PARENTAL ORIGIN OF MEIOTIC ERROR OF THE EXTRA CHROMOSOME 21 AS INDICATED BY SHORT TANDEM REPEAT (STR) POLYMORPHISMS IN DOWN SYNDROME

Ahmad Aleyasin PhD**, Shahla Mohammad-Ganji MSc**, Mohammad Ghazanfari MSc*, Faezeh Jahanshad MD***

Background – Down syndrome is the most common cause of mental retardation observed in approximately 1/230 pregnancies. This is the first epidemiological studies to categorize cases of Down syndrome by parent of origin of extra chromosome 21 meiotic error in Iranian Down syndromes.

Methods – We studied 100 Down families using conventional cytogenetic and chromosome 21-specific markers. Five specific DNA markers, related to chromosome 21, were used to determine the origin of meiotic error to each case.

Results – Chromosomal analysis revealed free trisomy 21 karyotype in 95%, mosaic trisomy karyotype in 5%, and none with chromosome translocation. The parent of origin of chromosome 21 trisomy was determined in 85% of the cases. Nondisjunctional error was determined as maternal in 86% and paternal in 14% of the cases.

Conclusion – Although the exact mechanism behind chromosome nondisjunction has not been very well described, but in the case of free chromosome 21 trisomy the exact reasons behind the nondisjunction may vary between female and male who passed this extra chromosome to their child. The knowledge of Down syndrome of parental origin is one of the basic steps to evaluate accurately the risk factors and etiology of meiotic nondisjunction.

Keywords • Down syndrome • nondisjunction • polymorphism • short tandem repeat (STR) • trisomy

Introduction

Down syndrome is one of the most common chromosome aneuploidies causing mental and growth retardation. Other problems which include increased incidence of heart abnormalities, predisposition to leukemia, and premature Alzheimer-like dementia.1 – 3 The incidence of Down syndrome is approximately 1 in 700 live births, even though 2/3 of Down syndrome pregnancies result in fetal death. It is mostly resulted from nondisjunction event of chromosome 21 at meiosis or with less frequency (5%) in postzygotic mitosis. Nondisjunction event leads to extra free chromosome 21 which is usually maternal in origin and occurs more frequently with advanced maternal age.4 – 10

Despite many years of research to identify risk factors associated with Down syndrome, only one factor, advanced maternal age, has been well established.7 There is no treatment available for Down syndrome, hence establishes etiology is important in reducing its occurrence in different populations. Previous studies of Down syndrome have pooled all cases regardless of the parent of

Authors affiliations: *Department of Medical Genetics, National Research Center for Genetic Engineering and Biotechnology, **Research and Science Campus, Islamic Azad University, ***Navid Institute of Infertility, Tehran, Iran.

Corresponding author and reprints: Ahmad Aleyasin, PhD, Department of Medical Genetics, National Research Center for Genetic Engineering and Biotechnology, P. O. Box: 14155-6343, Tehran, Iran. E-mail: sogand@nrcged.ac.ir.
origin of meiotic error. With new DNA technology and chromosome 21-specific genetic markers, the parent of origin of meiotic nondisjunction could be determined.

Short tandem repeats (STRs) are hypervariable regions of the genome with repeated units of 2 to 7 bp in length. The high-length polymorphism of STRs arises from variations in the number of tandem repeated units resulting in production of allele length variation in most parents. Through the process of polymerase chain reaction (PCR), polymorphic STR markers can be amplified and examined in a family to find out the progeny of specific chromosome 21 alleles. Informative loci representative of existing chromosome 21 in most cases would show either three or two alleles with a 2:1 ratio in dosage. The quantitative nature of this technique allows the amount of PCR product to be determined when parents share the same alleles.

The aim of this study was to determine the parental origin in the studied cases. This is the first population-based epidemiologic study in Iran to categorize Down syndrome cases by the parent of origin of the meiotic error. Assuming the different etiology of nondisjunction between father and mother of Down syndromes, parental origin determination could be useful in better identifying important environmental and/or genetic risk factors.

Materials and Methods

This study was carried out on approximately 250 samples taken from 100 families having a Down child who were invited to participate in this project from mentally retarded care centers or schools. A medical doctor, genetic specialist, consulted all selected families. Informed consent was obtained from all participants and the study was approved by the ethics committee of the center. In one-hundred Down cases, 57 had both parents and the rest had only a single parent. The control group consisted of 100 normal mothers who had a healthy child and was considered for comparison of Mendelian inheritance and density of molecular marker alleles. Five mL of blood samples were obtained from the cases and their parents, in 15-mL falcon tubes. They were subsequently divided into two tubes, one containing Na-heparin anticoagulant to be used in cytogenetic analysis and the other containing ethylenediaminetetraacetic acid (EDTA) anticoagulant for use in PCR analysis. The karyotype of a subgroup with no previous karyotype records were assessed by conventional cytogenetic analysis. For DNA analysis, standard phenol-chloroform DNA extraction procedure was used to extract DNA from collected blood samples. PCR amplification was performed using primer sequences for five STR markers (D21S11, D21S1414, D21S1440, D21S1411, and D21S1412) specific for chromosome 21. Two marker sequences were obtained from Pertl et al, and other primer sequences were obtained from the Genome Database (http://www.gdb.org/) (Table 1). These markers are polymorphic and have recently been used for Down syndrome diagnosis and parental origin determination in other populations. PCR amplification was carried out in separate assays in a total volume of 25 µL containing 50 ng genomic DNA, 200 µM dNTPs, 20 pmol of each primer, 2.5 µL of 10X Taq DNA polymerase buffer, 1.5 mM MgCl$_2$, and 0.5 U Taq DNA polymerase (Roche, Germany). PCR thermal was performed in 32 cycles. Each cycle consisted of 95°C denaturizing for 30 seconds, 60°C annealing for 1 minute, and 72°C extension for 1 minute. The thermal cycles were started with an initial denaturizing of 95°C for 30 seconds, 60°C annealing for 1 minute, and 72°C extension for 1 minute. The thermal cycles were started with an initial denaturizing of 95°C for 5 minutes and a final 72°C extension for 10 minutes.

The PCR products (5µL) were mixed with 1µL of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol); size marker ladder (Boehringer Mannheim DNA molecular weight marker X), and separated at 100 volts for two hours on 2% agarose gel, 0.5 XTBE buffer. After running gels, they were stained with 0.5 µg/mL ethidium bromide to visualize PCR bands on UV light transilluminator to determine the quality of PCR products.

Evaluation of polymorphism was carried out using denaturing polyacrylamide gel electrophoresis to better separate different alleles. The denaturing PAGE gel consisted of 8% polyacrylamide (29:1 acrylamide/bisacrylamide), 7 M urea, 1 XTBE, 500 µL 10% ammonium persulfate, and 50 µL TEMED. The gel was allowed to polymerize at room temperature for 1 hour and then was run at 100 Watts. Twenty-five µL of the PCR products were mixed with 45 µL of loading buffer and denatured at 95°C for 8 minutes. Denatured samples were cooled on ice and 8 µL of each sample was loaded. The gel apparatus was DNA sequencing unit (30 x 45 cm)
connected to 2000V power supply clamps. The gel was run at 50°C constant temperature and 100W limiting power for about 3 hours (BioRad Power/Pak 3000 power supply). Gels were stained and alleles were visualized using standard silver staining procedure. Ultrascan XL (Pharmacia LKB Biotechnology) was used to measure the density of the PCR product bands as a densitometry quantitative tool. The electrophoresograms of the PCR products were obtained for trisomic cases.

### Results

One-hundred Down families were examined to determine the parental origin of meiotic error in Iranian Down syndrome population. Free chromosome 21 was observed by conventional karyotyping and molecular analysis. In cyto genetic analysis, all cases had an extra chromosome 21 in which five cases showed chromosome 21 mosaics. Molecular diagnosis using five tested markers detected most of the Down syndromes in triallelic form, representative of free extra chromosome 21. From 100 cases, parental origin was determined for 85 meiotic cases of whom 86% were assigned as maternally-derived and 14% as paternally-derived cases. Each sample was determined to be homozygous or heterozygous for certain alleles, as a result of the number of bands appearing on the polyacrylamide gel. Trisomic samples exhibited either three STR bands in triallelic pattern or two bands with ratio of 2:1 in diallelic pattern. Each locus pattern was confirmed by subsequent tests for the other markers, whereas the normal case always showed a disomic pattern with a ratio near 1:1. The frequencies of triallelic, diallelic, and monoallelic for each marker are presented in Table 1. The markers, D21S1411 and D21S11 present a high degree of polymorphism, compared to other tested markers.

### Discussion

Using STR markers, we determined the parental origin of meiotic chromosome 21 nondisjunction in 85 out of 100 Iranian families with Down syndrome. Eighty-six percent of them were assigned as maternally-derived and 14% as paternally-derived Down syndrome. Chromosome 21 trisomy was confirmed by karyotype analysis. Several other studies reported parent of origin as maternally-derived approximately in 86 to 95% of the cases using similar molecular DNA marker technology.\(^5\)\(^,\)\(^10\)\(^,\)\(^20\)\(^–\)\(^23\) This indicates that our data of parental origin is consistent with the previous molecular analysis of Down syndrome. The selected markers used in this study were highly polymorphic in the human population and were well situated even in Down syndrome critical region (DSCR)\(^16\)\(^–\)\(^19\)

There is inadequate information on etiology of Down syndrome, for instance, about maternal age dependence. The frequency of birth of Down syndrome still remains high, and the high prevalence is mainly due to insufficient care delivered to 2/3 of Down syndrome pregnancies that are under 35 years of age. Categorizing cases of Down syndrome by parent of origin of the meiotic error allows for more precision in identifying Down syndrome risk factors.\(^11\)\(^–\)\(^13\)

Important advantage of new DNA technology is to correspond strongly to the etiology of Down syndrome in different population and allows a useful population-based epidemiologic study to categorize Down syndrome cases by the parental origin of the meiotic error. The information relating to maternal cause of Down syndrome may also be important for maternal health. It has been reported that the incidence of Alzheimer, a chronic neurodegenerative disorder, is higher among mothers of Down syndrome.\(^2\) This indicates that the cause of nondisjunction may not be the same between father and mother of Down syndrome, and in mothers it may relate basically to the metabolic factors that are counterpart with basic cellular biochemical functions.\(^24\) This data may contribute to extend the knowledge on mechanisms of chromosome nondisjunction and identifying important environmental and maternal health-related risk factors for chromosome nondisjunctions.

### Acknowledgment

This study was supported by project grants from National Research Center for Genetic

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Engineering and Biotechnology of the Ministry of Science, Research, and Technology in Tehran, Iran. We would like to thank cooperation of the mentally retarded care centers and schools personnel, patients and their families, and everybody who helped in taking blood samples. We also thank Nasrin Ataback, Mohammad Shahroei, and Shamila Darvishalipoor for their help.

References