymorphisms

Genomic DNA (15 μg/sample) was extracted from lymphocytes or long-term lymphoblastoid cell lines of family members (Monaco et al. 1985; Pericak-Vance et al. 1986) and cleaved with restriction enzymes according to conditions of the supplier (New England Biolabs). Samples were phenol extracted, redigested, reextracted, electrophoresed on 1% agarose gels, transferred to Gene Screen Plus® membranes (DuPont-NEN), and hybridized with ^{32}P-labeled DNA probes (Feinberg and Vogel 1984). The probes included (1) subclones of the DXS164 region (pERT 87-1, pERT 87-8, pERT 87-15, and pERT 87-J.Bir) (Monaco et al. 1985; Kunkel et al. 1986), (2) clones of the DXS41 (probe 99.6) and DXS84 (probe 754) regions (Bakker et al. 1985), and (3) the X;21 junction probe (XJ1.1) (Ray et al. 1985). Filters were washed twice at room temperature with 2 × SSC, twice at 65 C with 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate) and 0.2% sodium dodecyl sulfate (SDS), and twice at 65 C with 0.1 × SSC and 0.1% SDS and were autoradiographed for 2–3 days. The restriction enzymes employed were *Taq*I, *Xmn*I, and *Bst*NI.

Chromosome Analysis

Lymphoblastoid cell lines and 68-h PHA-stimulated peripheral blood lymphocyte cultures were harvested by adding 0.5 μg of colcemid and 10 μg of ethidium bromide for the last 1–2 h of culture (Ikeuchi 1984). The cells were treated with 0.075 M KCl, fixed in 3:1 methanol acetic acid, and GTG-banded.

RESULTS

The pedigree and the informative DXS164 haplotypes are shown in figure 1. On three separate testings, the proband's mother (II-1) had creatinine kinase (CK) levels within normal limits for our laboratory. She is heterozygous for two

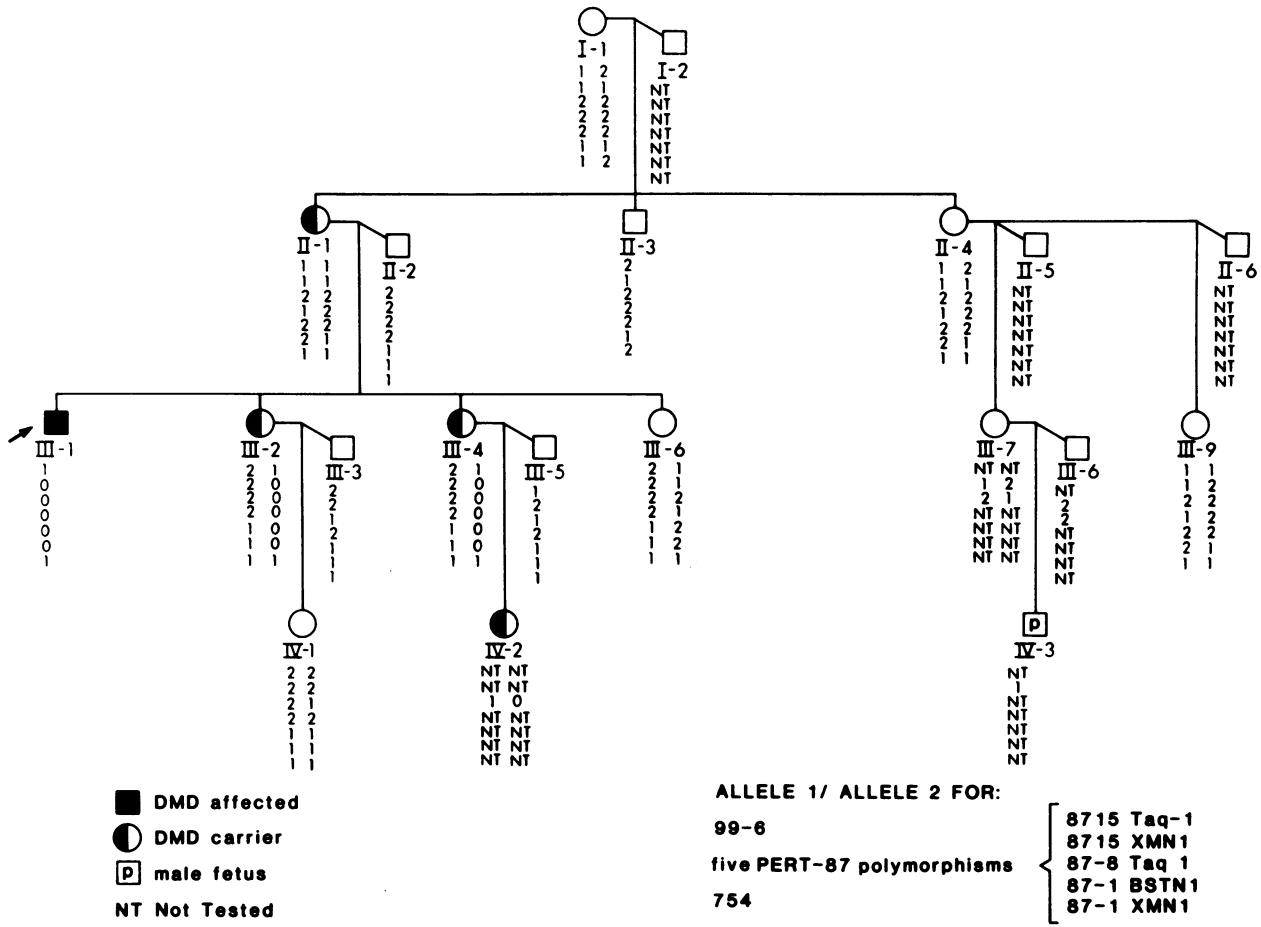

FIG. 1.—Pedigree of family BE. The alleles for the DXS164 probes, with fragment sizes (allele numbers), are as follows: 87-1/*Xmn*I, 8.7 kb (1) and 7.5 kb (2); 87-1/*Bst*NI, 3.1 kb (1) and 0.65 and 2.45 kb (2); 87-8/*Taq*I, 3.8 kb (1) and 1.1 and 2.7 kb (2); 87-15/*Xmn*I, 2.8 kb (1) and 1.2 and 1.6 kb (2); and 87-15/*Taq*I, 3.6 kb (1) and 3.3 kb (2). The alleles for 99-6/*Pst*I and 754/*Pst*I with fragment sizes (allele numbers) are 22 kb (1) and 13 kb (2) and 12 kb (1) and 9 kb (2), respectively.

*Taq*I alleles (1,2) at the pERT 87-8 locus and two *Xmn*I alleles (1,2) at the pERT 87-1 locus. She is homozygous for a *Taq*I polymorphism (1,1) and an *Xmn*I polymorphism (2,2) at the 87-15 locus and for a *Bst*NI polymorphism (2,2) at the pERT 87-1 locus. Figure 2 shows the Southern blot analysis of selected family members for *Taq*I-digested DNA hybridized to the pERT 87-8 and pERT 87-15 probes. The mother (II-1) was homozygous for *Bam*HI and *Taq*I alleles at the 87-J.Bir and XJ1.1 loci, respectively.

DNA from the proband (III-1) did not hybridize to probes pERT 87-1, pERT 87-8, and pERT 87-15. The proband's deletion had been documented previously (Bartlett et al. 1987). The extent of the deletion was examined by hybridizing restriction enzyme–digested DNA to probes within and flanking the DXS164 region. The proband's DNA hybridized to 87-J.Bir and XJ1.1 probes (data not shown), indicating that the deletion did not extend beyond these two loci.

Two daughters (III-2 and III-4) were identified as probable DMD carriers on the basis of elevated CK levels after repeated testing. The carrier daughters have the same hybridization patterns for the pERT 87 probes as their father (fig. 1). The absence of the maternal *Taq*I (fig. 2*B*) and *Xmn*I polymorphisms at the pERT 87-15 locus and the absence of the maternal *Bst*NI polymorphism at the pERT 87-1 locus indicate that the two daughters carry the deletion in their maternal X chromosomes. Dosage blot analysis of the proband's mother (II-1) and her descendants confirmed these results. One carrier daughter (III-4) transmitted the deletion to her fetus (IV-2), who was diagnosed prenatally as a deletion carrier. The fetus inherited the paternal *Xmn*I allele (i.e., the 2.8-kb fragment) at the pERT 87-15 locus, but she did not inherit the maternal X chromosome (Bartlett et al. 1987).

The third daughter (III-6) had a normal CK level, and she has two copies of the DXS164 region. She inherited her maternal grandfather's DXS164 haplotype.

Chromosome studies were done on both the proband's mother (II-1) and one of the carrier sisters (III-2). Both individuals had 46,XX chromosome complements, and no structural abnormalities (i.e., translocation, inversion, deletion, etc.) were observed in the p21 region of their X chromosomes. The X chromosomes from one of the carrier sisters (III-2) are shown in figure 3. The Southern blot and chromosome analyses indicate that the DXS164 deletion is not cytologically resolvable.

DISCUSSION

The coincidence of the DXS164 deletion and the expression of the DMD gene confirmed previous findings (Kunkel et al. 1986) that the DXS164 region may be important in expression of the normal allele at the DMD locus.

To explain the segregation of the DXS164 deletion and the DMD gene in the proband's sibship, we propose the following two hypotheses: the proband's mother (II-1) may (1) carry a familial X;autosome translocation in which the DXS164 region is deleted from one X chromosome and translocated (i.e., inserted) to an autosome or (2) be a mosaic both for cells with two normal X

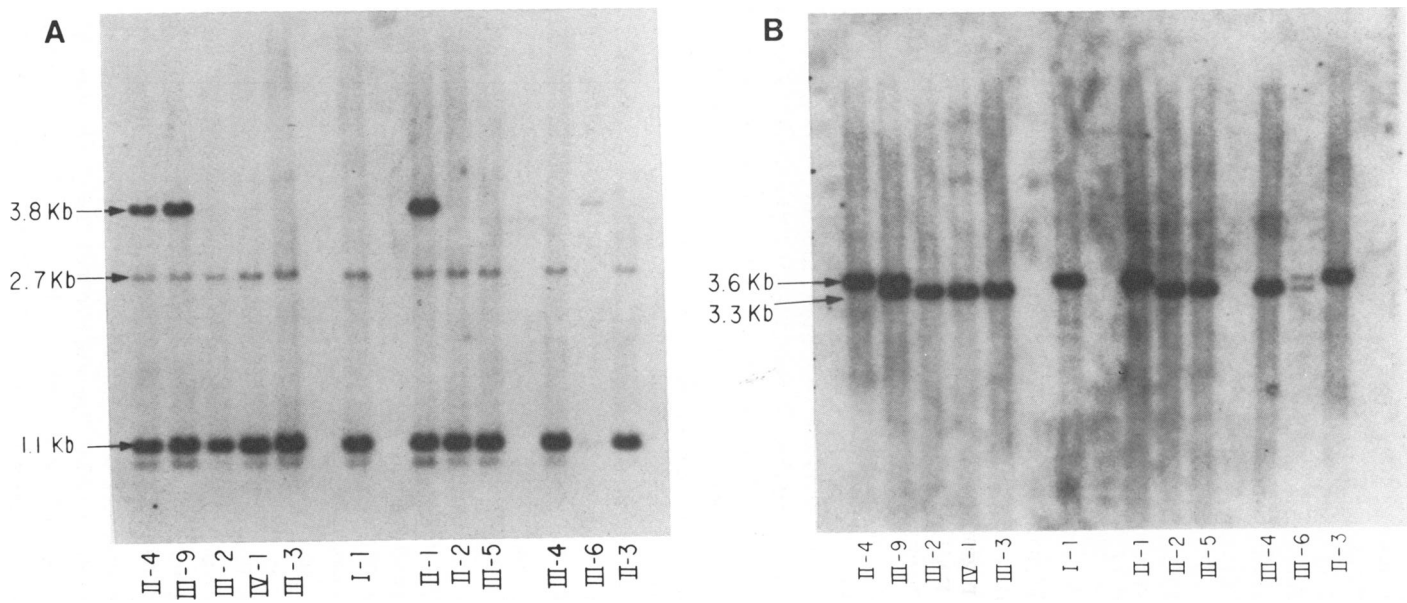

FIG. 2.—Southern blots of *Taq*I-digested DNA hybridized to two pERT 87 probes in family BE. *A*, Probe pERT 87-8 (allele 1 corresponds to the 3.8-kb fragment, and allele 2 corresponds to the 1.1- and 2.7-kb fragments); *B*, probe pERT 87-15 (allele 1 corresponds to the 3.6-kb fragment, and allele 2 corresponds to the 3.3-kb fragment). The lane numbers in *A* and *B* correspond to individual numbers on fig. 1.

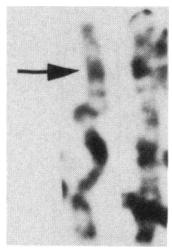

FIG. 3.—The X chromosomes from one of the proband's sisters (III-2), who carries a DXS164 deletion. Band Xp21 is denoted by the arrow.

chromosomes and for cells with one normal X chromosome and an X chromosome with a DXS164 deletion.

If the DXS164 region is inserted into an autosome in females carrying the DXS164 deletion, DMD carrier females could produce both affected males with one copy of the DXS164 region and carrier females with two copies of the DXS164 region. The chromosome studies on the proband's mother (II-1) provide no evidence that she received a structurally abnormal (i.e., deleted, translocated, or inverted) X chromosome from her mother (I-1); there is no previous family history of DMD; and there is no evidence that a DXS164 translocation or deletion is segregating in either her sister or her sister's descendants (see fig. 1). Gonadal mosaicism appears to be the most likely explanation for the segregation of DMD and the DXS164 deletion, but the possibility of a chromosomally undetectable translocation or inversion cannot be excluded.

This family illustrates the importance of family studies when RFLP analysis is used for prenatal diagnosis. Had the RFLP analysis been restricted to the proband (III-1) and the proband's mother (II-1), the proband would have appeared to be a new deletion mutation, as in the Monaco et al. (1985) study. If the proband's mother is a gonadal mosaic, her recurrence risk of transmitting the DXS164 deletion—and presumably DMD—to her next male child (Murphy et al. 1974) is estimated to be .496. This estimate is based on the posterior information that three of her four children have inherited the DXS164 deletion.

The data support the hypothesis that the DXS164 deletion did not result from unequal crossing-over between the X chromosomes during meiosis in the proband's mother. Kunkel et al. (1986) have demonstrated that there is significant heterogeneity among different deletions at the DXS164 locus (i.e., different deletion mutations involve different segments of the DXS164 region and surrounding loci). It is unlikely that the three separate meiotic events in the proband's mother would result in the three separate deletions of the same loci within and surrounding DXS164 region. In addition, the chromosome analysis and the restriction-enzyme analyses provide no evidence that the proband's mother received a structurally abnormal or deleted X chromosome from the maternal grandmother (I-1) (see fig. 1). With the availability of DNA probes in and adjacent to the DXS164 region, it should be possible to examine other DMD families and to determine (1) whether apparent de novo deletions in the

DMD gene occur premeiotically or during meiosis and (2) whether they are simple deletions or result from recombination (i.e., by unequal crossing-over, unequal sister chromatid exchange, or by translocation).

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