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DNA Studies in a Family with Duchenne Muscular Dystrophy and a Deletion at Xp21

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SUMMARY

We have performed Southern blot analysis on a large, four-generation kindred with Duchenne muscular dystrophy (DMD). Probes 754 (*DXS 84*), pERT87-1, pERT87-8, pERT87-15 (*DXS164*), and pXJ-1.1 did not hybridize to digested genomic DNA of affected males. Obligate-carrier mothers and unaffected brothers showed signals of a single X-chromosome copy intensity, and suspected noncarrier sisters demonstrated either a single band of two-copy intensity or informative polymorphisms. Uniform hybridization was seen with probes C7 (*DXS28*) and D2 (*DXS43*), which map distal to the *DMD* locus, and with *OTC*, which maps proximally. This deletion was present in six affected individuals and has been transmitted through 3 generations to date. On high-resolution chromosome analysis, a deletion within band Xp21 was consistently observed in one affected male studied and in one of the two X chromosomes in obligate carriers. This large molecular and cytogenetically visible deletion in affected DMD individuals without glycerol kinase deficiency, chronic granulomatous disease, retinitis pigmentosa (RP), or ornithine transcarbamylase deficiency is a very rare finding and should prove useful in specifically cloning additional probes within and flanking the *DMD* locus.

INTRODUCTION

Duchenne muscular dystrophy (DMD) has been localized to Xp21 by analysis of X-autosomal translocations in 13 unrelated girls reported to have muscular

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dystrophy and found to have the breakpoint in the same region of the short arm of the X (Jacobs et al. 1985; Dubowitz 1986). The restriction-fragment-length polymorphisms (RFLPs) recognized by DNA probes surrounding the *DMD* locus have already proved to be very useful tools in carrier detection of females and prenatal diagnosis of affected males (Bakker et al. 1985). Molecular deletions have been recognized in ~7% of patients with isolated DMD by using probes pERT87 and pXJ-1.1 (Kunkel et al. 1986). pERT 87-8, 87-1, and 87-15 were isolated from a DNA library enriched for human DNA from band Xp21 by using DNA isolated from BB, a male patient with DMD and two other X-linked disorders (Kunkel et al. 1985). BB has a visible deletion at Xp21 (Francke et al. 1985). Probe XJ-1.1 was isolated from the X-chromosome site of the t(X;21) junction in a female patient with muscular dystrophy (Ray et al. 1985). Sequences identified by pERT87 have been deleted in several patients with both DMD and the other closely linked X-linked disorders of glycerol kinase (GK) deficiency and adrenal hypoplasia (AH) (Wieringa et al. 1985; Dunger et al. 1986). We report here a Manitoba family with a molecular deletion spanning the sequences identified by pERT87, pXJ-1.1, and 754.

Although molecular deletions including pERT87 and 754 have been identified in patients with DMD and other X-linked disorders, there is only one recent report of such a large deletion in patients with DMD only (Wilcox et al. 1986). Following identification of this molecular deletion in the family that we studied, high-resolution chromosome banding identified a microscopic deletion in Xp21. Accurate carrier detection is possible in this family, and the visible chromosomal deletion offers the possibility to specifically clone additional probes for (1) studying this region of the X chromosome and (2) RFLP linkage studies in this heterogeneous disease.

MATERIAL AND METHODS

A. Family Data

The affected boys in this large four-generation pedigree (see fig. 1) demonstrate the signs and symptoms of classic DMD. All were nonambulatory and confined to wheelchairs by 11 years of age, and all but one were deceased before 17 years of age. The only surviving member is IV-14, now 12 years of age and nonambulatory. There is no history of visual impairment, suppurative infections, poor growth, or recurrent acidosis. All affected males have been slow learners. Physical examination of affected males revealed normal growth parameters, no pigmentary retinopathy, and the musculoskeletal findings typical of DMD. Pertinent biochemical findings included a normal hemogram and blood smear, normal serum ammonia, and normal triglycerides, cholesterol, and urinary glycerol, indirectly consistent with normal GK activity (Goossault et al. 1982). Creatine kinase (CK) measurements were performed on available females, and DNA was isolated from peripheral blood leukocytes, fibroblasts, or Epstein-Barr virus–transformed lymphoblastoid cell lines of all available individuals.

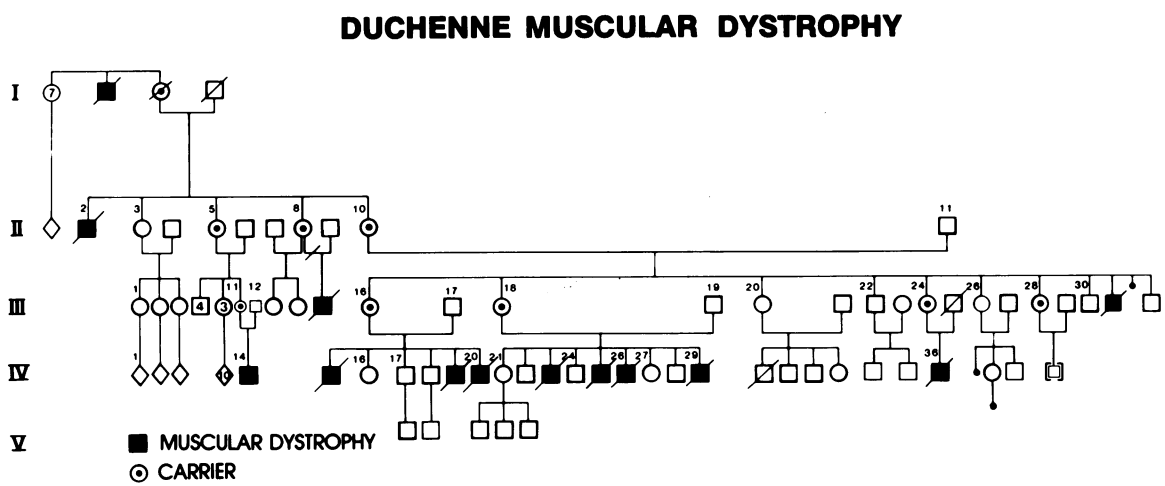


FIG. 1.—DMD pedigree. All females designated carriers (except II-5) had either (1) one or more affected sons or (2) elevated CK levels

TABLE 1

CLONED HUMAN CHROMOSOME SEQUENCES USED

Clone (Human Gene Mapping Symbol)	RFLP	Reference
pD2 (DXS43)	*Pvu*II	Kunkel et al. 1982
pC7 (DXS28)	*Eco*RV	Dorkins et al. 1985
pERT87-15 (DXS164)	*Xmn*I	Kunkel et al. 1985
pERT87-8 (DXS164)	*Bst*XI	Kunkel et al. 1985
pERT87-1 (DXS164)	*Xmn*I	Kunkel et al. 1985
pXJ-1.1 (DXS206)	*Taq*I	Ray et al. 1985
p754 (DXS84)	*Pst*I	Hofker et al. 1985
pHOC3 (OTC)	*Msp*I	Horwich et al. 1984
pX83 (DXS47)	...	Riddell et al. 1986
pSW50 (D8MGV1)	*Eco*RI	Wood et al. 1986

B. DNA Isolation

For preparation of DNA from whole blood, red cell lysis was achieved using ammonium chloride, and the recovered nucleated cells were washed with 0.85% saline and lysed using a solution of 100 mM Tris-HCl, pH 8, 40 mM ethylenediaminetetraacetate (EDTA) and 0.2% sodium dodecyl sulfate (SDS). Proteins were removed by means of phenol, followed by choloroform extractions; and the nucleic acids were then precipitated in the presence of 100 mM NaCl by means of isopropanol and washed, dried, and resuspended in 10 mM Tris-HCl 1 mM EDTA (D. Hoar, personal communication). Extraction of DNA from lymphoblastoid lines was achieved using the same procedure, omitting the ammonium chloride lysis. Fibroblast DNA was extracted from cultured fibroblasts following harvesting by means of trypsin digestion (Maniatis et al. 1984).

C. X-Chromosome Probes

Ten DNA probes and the RFLP-detecting restriction endonucleases used in this study are listed in table 1.

D. Southern Blot Analysis

Five to 10 micrograms of genomic DNA were digested to completion using the appropriate restriction enzymes, size separated by electrophoresis through 0.8% agarose gels, and transferred overnight to nitrocellulose membranes by means of Southern (1975) blotting. Prehybridization and hybridization solutions contain 6 × SSC (1 × SSC = 150 mM NaCl, 15 mM trisodium citrate), 10 × Denhardt's solution (1 × Denhardt's = 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 0.02% Ficoll 400), 0.5% SDS, 10 mM EDTA, 50 mM sodium monophosphate, 50% formamide, and 200 μg of denatured heterologous salmon sperm DNA/ml. Hybridizations containing ^{32}P-labeled probes at 10–20 ng/ml proceeded overnight at 42 C. Probes were labeled using the Amersham Nick Translation Kit. Each reaction contained 50 μC ^{32}-P-dCTP (3,000 Ci/mM), 50 μC ^{32}P-dATP (3,000 Ci/mM), and 50–100 ng DNA. Blots were washed in 2 × SSC, 1% SDS at room temperature for a total of 20 min and then

at 55–60 C in 0.5 × SSC, 0.5% SDS for 15 min. Further washings were done in 0.2 × SSC, 0.1% SDS at 60 C as required. Autoradiography with intensifying screens at −70 C proceeded for 12 h–7 days.

E. Chromosome Analysis

Methotrexate-synchronized early-metaphase chromosomes were prepared from peripheral lymphocytes or lymphoblastoid lines from III-11, III-16, and IV-14 according to the method of Yunis (1976).

RESULTS

The deletions of DNA markers pERT87-8 and 754 are shown in figures 2*a* and 2*b*. In figure 2*a*, probe pERT87-8 did not hybridize to the DNA of IV-14; and (1) the subject's mother (III-11) has a single 2.2-kb band (presumably of paternal origin), (2) his maternal grandmother (II-5) has a single 4.4-kb band, and (3) his father (III-12) has a single 2.2-kb band. His maternal grandfather was not available for study. Dosage analysis with use of an autosomal probe pSW50 confirmed that the signal intensity in each lane represents a single X-chromosome copy (Dorkins et al. 1985). The more proximal probe 754 recog-

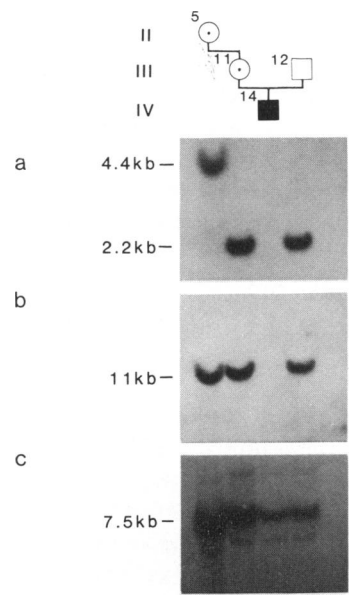


FIG. 2.—*a:* Southern blot analysis of DNA from IV-14, his parents (III-11 and III-12), and his maternal grandmother (II-5). In the *Bst*XI-digested DNA, no hybridization is seen with ^{32}P-labeled probe pERT87-8. The single 2.2-kb band seen in III-11 is presumably of paternal origin, and II-5 has a single hybridizing 4.4-kb band and a deleted, nonhybridizing 2.2-kb band. Dosage analysis with use of densitometry with an autosomal probe pSW50 confirmed that each signal, although of different intensity, represents a single X-chromosome copy. *b:* In *Pst*I-digested DNA, no hybridization is seen with ^{32}P-labeled probe 754 in IV-14, and an 11-kb signal of single-X copy intensity is present in II-5, III-11, and III-12. *c: Eco*RV-digested DNA of IV-14 shows hybridization with ^{32}P-labeled C7 and a two-copy signal in his mother (III-11) and maternal grandmother (II-5).

nizes a molecular deletion in IV-14 (fig. 2*b*), and, distal to the pERT locus, there is hybridization with marker C7 to *Eco*RV-digested genomic DNA of IV-14 and a signal of two-copy intensity in his mother and grandmother (fig. 2*c*). Extended family studies confirmed that pERT87-8 recognizes a molecular deletion in the affected males and that obligate carriers III-16, III-18, and III-24 are hemizygous for this region of the X chromosome. In addition, on the basis of DNA analysis and CK values, female III-28 was identified as a carrier, whereas individuals III-26, IV-16, IV-21, and IV-27 are presumably noncarriers. The three sons of IV-21 are unaffected and have normal CK values. Markers OTC, pX83, and pD2 were present in IV-14, whereas pERT87-1, 87-15, and pXJ-1.1 were absent (data not shown).

Because of the extensive molecular deletion identified, high-resolution chromosome analysis was performed on obligate carriers III-11 and III-16 and on one affected male (IV-14). These studies revealed a deletion within Xp21 (fig. 3). The lightly staining band Xp21.2, which normally separates Xp21.1 and Xp21.3 (both of which are darkly staining bands), is nowhere observed. The lightly staining flanking bands Xp11.4 and Xp22.1 appear to be of normal size.

DISCUSSION

In this large kindred with isolated DMD, a molecular deletion involving DNA probes 754, pXJ-1.1, and pERT87 has been detected. Since this large deletion can be assumed to be the cause of DMD in this family, very accurate genotype assignment is possible in this family and is not hampered by recombination, which occurs to a significant degree even with probes mapping apparently within the *DMD* locus (Kunkel et al. 1986). Individual II-5 was studied only at the age of 60 years, following the identification of DMD in her grandson, IV-14. Her CK was normal at this time, and her calculated risk of being a carrier, as based on Bayesian analysis and CK measurements, was only 5%. DNA studies confirmed that she is indeed a carrier of the molecular deletion, and her other three daughters therefore have been offered DNA-analysis carrier testing. This emphasizes the importance, prior to genetic counseling of families with DMD, of using DNA markers in addition to CK and Bayesian analysis.

Wilcox et al. (1986) recently reported two cousins with isolated DMD, a small microscopically visible deletion in Xp21, and a molecular deletion of *DXS164* and *DXS84* in which flow cytometry showed that ~6,000 kb of DNA were deleted. We presume that the deletion in the family that we studied is of a size similar to that in Wilcox's patients—and that it highlights the importance of pursuing high-resolution chromosome analysis in patients in whom a molecular deletion may be first identified. The molecular and cytogenetic findings in the family that we studied are also similar to those for patient BB, in whom flow-cytometric analysis has also revealed a 6,000-kb deletion (Wilcox et al. 1986). How, then, does one resolve the clinical differences between the boys in the family that we studied, who were affected with DMD alone, and BB, who had 3 X-linked disorders? The order of Xp21 loci, as based on patients with

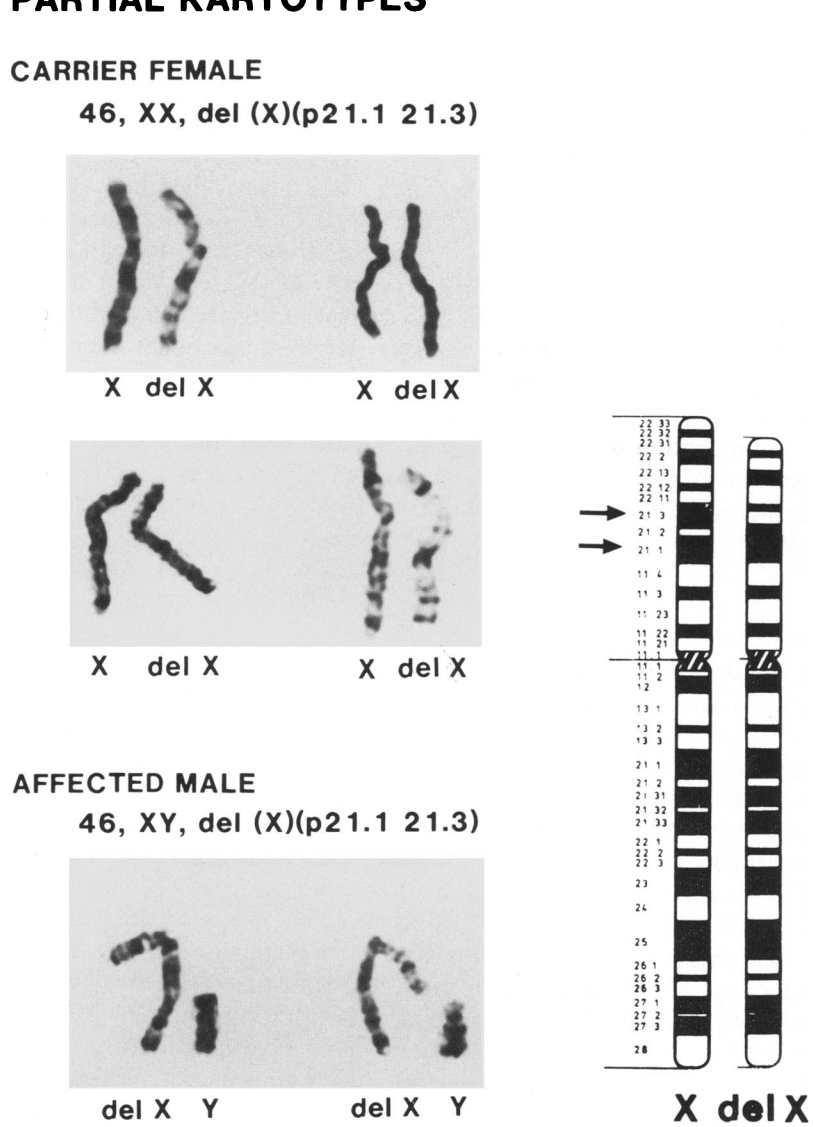
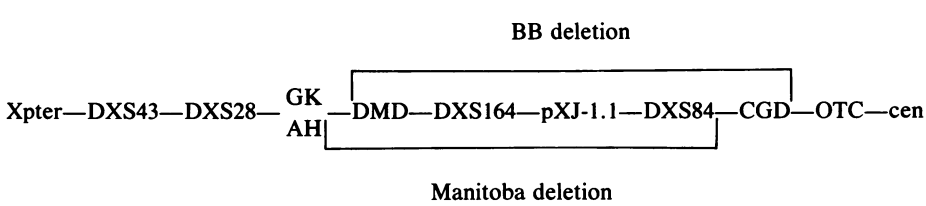


FIG. 3.—Partial karyotypes of a carrier female (III-11) and her affected son (IV-14) are shown on the left, and ideograms of a normal human X and a deleted X at the 850-band stage are depicted on the right. In these high-resolution chromosome preparations, band Xp21.2, which normally separates dark subbands Xp21.1 and Xp21.3, is never observed. The arrows indicate the location and approximate size of the deletion.

molecular deletions in the Xp21 region, is presumed to be as follows (Baehner 1986; Dunger 1986; Wilcox 1986):



Given the large segment of DNA missing in both the family that we studied and BB, different breakpoints can explain some of the clinical differences depicted above. The precise genetic location of retinitis pigmentosa remains unresolved at present.

The identification of molecular deletions has been seen in ~7% of patients with isolated DMD, both in familial and sporadic cases (Kunkel et al. 1986). In such families, more accurate carrier identification and prenatal diagnosis are possible as compared with RFLP linkage findings. The large deletion including *DXS84* (754) in the DMD family that we studied is a very unusual finding and offers the opportunity to isolate additional probes from this area by subtraction-hybridization cloning (Kunkel et al. 1985). Cells from affected males in this family will be made available to interested investigators. As more probes become available, particularly from the region between (1) *GK* and *DMD*, (2) *GK* and C7, and 754 and *OTC*, the locations of the presumed individual gene loci for these disorders will be more precisely assigned. Since the molecular lesions resulting in DMD appear to be very heterogeneous, a universal DNA test for the *DMD* locus probably cannot be developed in the near future. Rather, RFLP linkage studies with a battery of intragenic and flanking probes will be required for carrier detection and prenatal diagnosis.

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