Somatic Mosaicism at the Duchenne Locus

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Results of testing a family for carrier status and prenatal diagnosis for Duchenne muscular dystrophy (DMD) are best explained by somatic mosaicism in the maternal grandfather. This genetic situation was identified using segregation analysis of intragenic DNA polymorphisms, a serum creatine phosphokinase assay, and physical examination of the patient. This event at the DMD locus represents one more potential source of error in carrier testing and prenatal diagnosis.

KEY WORDS: carrier testing, prenatal diagnosis, Duchenne muscular dystrophy

INTRODUCTION

Duchenne muscular dystrophy (DMD) and the less severe Becker muscular dystrophy (BMD) resulting from mutations at the same gene locus are X-linked recessive disorders affecting approximately 1/3,300 males [Moser, 1984] with BMD representing about 1/10 of this total. Because one-third of the males with this lethal X-linked disease represent new mutations [Murphy and Mutalik, 1969], the DMD gene mutation rate is quite high (about 1/10,000). Unlike hemizygous males, heterozygous females are functionally mosaic as a result of random inactivation of their X chromosomes [Lyon, 1961] and as such are generally asymptomatic even with elevated serum creatine phosphokinase (CPK) levels. Cytogenetically detectable chromosome abnormalities have localized the DMD gene to band Xp21 [Verellen-Dumoulin et al., 1984; Francke et al., 1985; Ray et al., 1985]. The DMD gene spans about \(2 \times 10^6\) bp of chromosomal DNA and encodes the dystrophin gene product [Monaco et al., 1986; Koenig et al., 1987, 1988; van Ommen et al., 1987; Hoffman et al., 1987]. The dystrophin gene product is found in the muscle cell membrane [Chelly et al., 1988; Arahata et al., 1988; Watkins et al., 1988]. Muscle enzymes including CPK leak into the serum when dystrophin is abnormal or absent.

Several DMD disease characteristics must be considered when diagnosing fetuses and at-risk women: 1) one-third of all DMD males represent new mutations, 2) at least one-half of the DMD mutations are intragenic deletions [den Dunnen et al., 1987; Darras and Francke, 1988; Chen et al., 1988], and 3) recombination between intragenic polymorphisms and mutations in the DMD gene occurs in about 5% of meioses [5%—Kunkel et al., 1986; 6%—Fischbeck et al., 1986; 0%—Mulley et al., 1988; 4%—our laboratory]. Very high serum CPK activity in at-risk women indicates an at-risk woman is a carrier [Katayama et al., 1988]. In contrast low CPK activity does not indicate non-carrier status because normal women and carriers have overlapping enzyme activities. We report somatic mosaicism in a male carrier of DMD which is a potential source of diagnostic error.

MATERIALS AND METHODS

DNA is extracted from appropriate family members' peripheral blood cells [Goossens and Kan, 1981]. The RNase step is omitted and 10 μg yeast tRNA is added to improve DNA recovery from fetal cell cultures, villi, or amniocytes. DNA (10 μg) is digested with 35 U restriction endonuclease (Bethesda Research Labs, Gaithersburg, MD, or New England Biolabs, Beverly, MA) for 16-20 hours according to manufacturer's recommended conditions. DNA fragments are electrophoresed, depurinated, denatured, neutralized, and then transferred to nitrocellulose [Goossens and Kan, 1981], with 20 × SSC overnight. Then the filters are baked, prehybridized, hybridized to pert 87 probes from Dr. Kunkel, p20 from Dr. Pearson, or C7 probe from Dr. Mandel. Nick-translated radiolabeled probes (S.A. = \(5 \times 10^7\) to \(5 \times 10^8\)) are hybridized at \(2 \times 10^6\) cpm/ml, washed and autoradiographed [Lebo et al., 1985].

RESULTS AND DISCUSSION

We have tested 8 intragenic DMD polymorphisms and one flanking polymorphism (C7) for carrier testing and prenatal diagnosis (Table I). The eight intragenic polymorphisms, predicted to be informative in 98.9% of at-risk women (Table I), have been informative in 59 of 60 families. The pert 87 and p20 polymorphic sites have been used preferentially for DMD diagnosis because each has been shown to reside at one of the 2 deletion
hotspots in the DMD gene. P20 has been mapped to a noncoding intragenic region (intron) and exhibited a large spontaneous deletion when the cosmid was first isolated [Wapenaar et al., 1988]. This p20 probe has been particularly useful to test recombination. We found a new useful p20 MspI polymorphism during this study. Subsequently each restriction fragment length polymorphism (RFLP) will be referred to according to the probe name followed by the restriction enzyme (c.f. p20-EcoRV).

Three explanations can be advanced to explain the data obtained in one of the pedigrees we studied (Fig. 1). First, spontaneous mutation may have caused 3 independent events in 3 first cousins. This explanation is highly improbable [probability \( P = (1 \times 10^{-4})(1 \times 10^{-4})(1 \times 10^{-4}) = 10^{-12} \)]. Second, if the common female ancestor (II-2) carries the DMD gene, 3 recombinations would have had to occur to result in the DMD phenotypes and polymorphic genotypes in this pedigree (probability = \( 0.04 \times 0.04 \times 0.000064 = 6.4 \times 10^{-5} \)) (Fig. 1). Most pedigrees with this pattern of DMD segregation arise from a gene carried by a common female ancestor (II-2). The informative polymorphism using XmnI restriction enzyme analysis with the pert 87 has demonstrated that both tested DMD males received the 7.5 kb grandpaternal restriction fragment. In addition the first obligate carrier III-1 received a 4.4 kb pert 87-8 BstXI allele from her mother, while her 2 obligate carrier sisters received the 2.2 kb allele. These results would be observed only if the grandmother carried a DMD mutation in a gene region flanking the intragenic pert 87 and p20 loci (Fig. 1), and all 3 meiotic recombinations occurred between the DMD mutation and the pert 87 polymorphic loci: 2 between III-2 and III-5 and their sons, and a third between II-2 and her daughter III-1. Note that the direction of the DMD mutation from pert 87 has not been established but was illustrated upstream for descriptive purposes.

A third and much more probable explanation is that grandfather (II-1) carries the DMD allele (Fig. 1). The grandfather’s elevated serum CPK activity and clinical history distinguished the actual genetic event in this pedigree. His serum CPK activity was 1,080 U/L and 1,350 U/L on 2 occasions 2 years apart, while his wife had a lower CPK value (16 U/L) than any of the 38 obligate DMD carriers we have tested. Physical examination revealed the grandfather had striking muscular weakness and atrophy in his right arm and shoulder in the C5-6 innervated myotomes which he reports began about ten years ago. He declined an EMG and muscle biopsy. These relatively mild symptoms contrast to those of his 3 grandsons who have CPK values of 9,800, 5,570, and 9,600 and who are already experiencing significant motor difficulties at the ages of 3 to 4 years old consistent with a Duchenne’s mutation. These data indicate II-1 is mosaic for the DMD mutation. The CPK activity is significantly higher in III-4 than any of the values on our normal CPK curve which indicates she is a DMD carrier. Whether III-3 is a DMD carrier depends upon whether her father’s germinal cells are mosaic and, if so, whether she received a normal or DMD allele. Because 4 of 5 daughters received their father’s DMD allele, a significant fraction of their father’s germ cells probably carry the DMD allele and III-3 has a significant risk of also carrying this DMD allele. Although this family has refused to cooperate further and all available DNAs have been exhausted, we conclude the most likely explanation is that grandfather II-1 is a somatic mosaic.
Fig. 1. Grandpaternal somatic mosaicism. XmnI restriction enzyme analysis with the pert 87-1 probe revealed both affected males IV-2 and IV-3 received the grandpaternal 7.5 kb pert 87-1 XmnI allele. Obligate carrier III-1 received the 4.4 kb pert 87-8 allele from her mother in contrast to her obligate carrier sisters III-2 and III-5, who received the 2.2 kb pert 87-8 allele. CPK activity and medical history in the grandfather is consistent with a mild form of DMD as a result of somatic mosaicism involving his muscle cells. The grandfather's mosaicism is indicated by fewer cross-hatch marks in the opposite direction from his severely affected grandsons. ● = genetic obligate carrier; ○ = carrier by serum CPK level. Numbers to the upper right of each symbol are CPK activities in international units/liter. Polymorphic probe names and their polymorphic fragment lengths are listed in order within the gene locus. The brackets at each pert 87 sublocus indicate that the order within the sublocus has not been established.

with a DMD mutation expressed in his muscle tissue as indicated by his clinical features and high serum CPK and in his germ line cells as indicated by his 4 carrier daughters and 3 affected grandsons.

In addition to the somatic mosaic reported here, 9 examples of DMD germinal mosaicism have been reported in about 1400 DMD families studied [Bakker et al., 1987; Edwards, 1986; Darras and Francke, 1987; Bech-Hansen 1987; Lanman et al., 1987; Monaco et al., 1987; Wood and McGillivray, 1988]. In contrast, proven mosaicism at other gene loci is very rare. For example, only a single case of confirmed somatic mosaicism has been reported at the OTC gene locus [Maddalena et al., 1987]. Germline mosaicism at other loci has been reported in 10–15 individuals [Hall, 1988]. When a new mutation or autosomal recessive muscular dystrophy [Salih et al., 1983; Somer et al., 1985] is suspected, care should be taken to rule out somatic mosaicism in male ancestors. One implication of mosaicism is the possible significant risk of recurrence in what had been thought to represent an apparent new mutation with a low risk. Our results of a case of somatic mosaicism at the Duchenne locus emphasize the importance of obtaining thorough histories of DMD pedigrees, examining as many suspected carriers as possible, and testing CPK levels on common male ancestors.

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REFERENCES


