VOLUME - 11, ISSUE - 01, JANUARY - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Genetics

RAPID DETECTION OF DOWN SYNDROME BY SHORT TANDEM REPEAT ANALYSIS FROM EASTERN INDIA

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KEYWORDS : Down syndrome, Short Tandem Repeat Analysis, Indians

INTRODUCTION:

Down syndrome (DS) is the most common chromosomal disorder in human, caused by an extra copy of chromosome 21(Ward et al. 1999). It constitutes the most frequent form of intellectual disability. The cytogenetic profile of down syndrome includes trisomy 21, Robertsonian translocations, mosaicism, duplication of the critical region and other structural rearrangement involving chromosome 21(Verma et al. 1998,). India has the highest number of people suffering from DS in the world (Sherman et al 2007). The high prevalence is mainly due to lower level of care considered for 2/3 of Down syndrome pregnancies that are under 35 years of mother age and low level of information behind etiology of Down syndrome for instance about advanced maternal age(Van Montfrans et al. 2002). To reduce significantly the birth prevalence of Down syndrome, a wide-ranging screening of pregnant women has been suggested (Pertl et al. 1996). However, conventional methods such as cytogenetic analysis for diagnosis of chromosomal abnormalities often need lengthy laboratory procedures, and expertise, are expensive as well as significant delay in obtaining a diagnosis (Patterson et al. 2005). Applying of fluorescence *in situ* hybridization technique from late 1980s using fluorescently labeled DNA probe has facilitated analysis of chromosome abnormalities. However, genotyping of short tandem repeats (STR) on chromosome 21 is an alternative rapid inexpensive & reliable method for the identification of DS child and is also even suitable for large scale screening of pregnant women (Rahil et al. 2002). The STRs are the hypervariable regions of the genome with variable repeat length and can be used for the quantitative analysis of extra chromosome of 21. This study aims to identify the DS child using simple PCR based analysis of STR markers on chromosome 21 among the eastern Indian population.

MATERIALS AND METHODS:

A total of 85 Down syndrome cases (Male: 55; Female: 30; Mean age: 43.74±82.85 months) were recruited in this study with their written informed consent as per guidelines of Indian Council of Medical research (ICMR). The subjects were referred to the Genetics department at Ramakrishna Mission Seva Prathisthan, Kolkata, India. In addition to that 85 age and sex matched controls (Mean age: 55.65±64.74 months) with no personal or family history of any developmental disorders were recruited in the present study from Kolkata. Approximately, 4-5 ml of blood was collected inethylene diamine tetra acetic acid and heparin vial. Genomic DNA was extracted using QIAmp DNA blood Mini Kit (Qiagen, Germany) as per manufacturers protocol. For molecular detection of Down syndrome, D21S11, located at 21q21.1, were PCR amplified using the primer pair: forward primer 5'-GTGAGTCAATTCCCCAAG-3' and reverse primer, 5'-GTTGTATTAGTCAATGTTCTCC-3'. The fluorescence labelled PCR products (forward primer was fluorescence tagged at the 5' end with FAM) were then genotyped in AVANT 3130 DNA sequencing System (Applied Biosystem Inc., Foster City, CA USA) using Genescan-500 ROX size standard (Applied Biosystem Inc., Foster City, CA USA).

RESULTS:

A total 85 DS patient was genotyped for STR marker analysis [Table 1]. Out of 85 cases studied, 79 patients were exhibited tri-allelic (trisomy is represented by the presence of three alleles) for the D21S11 which represent ~92.94% cases. Six individuals were either bi-allelic or mono-allelic. The Karyotyping analysis revealed trisomy at chromosome 21, Mosaicism & translocation were 78 (91.76%), 5 (5.88%) & 2 (2.35%) cases respectively. All the control individuals showed tow peaks with ratio 1:1(Fig.1).

DISCUSSION:

Genotyping of D21S11 STR marker were able to detect trisomy at chromosome 21 in 92.94% of Down syndrome cases among the study cohort. Our results were also validated by Karyotyping analysis. Regarding trisomy 21 cases with 2 uneven peaks, it is assumed that 2 possibilities exist: (a) the nondisjunction of chromosome 21 could have occurred during meiosis II so that 2 of the 3 chromosome 21 had identical numbers of STRs, or (b) meiosis II nondisjunction did not occur but the STR numbers were actually the same in 2 of the 3 chromosome 21s (Vogt et al. 2008). Such trisomy cases with 2-peaks can be distinguished from those of healthy control by comparing their peak area ratios.

Detection of Down syndrome by chromosomal analysis (Karyotyping) involved considerable time and manpower. In contrast, STR marker analysis is reliable and less time consuming. The presence of three alleles with the peak ratio 1:1:1(Fig.1), two alleles with peak ratio approximately 2:1 and one allele with peak ratio 3:0 can begood marker for the detection of trisomy at chromosome 21 (Verma, 1998). Rapid PCR based amplification of STR markers may reduce the paternal anxiety and medical termination of pregnancy is also feasible for an antenatal diagnosis. On the other hand, Fluorescent *in situ* hybridization (FISH) technique also requires skilled personnel and proper experimental set up.

In summary, it can be concluded that we can rapidly detect the Down syndrome cases by genotyping of D21S11 marker. Therefore, this method should get the priority for the rapid, cost effective identification of Down syndrome cases among eastern Indian population. Our result should be validated in larger number of cases from different ethnic population of India.

Acknowledgement

We are grateful to the Secretary, Ramakrishna Mission Seva Pratisthan, Vivekananda Institute of Medical Sciences, Kolkata for the necessary permission to carry out this study.

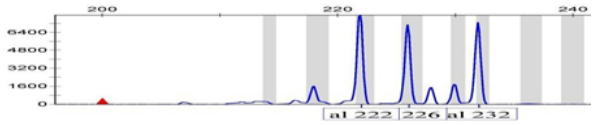
Funding

This study was financially supported by the grants from

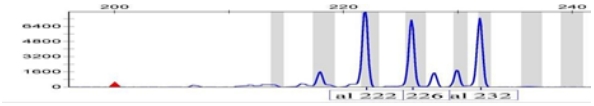
Department of Science & Technology (DST), Govt. of West Bengal.

Table 1: Genotypic Frequency Distribution Of D21s11

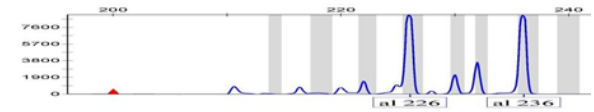
	Tri-allelic	Bi-allelic	Mono-allelic	Trisomy	Mosaicism	Translocation
Patients (n = 85)	79 (92.94%)	5 (5.88%)	1 (1.18%)	78 (91.76%)	5 (5.88%)	2 (2.35%)
Male (n = 55)	50 (58.82%)	4 (4.7%)	1 (1.18%)	49 (57.65%)	4 (4.7%)	2 (2.35%)
Female (n = 30)	29 (34.12%)	1 (1.18%)	0 (0)	29 (34.12%	1 (1.18%)	0 (0)



(A)



(B)



(C)

Figure.1. Electrophoretograms of fluorescent D21S11 amplified STR markers. (A) & (B) Trisomy 21 affected patients with a triallelic pattern with peak height ratio of 1:1:1; (C) A normal individual with a diallelic pattern with peak hight ratio of 1:1

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