

DNA Diagnosis of Down Syndrome Using Polymerase Chain Reaction and Polymorphic Microsatellite Markers

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ABSTRACT For antenatal diagnosis of Down syndrome (trisomy 21) and for the determination of parental origin of nondisjoined chromosome, we have evaluated a simple PCR based DNA diagnostic method where polymorphic allelic fragments were resolved in 6% polyacrylamide gel. Three families, DS5, DS7 and DS9, each with a single child with trisomy 21 were studied, using two polymorphic microsatellite markers D21S2055 and D21S11 located on the long arm of the chromosome 21. Trisomy 21 was identified by the presence of three distinct alleles while the origin of nondisjunction was found to be maternal in all three families. The PCR based DNA diagnostic method was found to be sensitive, reproducible and most efficient not only for diagnosis of trisomy 21, but also for tracing allelic transmission from parents to the offspring.

INTRODUCTION

Down syndrome is one of the most frequent live born aneuploidy in human and its frequency was one in 700 live births (Mueller and Young 1995). Trisomy 21 produces Down syndrome which includes a variety of developmental anomalies including facial dysmorphism, congenital defects of heart and gut, infertility, immuno deficiencies, increased incidence of leukemia and mental retardation (Epstein 1986). Chromosome analysis and karyotyping were the only available methods for diagnosis of Down syndrome patients till 1980s. In the late 1980s, the introduction of fluorescence *in situ* hybridization (FISH) has enabled the identification of trisomy 21 in interphase cell nuclei (Li et al. 1999; Acar et al. 2002; Witter et al. 2002). The development of PCR technology and human genome mapping are landmark

contributions that have facilitated the initiation of an alternative approach for the detection of trisomy 21. It involves PCR amplification of small tandem repeat (STR) markers located on human chromosome 21 and analysis by fluorescence based method to identify the presence of an additional allele on the third copy of the chromosome. This DNA approach was first used for the diagnosis of the X-chromosome aneuploidy (Lubin et al. 1991) and has since been modified to include identification of other common trisomies. Quantitative fluorescence PCR was the method of choice for many workers (Mansfield 1993; Pertl et al. 1999; Yang et al. 1999; Valero et al. 1999) while multiplex PCR was also found to be very useful for rapid detection of trisomy 21 (Blake et al. 1999; Findlay et al. 1998).

The aim of the present study was to investigate the sensitivity, reproducibility and reliability of PCR based DNA diagnostic method and usefulness of microsatellite markers for routine diagnosis of trisomy 21 and also for the determination of parental origin of nondisjoined chromosome.

MATERIALS AND METHODS

Three families, DS5, DS7 and DS9, each with a clinically suspected Down syndrome proband and a control family consists of normal individuals were included in this study. Heparinised blood samples were received from the Department of Paediatrics, Calcutta Medical College. Chromosome preparation was made from lymphocyte culture by the conventional air drying method.

Genomic DNA was isolated, using salting out procedure of Miller et al. (1988). DNA polymorphism was studied by using two tetranucleotide microsatellite markers D21S2055 situated at 21q22 and D21S11 situated at 21q21. The PCR conditions for first primer were

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as follows: after the initial denaturation at 94°C for 3 min, 30 cycles of PCR amplification were done-94°C for 30 s; 56°C for 30s; 72°C for 1 min and final extension for 3 min at 72°C. For 2nd primer, after the initial denaturation at 95°C for 5 min, 30 cycles of PCR amplifications were done-95°C for 30s; 60°C for 30s; 72°C for 30s and final extension for 9 min. at 72°C in a Perkin-Elmer 2400 Gene Amp. Thermal cyclers. The sequences of two primers of D21S2055 and D21S11 used for amplification were FP, 5' - AACAGAACCAATAGGCTATCTATC-3', RP, 5' - TACAGTAAATCACTTGGTAGGAGA-3' and FP, 5' - GTGAGTCAATTCCCCCAAG-3', RP, 5' - GTTGATTAGTCAATGTTCTCC-3' respectively. PCR products were analysed in 6% polyacrylamide gel and analysed in UV transilluminator after staining with ethidium bromide. The parental origin of supernumerary chromosome 21, and therefore, the parental origin of nondisjunction, was determined after scoring of the polymorphic alleles in the parents, the proband and siblings if available by following the method described by Antonarakis et al. (1991, 1993).

RESULTS AND DISCUSSION

Cytogenetic analysis

Analysis of chromosomes and karyotype revealed the diploid count of 2n=47 with free trisomy 21 in probands of DS5, DS7 and DS 9 while in control the children have normal chromosome count 2n=46 and have normal karyotype.

Molecular Analysis

Analysis of DNA polymorphism in three families DS5, DS7 and DS9 is shown in figure 1, Fig. 2 and Fig 3 respectively. DS5 and DS9 were analysed by D21S2055 while DS7 was analysed by both D21S2055 and D21S11. In DS5 (Fig. 1), mother has two alleles 1 and 2 while proband with trisomy 21 revealed three different alleles 1, 2 and 5. The father has two alleles 4 and 5 and the following lane shows a control individual having two alleles 3 and 5. The last lane contains a size marker pBR 322/Alu 1 digestion (90bp-403bp). In DS7 (Fig. 2), lane 1 contains size marker. Father shows two alleles 1 and 4. Lane 3

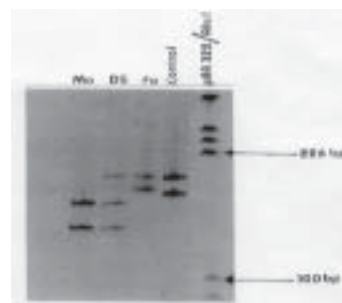


Fig 1. Proband with three different alleles 1, 2 and 5 (allele 1 is the shortest and allele 5 the longest in this case). Alleles 1 and 2 originate with the mother and allele 5 originates with the father. Control individual shows two alleles 3 and 5 (D21S2055). DS = Down Syndrome, Fa = Father, Mo = Mother

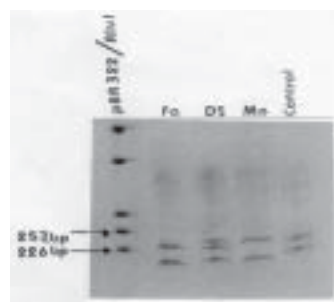


Fig. 2. Proband with three different alleles 2, 4 and 5. Alleles 2 and 5 originate with the mother and allele 4 originates with the father. Control individual revealed two alleles 3 and 5 (D21S11). DS = Down Syndrome, Fa = Father, Mo = Mother

contains trisomic proband with three distinct alleles 2, 4 and 5 while mother has two alleles 2 and 5. The last lane contains control individual with two alleles 3 and 5. On the other hand, in DS9 (Fig. 3), lane 1 contains size marker and proband with trisomy 21 shows three different alleles 1, 5 and 6 in lane 2. The two following lanes show father and mother, one having two alleles 2 and 7 and other having two alleles 1 and 6. Lanes 5, 6 and 7 show a normal family consists of child, father and mother respectively. Child has two alleles 4 and 7 while father revealed two alleles 4 and 6 and mother has alleles 3 and 7. These alleles were scored visually and were within the size range of 100-126 base pairs and 100-257 base pairs for D21S2055 and D21S11, respectively.

The parental origin of supernumerary 21 chromosome was determined in all three families DS5, DS7 and DS 9 and was found to be maternal

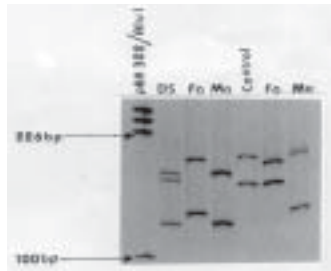


Fig. 3. Three different alleles 1,5 and 6 of which alleles 1 and 6 originate with the the mother The paternal origin of allele 5 could not be determined from DNA sample of father. In the control family, normal child shows two alleles 4 and 7. Allele 4 originates with the father and allele 7 originates with the mother (D21S2055).

DS = Down Syndrome, Fa = Father, Mo = Mother

in origin. On the other hand, in control family, the normal child received one allele each from father and mother. Genotypes of Down syndrome (trisomy), father, mother and normal individuals and origin of nondisjunction are shown in Table 1.

Table 1: Genotypes of DS family, control individual, control family and Parental origin of Nondisjunction

Family (code no.)	Genotype			Origin of non-disjunction
	DS	Fa	Mo	
DS5	1,2,5	4,5	1,2	Maternal
Control individual	3,5	-	-	-
DS7	2,4,5	1,4	2,5	Maternal
Control individual	3,5	-	-	-
DS9	1,5,6	2,7	1,6	Maternal
Control family	4,7	4,6	3,7	-

DS=Downs syndrome, Fa=Father, Mo=Mother

Analysis of genotypes of the members of three families and control revealed that in all three families nondisjunction was maternal in origin (Table 1). In DS5, alleles 1 and 2 of trisomic proband were originated with the mother while alleles 2 and 5 of the proband were also originated with the mother in DS7. Further, in DS 9, the trisomic proband consists of three alleles 1, 5 and 6 of which 1 and 6 were transmitted from mother. However, the allele 5 of the proband did not originate with the father who has two alleles 2 and 7. Therefore, it seems that person was not the biological father of the proband in DS9. On the other hand, in control family, normal

individual whose genotype consists of two alleles 4 and 7 received one allele each from father and mother. Similarly, in both DS5 and DS7, genotypes of control individuals against trisomic proband consist of only two alleles (Table1). There is a well-recognized association between advanced maternal age and the incidence of free trisomy 21 which has been studied for past twenty years (Hassold and Jacobs 1984; Antonarakis 1991, 1998). Initially, the method of study was by determination of the chromosomal heteromorphism in a given family which resulted in erroneous assignment of parental origin of supernumerary chromosome 21 and it has been observed by several workers that there were discrepancies between the results of the DNA analysis and those of cytogenetic analysis (Carothers 1987; Antonarakis et al. 1991, 1993). In recent years, the study of the mechanism of meiotic nondisjunction and determination of parental origin of supernumerary chromosome 21 have been done more accurately and rapidly by the development and utilization of polymorphic microsatellite markers on HC 21 (Antonarakis 1998; Zittergruen et al. 1995). In the present study we have used a simple PCR based method where polymorphic allelic fragments were separated in polyacrylamide gel and this method was found to be as efficient as quantitative fluorescence technique where fluorescence labelled primer, DNA sequencer and genescan software are usually required for genotyping (Findlay et al. 1998; Valero et al. 1999; Blake et al. 1999).

We have also determined the parental origin of extra 21 chromosome in three Down syndrome families DS5, DS7 and DS 9 and in all three cases the extra 21 chromosome was maternal in origin. In case of DS7 parental origin could not be determined by using the primer D21S2055. Therefore, we have used another polymorphic marker D21S11 to resolve the parental origin in DS7. Clearly, the judicious choice of a few highly polymorphic markers is very essential for investigating the parental origin of trisomy 21 (Chakravarti 1989). In the present study the mean maternal age was 32 years and paternal age was 37 years. However, more data are required to prove the hypothesis that the advancing maternal age was responsible for the origin of nondisjunction. Moreover, another advantage of PCR based method was that the translocation Down syndrome could be detected by using

DNA markers on the short arm of the chromosome 21 while cryptic structural rearrangements involving critical DS region which was missed by current chromosome diagnosis method could also be identified by the PCR based method (Nakamura et al.1997).

The simple PCR based DNA diagnostic method which we have evaluated in the present investigation is found to be an efficient and rapid alternative for quantitative fluorescent PCR method and can be used for antenatal, prenatal and preimplantation diagnosis not only for Down syndrome but also for other chromosomal aneuploidies. Our results further demonstrate the usefulness of highly informative microsatellite markers for the study of nondisjunction in Down syndrome.

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