Original Article

Short Tandem Repeats (STR) Pak Armed Forces Med J 2011; 61 (4): 537-41

FEASIBILITY OF SHORT TANDEM REPEATS (STR) ANALYSIS FOR CARRIER DETECTION AND PRENATAL DIAGNOSIS IN FAMILIES WITH DUCHENNE MUSCULAR DYSTROPHY (DMD)

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ABSTRACT

Objective: To determine the feasibility of Short Tandem Repeats (STR) based linkage analysis for carrier detection and prenatal diagnosis (PND) in families having children affected with Duchenne Muscular Dystrophy.

Study design: Case series

Settings: Department of Chemical Pathology and Endocrinology in collaboration with Department of Molecular Biology, Armed Forces Institute of Pathology (AFIP), Rawalpindi.

Duration: From February 2007 to January 2008.

Subjects: Six unrelated families with at least one affected child in each family who had characteristic features of DMD (index case).

Materials and Methods: PCR for Duchenne Muscular Dystrophy was carried out with STR based linkage analysis at introns 44, 45, 49 and 50 of DMD gene .Thermal cycling in TC-480 (Perkin Elmer) included 25 cycles each comprising 30 sec denaturation at 94°C, annealing at 62°C for 30 sec, extension at 65°C for 2 min. The final extension was done for 3 min. The amplified products were run on 8% nondenaturing polyacrylamide gel electrophoresis (PAGE) carried out at 200V for three hours on electrophoresis apparatus (Bio-Rad UK). The gels were stained in silver nitrate. By comparing STR pattern of X-chromosome allele of index case with X-chromosome alleles of the mother, the diseased or affected X-chromosome was ascertained.

Results: Carrier detection and prenatal diagnosis was feasible with STR marker at intron 44 in DMD families. It was informative in 5 out of 6 DMD families.

Conclusion: Carrier detection and PND by STR based linkage analysis is technically feasible in Pakistani families with DMD.

Keywords: Carrier detection, Duchenne muscular dystrophy, prenatal diagnosis, Short Tandem Repeats.

INTRODUCTION

Duchenne muscular dystrophy is caused by deletion, duplication or point mutation of the DMD gene[1] located on short arm of X chromosome (Xp21), which encodes dystrophin[2]. DMD is named after French neurologist Guillame Benjamin Amand Duchenne who in 1861 provided the first detailed description about the disease in which he explained most of the features like hypertrophy of the calves, progressive muscular weakness, intellectual impairment and connective tissue proliferation in the muscles[3]. DMD typically presents as proximal limb girdle weakness between 2 to 3 years[4]. Most affected males' exhibit retarded motor development[5]. The dystrophin is normally found in skeletal muscles, smooth muscles, cardiac muscles, and brain[6]. The loss of the dystrophin associated proteins in DMD is associated with influx of extracellular calcium triggering calcium activated proteases and fiber necrosis[7]. Dystrophin deletions involving the brain distal isoform Dp140 are associated with intellectual impairment[8]. Disruption of heart sarcoglycan complex due to β sarcoglycan mutations cause severe cardiomyopathy[9] leading to reduced ejection fraction and myocardial dysfunction[10].There may be chest deformity due to scoliosis[11]. Serious and fatal pulmonary infections occur by the age 16 to 18

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Received: 03 July 2009; Accepted: 20 Jan 2011

years. Death due to respiratory insufficiency occurs in second or third decade[1].

Polymerase chain reaction (PCR)[12] provides a key component in the diagnosis of DMD and carriers of the disease[13]. In potential carriers, PCR performed on Chorionic Villous Samples (CVS) is a reliable option for prenatal diagnosis[14]. PCR based diagnosis of DMD can be done either by mutation detection or linkage analysis[15]. Mutation detection technique is labor intensive, expensive and sophisticated, so alternative, reliable and feasible technique is linkage analysis by using STR in the introns of dystrophin gene. One of the most frequent uses of STR analysis in DMD families is to determine the carrier status of females. A prerequisite for the linkage analysis is the presence of an affected child with DMD in the family[16]. By comparing the STR allele pattern of the affected child it is possible to ascertain the presence or absence of the affected X-chromosome allele in the mother and other siblings. The knowledge of the allelic association and the determination of carriers is an important aspect in providing prenatal diagnosis and genetic counseling to DMD families[17]. After thorough search of literature and internet we did not find any local study on the subject. Hence a study was planned to determine the feasibility i.e., informativeness of STR based linkage analysis at introns 44,45,49 and 50 of DMD gene, for carrier detection and prenatal diagnosis in families having children affected with Duchenne Muscular Dystrophy.

MATERIALS AND METHODS

It was a case series study carried out after the approval from ethical committee of AFIP. The study was conducted from February 2007 to January 2008 in the Department of Chemical Pathology and Endocrinology in collaboration with Department of Molecular Biology, AFIP Rawalpindi.

It included six unrelated families having index cases of DMD. The sampling method was non probability purposive.

Informed written consent was taken from each family who participated in the study. History of (H/O) onset of disease in the index case, family history of DMD, ethnicity and H/O consanguineous marriage was noted on a predesigned proforma. Index case in each family was selected on the basis of H/O early onset symmetrical MD (at the age of 2 to 3 years) having characteristic clinical features of DMD, positive Gower's sign and with markedly elevated S.CK level (more than 20 to 50 times upper normal limit of 171 IU/L at 37°C).The S.CK estimated by International Federation of Clinical Chemistry (IFCC) recommended method with Teco Diagnostics Kit (USA made). Index cases with MD of adult onset, post viral myositis, polymyositis, neurological ailment leading to MD and unilateral muscular disorders were excluded. Venous blood samples of all family members were taken in K-EDTA bottles. The DNA analyses were carried out by using Puregene DNA Purification system (blood kit by GENTRA system).

During this study, one family reported for prenatal testing of DMD.This family with an affected child had positive family history of DMD on maternal side. The Chorionic villous sampling (CVS) was done at 12 weeks of gestation; by transabdominal ultrasound guided aspiration technique. After collection, the CVS was meticulously cleaned under a dissecting microscope to remove any maternal tissue contamination. DNA extraction of Chorionic villous sample was done in duplicate by Phenol Chloroform extraction Technique[18].

After DNA extraction, PCR for DMD was carried out with STR based linkage analysis at the introns 44, 45, 49 and 50 of DMD gene [19]. Thermal cycling was done in TC-480 (Perkin Elmer), and it included 25 cycles each comprising 30 sec denaturation at 94°C, annealing at 62°C for 30 sec, extension at 65°C for 2 min. The final extension was done for 3min. The amplified products were run on 8% nondenaturing Polyacrylamide gel electrophoresis (PAGE) carried out at 200V for three hours on electrophoresis apparatus (Bio-Rad UK). The gels were stained in silver nitrate. By comparing STR pattern of X-chromosome allele of index case with X-chromosome alleles

of mother, the diseased or affected X-chromosome was ascertained.

RESULTS

Six families of DMD were included in the study. Fig shows PAGE of amplified STR products at intron 44 of DMD gene in the family No. 4 (reported for prenatal testing). STRs analyses showed that Intron 44 was informative for carrier detection and prenatal diagnosis in DMD families while introns 45, 49 and 50 were noninformative in all families. Almost 5/6 families were informative with intron 44 while one family was non informative with all introns. Almost 5/6 families were Punjabi by ethnic origin (Table).

Fig: **PAGE of amplified STR products at Intron 44 of DMD gene in the family No. 4 (reported for prenatal testing of DMD). From right to left;**

* Father's sample
**Mother's sample with two X-chromosome alleles denoted by allele a and allele b
*** Index child with affected X-chromosome allele a
NC@ Non Carrier sister
CVS sample run in duplicate. Comparison of the result in lane 3 (index child) and lane 2 (mother) suggested that out of the two maternal X-chromosome alleles, the allele a was affected while the allele b was normal. The sample of the female sibling in lane 4 was suggestive of non carrier sister as out of two maternal alleles she inherited normal allele b. CVS sample revealed female sibling as a carrier female as out of two maternal alleles she inherited affected allele a.

DISCUSSION

Duchenne Muscular Dystrophy is an X-linked recessive, fatal genetic disorder resulting from mutations in the dystrophin gene located at Xp21 region[2]. Advances in laboratory techniques now focus direct mutational analysis as the most reliable and indirect analysis using Short Tandem based linkage analysis as feasible, inexpensive and efficient method for carrier detection and prenatal diagnosis.

This study has determined the feasibility of STR in DMD families having at least one affected member (index case). The result showed that STR based linkage analysis at Intron 44 is an excellent molecular tool for carrier status identification and PND in Pakistani families and the finding matched with the study carried out in Hemophiliacs[20].

The result with intron 44 showed that carrier screening in targeted DMD families is feasible in our setup and matched with studies conducted in Spain[21], Mexico[22], Korea[23] and Taiwan[24] which determined informativeness of STRs in DMD gene for carrier detection and prenatal diagnosis.

In the present study 5/6 DMD families were Punjabi by ethnic origin with a strong history of consanguineous marriages and the findings matched with a local study, which showed high prevalence of β thalassemia and consanguinity in Punjabi families[25]. The limitation of this study was small sample size so acceptability of PND in DMD families is yet to be seen in our target population and require further studies.

In this study the prenatal diagnosis was successfully carried out with STR at intron 44 in a Punjabi family. The fetal sampling was done at AFIP by transabdominal chorionic villous

Table: Salient features in history and STR analysis at intron 44 of six DMD families

Family No	Ethnic origin of families	Consanguineous marriage	STR analysis at intron 44		
			Mother	Female sibling	Chorionic villous sample
1	Punjabi	Yes	Carrier	-	-
2	Punjabi	No	Non informative	Non informative	-
3	Pathan	Yes	Carrier	Non carrier	-
4	Punjabi	Yes	Carrier	Non carrier	Carrier fetus
5	Punjabi	Yes	Carrier	Carrier	-
6	Punjabi	Yes	Carrier	Carriers (two)	-

biopsy and in experienced hands the procedure is extremely safe and useful for PND[26]. In AFIP there is well established setup of PND which is functional since 1994[27] and over 2000 prenatal diagnoses have been carried out for various genetic disorders in past 12 years[28].

The accurate selection of index case of DMD was mandatory, as a prerequisite for linkage analysis. The confounding variables were excluded like adult onset MD, unilateral myopathy, patients with post viral myositis and neurological ailments leading to MDs.

Out of six families of DMD only one family was noninformative with all four STR introns. This finding was in contradiction to a study which reported informativeness with all STR markers[15]. But the result matched with a Hungarian study[16] in which 13 out of 41 families were noninformative with introns 45 and 49. In a Polish study 8 out of 40 families were non informative with the STR analysis for carrier detection in families with an isolated case of DMD/BMD[29]. An Indian study reported 58-70% informativeness with these four STRs[30]. Another Indian study reported 80% informativeness[31] and these studies have considered the analysis with STRs in DMD families as a cornerstone for carrier detection and prenatal diagnosis.

CONCLUSION

Carrier detection and prenatal diagnosis with STR based linkage analysis is technically feasible in Pakistani families with Duchenne Muscular Dystrophy.

Recommendation

Prenatal diagnostic facilities for genetic disorders should be made available in specialized Molecular Diagnostic Centers.

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