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DNA DIAGNOSTIC TESTS IN Xp21 DYSTROPHY FAMILIES FOR PRENATAL DIAGNOSIS*

*Pervin Dinçer PhD**, Haluk Topaloğlu MD***, Şükrüye Ayter PhD*****

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Duchenne and Becker muscular dystrophies are X-linked genetic disorders characterized by dystrophin gene defects. We have studied 250 families with Duchenne and Becker muscular dystrophies (D/BMD) by molecular genetic methods since 1992. Nineteen exons of the dystrophin gene were analyzed for deletion. In families with no deletion, linkage analysis was performed to follow the inheritance of mutant alleles in the affected families. Twenty of these families requested prenatal diagnosis. Six mothers were found to be non-carriers (99% accuracy), thus fetuses were examined in the remaining 14. Two fetuses were affected and terminated. We report our experience and our current clinical practice in providing prenatal studies for D/BMD. *Key words: Duchenne/Becker muscular dystrophies, deletion, linkage analysis, prenatal diagnosis.*

Duchenne and Becker muscular dystrophies (D/BMD) are allelic X-linked genetic disorders[1]. These disorders arise as a result of mutations affecting the dystrophin protein. The majority of gene mutations consist of deletions (65%) or duplications (5%) which are clustered in two hot spots[2-3]. Ninety-eight percent of these deletions can now be detected by polymerase chain reaction (PCR)[4]. For the remaining DMD cases, carrier detection and prenatal diagnosis depend on DNA linkage analysis[5-6].

Genetic counseling and prenatal diagnosis in families with D/BMD are crucial. Females at risk of carrying D/BMD should be counseled before they undertake pregnancies. However, it depends on the availability of information on the carrier status. To date, linkage analysis in D/BMD families without detectable deletions has been performed using diallelic RFLP markers flanking and within the DMD gene by Southern blot analysis[6]. Today, not only RFLPs[7] but also other linkage markers[8-10] can be easily assayed by PCR.

In this study, we report our experience in the molecular approach of D/BMD families who applied for prenatal diagnosis and who were studied at the Medical Biology Department, Hacettepe University Faculty of Medicine, Ankara.

* From the Department of Medical Biology, Hacettepe University Faculty of Medicine, Ankara.

** Assistant Professor of Medical Biology, Hacettepe University Faculty of Medicine.

*** Professor of Pediatrics, Hacettepe University Faculty of Medicine.

**** Professor of Medical Biology, Hacettepe University Faculty of Medicine.

Material and Methods

Patients

Patients were diagnosed at the Child Neurology Department of Hacettepe University. Diagnosis for DMD was based on family history and pedigree analysis, clinical findings, high creatine phosphokinase levels (CPK), electromyographic findings, DNA studies, and dystrophin immunocytochemistry of muscle biopsy where necessary.

DNA Studies

DNA was isolated by standard methods from leukocytes[11, 12], or from amniotic[13] or chorion cells[14]. PCR deletion analysis of different exons of the dystrophin gene was performed according to Chamberlain et al.[15-16] and Gibbs et al.[17], with modifications described by Abbs et al.[18].

5'DYS1-5'CA[9], short tandem repeats (STR 44-STR 45-STR49-STR50[8], J66 (Yau SC. unpublished data), and 3'CA[10] markers were analyzed at DMD locus in families to detect the mothers' haplotype, for carrier detection of females and to determine the genetic status of the fetus. The methodology for the molecular analysis of D/BMD cases was applied for both pre-and postnatal diagnostic approaches.

To date, we analyzed 250 D/BMD families. In 145 of these (58%), deletion was detected[19]. We studied 105 families by linkage analysis.

Prenatal Diagnosis

We used the X-Y homologous amelogenin gene primers (AMEL) (20) to detect the sex of the fetus for noninformative families. Multiplex PCR and linkage analyses were performed on the DNAs extracted from chorionic villus sample (CVS)[13] or amniocytes[14].

Only a few of our D/BMD families applied to our laboratory for genetic analysis prior to pregnancy; the majority of mothers came eight to ten weeks pregnant. Under these circumstances, deletion screening was done in the male patient. If deletion was detected, a chorionic villus sample was taken for determination of sex and of deletion status of the fetus. If there was no deletion in the male patient, we proceeded with linkage analysis of the family. Twenty families requested prenatal diagnosis. Deletions within the D/BMD gene were found in 12 of the index cases of these families; linkage analyses were carried out in the remaining eight families with an accuracy of 99 percent. Six pregnant women were found to be non-carriers (99% accuracy), thus fetuses were examined in the remaining 14.

Results

Over the past four years, 20 families asked for prenatal diagnosis. We applied the same methods used for the molecular diagnosis of 250 D/BMD families. Deletions were identified in 12 of them; eight families were studied by linkage analysis (Fig. 1). Six mothers were diagnosed as non-carriers, thus we did not proceed with prenatal diagnosis. In the remaining 14 families in which prenatal diagnosis was carried out, two fetuses were found to be affected and were terminated.

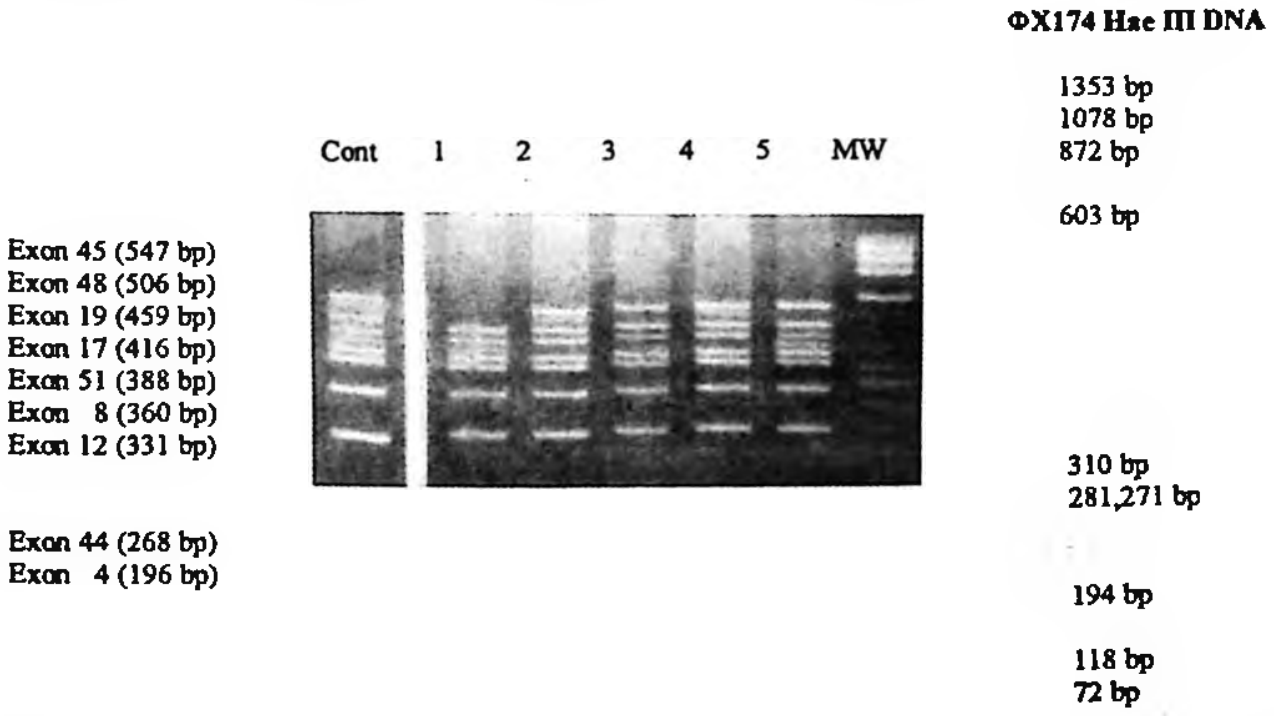

Fig. 1: Deletion analysis of the dystrophin gene by multiplex PCR. The exons represented by each PCR product and product sizes are indicated on the left and MW marker is on the right. Lane 1: deletion of exons 45-48; lanes 2-4: deletion of exon 51; lane 3: deletion of exons 48-51; lane 5: deletion of exon 48. Cont: indicates nonaffected male control subject. MW: molecular weight marker (ϕX174 Hae III DNA) 3% Nu Sieve + 1% agarose gel (05 µg/ml etidium bromid), 120 V, 1.5 hour.

Here, we present several examples of the combined use of dystrophin flanking and intragenic PCR based markers in DMD families, to whom we have provided prenatal diagnosis.

Family 1 : Here, the index case is sporadic. His pregnant sister is asking whether she is a carrier. Highly polymorphic markers showed that she is not a carrier. The affected male (II3) was shown to be deleted for exon 50. STR haplotyping confirmed the deletion, since STR49 failed to amplify while STR 50 was detected. The mother (I1) was heterozygous at STR49 so her carrier risk was therefore reduced to the level of probable mosaicism. Proband's sisters (II2, II4) were heterozygous at STR49, therefore, pregnant sister (II2) did not need to apply for prenatal diagnosis (Fig. 2).

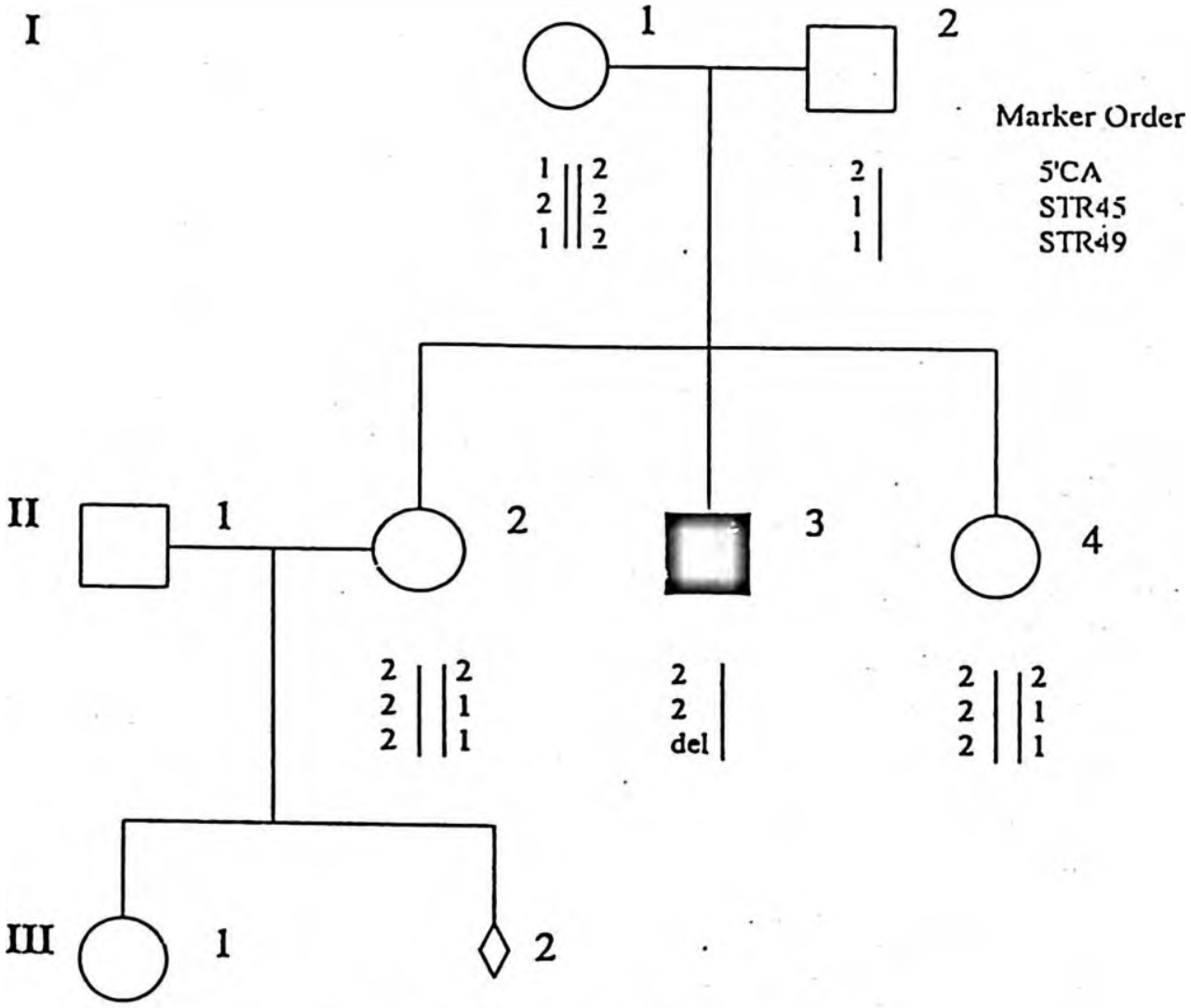

Fig. 2: Pedigree of DMD family 1. del: deletilon.

Family 2: The index case has a deletion. The abnormal X chromosome has a grandpaternal origin which is demonstrated by linkage analysis. This male patient (III4) was shown to have no deletion in the dystrophin gene using the multiplex PCR. He inherited grandpaternal X chromosome at the intragenic marker STR45-9, 5' flanking marker 5'CA and 3' flanking marker 3'CA (recombination error about 1%). His sisters (III3, III5), who inherited the same X chromosomes at these markers and have very high CPKs, are carriers of DMD. The mutation is therefore either grandpaternal in origin or it arose in his mother (II4). A prenatal diagnosis for the exclusion of the grandpaternal X chromosome was offered. After sex determination and genetic analysis, the fetus (III6) was found to be a carrier female (Fig. 3).

Family 2

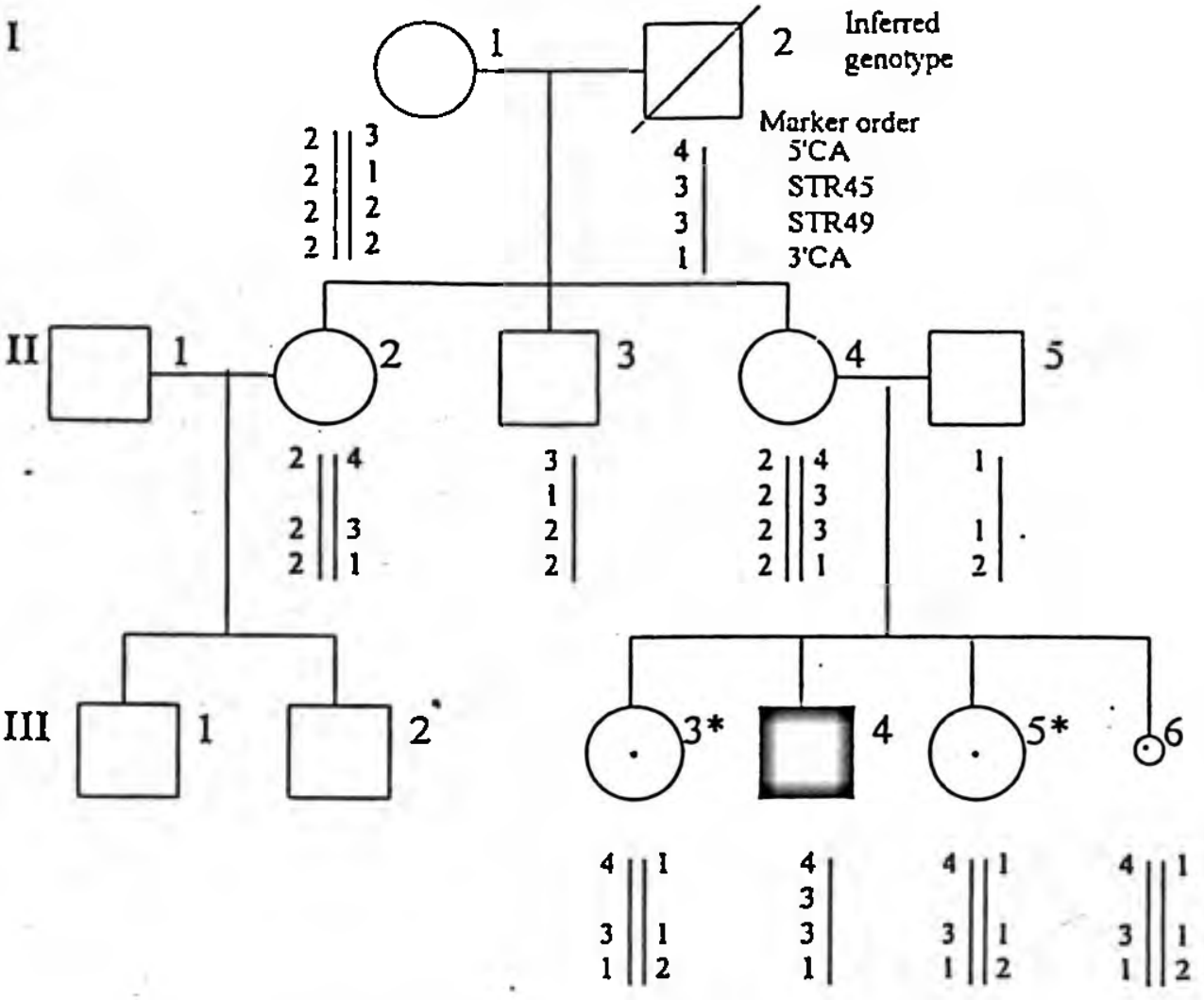

Fig. 3: Pedigree of DMD family 2. *elevated CPK.

Family 3: This example shows the correlation between the biochemical and genetic data. Deletion could not be found in the affected son (III5). The PCR results for the polymorphisms of the whole family are shown in Figure 4. The male patient (III5) inherited the opposite maternal allele from his sisters (III2, III4). This result, combined with the mother's high CPK results, shows that the mother (II4) is a carrier. The CPK results of the sisters were normal. The pregnant sister (III2) is therefore not a carrier (less than 1% recombination error).

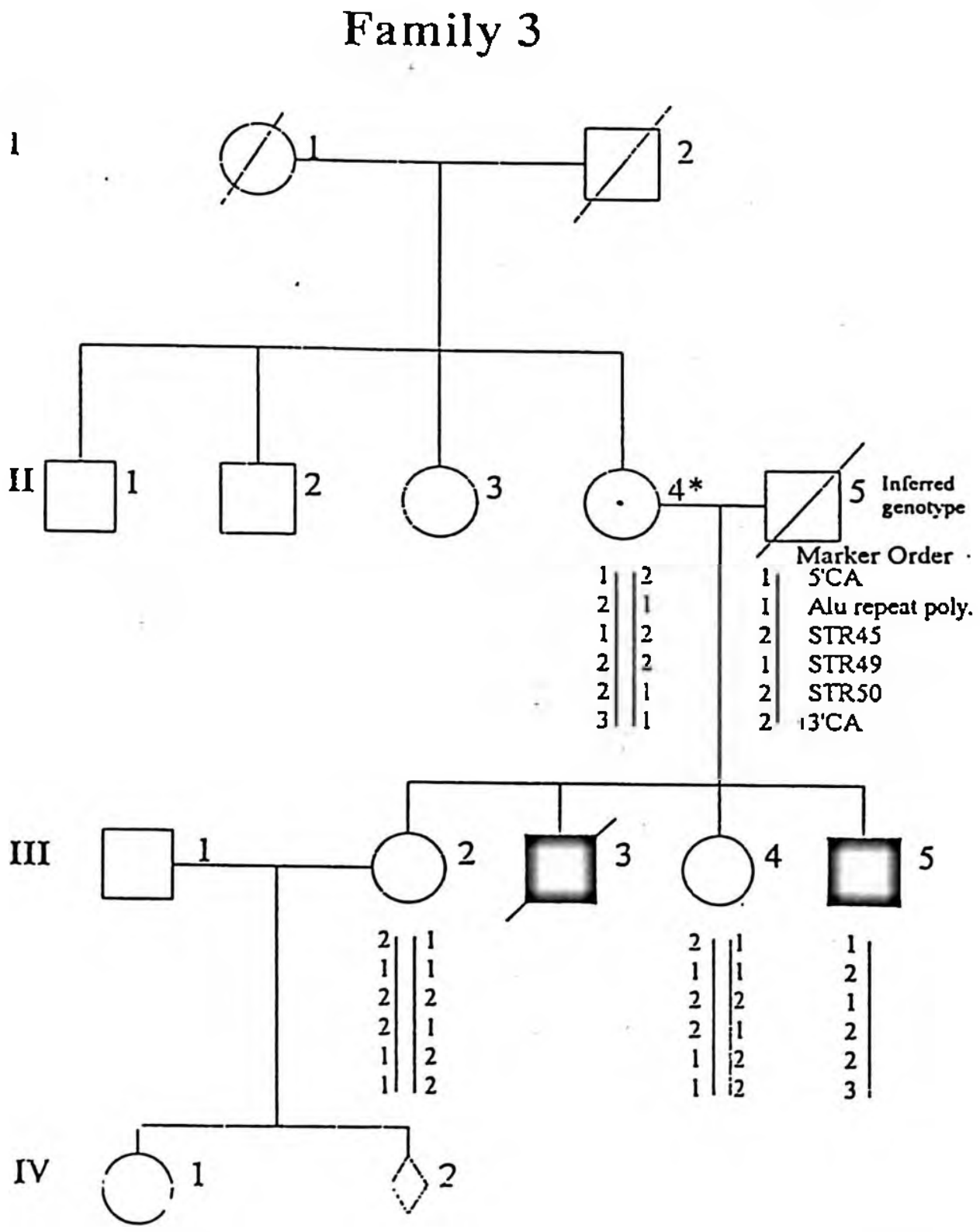

Fig. 4: Pedigree of DMD family 3. * elevated CPK.

Family 4: Deletion studies in the male patient of this family were negative. The two sons (II1, II2) of the pregnant mother (I1) had inherited the opposite alleles from their mother. This increases the mother's carrier risk. Prenatal diagnosis

could be performed for this mother using 5'CA, STR45, STR49, STR50 markers with a one-to-two percent recombination error. For 3' flanking marker of the gene, none of the markers was informative. There is only a one-to-two percent recombination error between intron 50- 3' end of the dystrophin gene[21] (Fig. 5). Fetus has not been worked yet. If the fetus is a male and if he is inheriting the same allele as his unaffected brother, he will be healthy (99% accuracy).

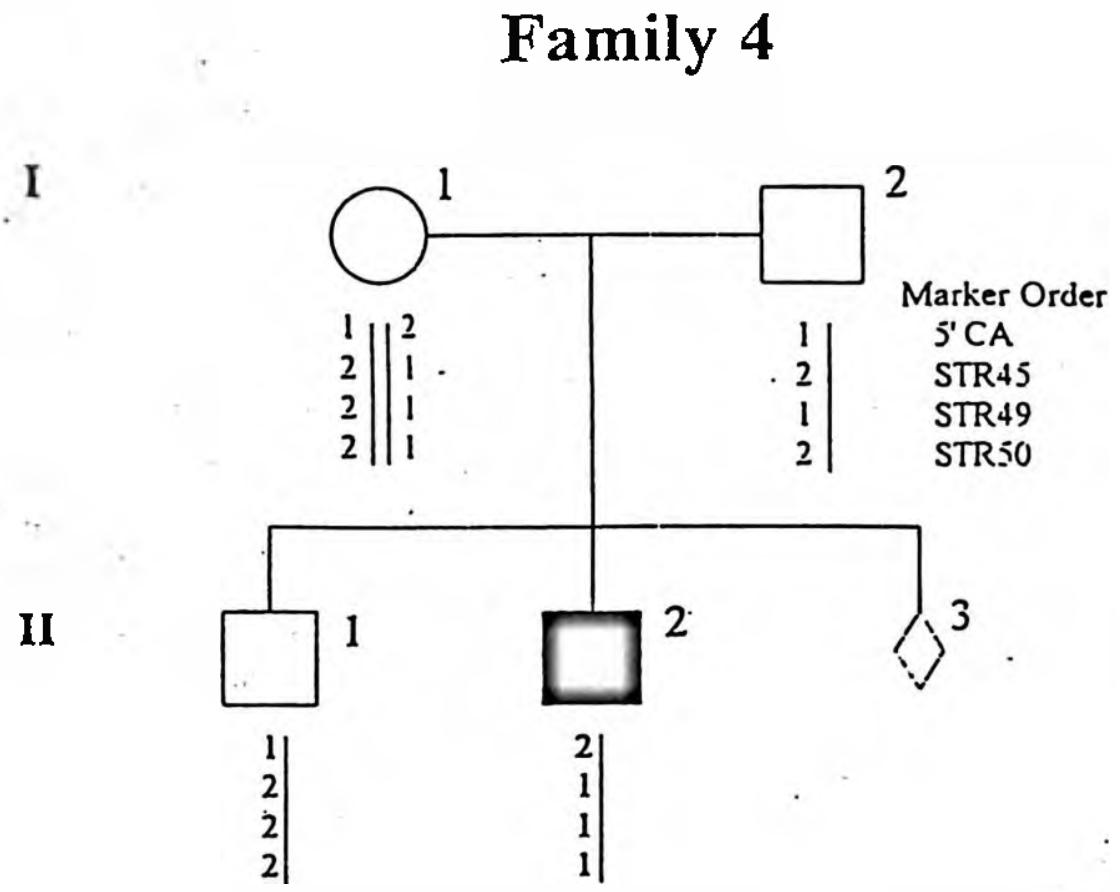

Fig. 5: Pedigree of DMD family 4.

Discussion

There is still no treatment for Duchenne and Becker muscular dystrophies. Genetic counseling is therefore very important to females at risk. There are two means for pre- and postnatal diagnosis. These methods are universal. First, deletion studies must be done. In cases where deletion cannot be demonstrated, a linkage analysis should follow. In our routine experience, we use the same approach. Detection of a deletion in affected children provides a very accurate prenatal test for the future pregnancies. Our deletion rate of 58 percent is nearly the same as other major laboratories[19]. When a deletion is not recognized, we try to track the disease allele through a family by linkage analysis. The problems in using linkage analysis for prenatal diagnosis are the same as for genetic

counseling. The mother must be informative at a suitable locus, and family studies are needed, preferably before prenatal diagnosis. However, we occasionally see mothers already pregnant with no initial DNA studies done. Thus, rapid detection methods should be available, and highly polymorphic and easy-to-study markers should be tried.

Due to the very large size of the gene, analysis of flanking markers is required to avoid diagnostic errors due to intragenic meiotic recombination. When the 5'-3' flanking markers and an intragenic marker are informative and show inheritance of an intact haplotype, the error rate due to recombination is less than one percent[22-23].

As a standard, highly polymorphic microsatellite markers are used in most laboratories. These markers (5'-3' CAs, STRs, DMD1c-2) seem to be efficient for nearly all D/BMD families. In this way, we offer carrier detection and prenatal diagnosis with an accuracy of 99 percent. STR markers lie within the distal deletion hot spot of the dystrophin gene. They provide direct diagnosis of deletion carriers and help to define the mutant X chromosome in affected individuals. These advantages make STRs an ideal intragenic marker for the analysis of D/BMD families. For a single STR marker, the heterozygosity frequency was around 50 percent in our population. When four markers are used in combination, the chances of being informative are tremendously increased. Among the 105 families in which we carried out linkage analysis, only one was found to be non-informative with the STR markers we used. This is very helpful during routine practice of analyzing families.

In conclusion, this combined approach, i.e deletion and linkage analysis, is a safe and reliable method to study D/BMD families in busy clinics serving a large number of families.

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