A comparative analysis of the effectiveness of cytogenetic and molecular genetic methods in the detection of Down syndrome

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Abstract

The goal of this study was to examine the effectiveness of 6 STR markers application (D21S1435, D21S11, D21S1270, D21S1411, D21S226 and IFNAR) in molecular genetic diagnostics of Down syndrome (DS) and to compare it with cytogenetic method. Testing was performed on 73 children, with the previously cytogenetically confirmed Down syndrome. DNA isolated from the buccal swab was used. Previously mentioned loci located on chromosome 21 were simultaneously amplified using quantitative fluorescence PCR (QF PCR). Using this method, 60 previously cytogenetically diagnosed DS with standard type of trisomy 21 were confirmed. Furthermore, six of eight children with mosaic type of DS were detected. Two false negative results for mosaic type of DS were obtained. Finally, five children with the translocation type of Down syndrome were also confirmed with this molecular test. In conclusion, molecular genetic analysis of STR loci is fast, cheap and simple method that could be used in detection of DS. Regarding possible false results detected for certain number of mosaic types, cytogenetic analysis should be used as a confirmatory test.

KEY WORDS: Down syndrome, cytogenetic analysis, STR markers, QF PCR

INTRODUCTION

Down syndrome (DS) is the most common disorder that prevents normal physical and mental development of children. The higher incidence of DS is detected within group of children whose mothers were older than 35 at the time of conception [1]. It occurs as a result of chromosomal aberrations. The 95% of all detected DS types is typical chromosome trisomy 21, while 5% of detected DS could be described as "partial trisomy". It is caused by chromosome 21 translocation to another chromosome, usually some of acrocentric type. The lowest number of persons with DS have mosaic type that occurs in 2-3% of the cases [2]. Basic cytogenetically screening ("karyotyping") is accepted as standard diagnostic procedure for DS detection, as prenatal and postnatal diagnostics. Karyotyping is based on analysis of numerical and structural changes of all observed chromosomes. However, this method could not be used for detection of microdeletion and microduplication. In addition, karyotyping it time consuming method which requires significant time period for sampling, preparation and analysis of metaphase chromosomes. Therefore, last few years, with more or less success, various molecular-genetic analysis were introduced as alternative method. Detailed examination and extensive application of the microsatellite regions, especially short repetitive DNA sequences (*Short Tandem Repeat - STR*) promote this markers as possible solution as fast, accurate, cheap and simple genetic tool that could be used in DS detection [3]. Short tandem repeats (STRs) are easily typed, ubiquitous and polymorphic loci with high mutation rates. The tendency of the mass application of STR markers has clearly defined them as the molecular polymorphisms which are widely used in population, forensic and medical genetics. STRs are short sequences of DNA, normally of length 2-5 base pairs (according to some sources 2-10) that are repeated numerous times at the particular locus. Number of repetitions varies from person to person. The real value of the application of these markers lies in the simplicity and rapidity of the process and the possibility of simultaneously testing of a large number of STR markers in the so-called multiplex STR systems, enabling an extremely high degree of individualization in identifying biological evi-

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dence. Also, these sequences, in addition to its wide application in forensic DNA analysis, have become very attractive as a subject of genetic research from a medical point of view, because it could be performed on the bucal swab sample and it could be associated with certain genetic disorders [4]. For that reason, we have decided to test 6 previously described STR loci, located on the chromosome 21 as possible DS diagnostic method. Additional intention of this study was optimization of the existing protocols and detection of all advantages and disadvantages of STR loci application as possible fast and routine screening method in molecular diagnostics of Down syndrome.

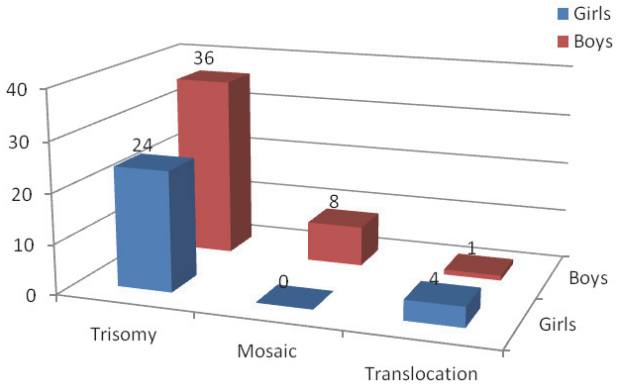

FIGURE 1. Frequency of Down syndrome by type syndrome and sex of the child

MATERIAL AND METHODS

Sample

This study included 73 individuals from the Bosnia and Herzegovina. All tested individuals were voluntary donors which parents gave consent in compliance with ethical norms set by international conventions. These 73 individuals were selected according the fact that, based on the phenotype, DS was suspected. Molecular genetic testing was performed after cytogenetic confirmation of Down syndrome.

Procedures

All the samples which underwent cytogenetic analysis were processed according to standardized protocols for processing and analysis of peripheral blood samples. Karyotype description is done according to the ISCN nomenclature (*International Standard Committee on Human Cytogenetics Nomenclature*) [5]. In order to perform molecular-genetic analysis the buccal swab samples were taken. Optimized salting out procedure by Miller [6] was used for DNA extraction. Six STR loci (D21S1435, D21S11, D21S1270, D21S1411, D21S226 and IFNAR) located on chromosome 21 (Table 1) were simultaneously amplified using quantitative fluorescence PCR (QF PCR). The total volume of polymerase chain reaction (PCR) was 15 µl. It contained 7.5 µl *2xQiagen Multiplex PCR Master Mix and 1.5* µl *Primer Pair Mix-21 Assay* (*Assay 21* consists of a primers system, 0.5 µl *Forward* and 0.5 µl *Reverse* concentration of 0.20 mM, and 19 µl of deionized water) with 6 µl of DNA sample. The PCR reaction consisted of 26 cycles, after heating for 15 minutes at 95°C, followed by denaturation at 94°C for 30 seconds, hybridization for 1:30 minutes at 57°C and elongation for 1:30 minutes at 72°C. The last cycle was extended for 20 minutes at 72°C. PCR reaction was performed on AB Gene Amp PCR System 9700 Thermal Cycler (Applied Biosystems). Following amplification, detection of results is carried with the DNA genetic analyzer *ABI 3130*.

TABLE 1. Characteristics of STR markers separated by capillary electrophoresis

STR marker	Labeling	Allele range (bp)	Chromosome location	Primer sequences
D21S1435	6-FAM	160-200*	21q21.3	F-CCCTCTCAATTGTTTGTCTACC** R-ACAAAAGGAAAGCAAGAGATTTCA
D21S11	6-FAM	225-280*	21q21.1	F- TTTCTCAGTCTCCATAAATATGTG** R-GATGTTGTATTAGTCAATGTTCTC
D21S1411	NED	256-340*	21q22.3	F-ATAGGTAGATACATAAATATGATGA** R-TATTAATGTGTGTCCTTCCAGGC
D21S1270	6-FAM	285-340*	21q21-q22.1	F-CTATCCCACTGTATTATTCAGGGC** R-TGAGTCTCCAGGTTGCAGGTGACA
D21S226	6-FAM	440-470*	21q22.1	F-GCAAATTTGTGGATGGGATTAACAG** R -AAGCTAAATGTCTGTAGTTATTCT
IFNAR	NED	370-410*	21q22.1	F- CATTTGATCTTAGCCATCTATTGC** R-ACTATGCAGCCATTTGAAAGACTA

*/ ** size of the fragment and primers [7]

RESULTS

Based on cytogenetic findings, 60 karyotypes of 73 children have a regular type of trisomy, five have translocation and eight a mosaic type DS (Figure 1.). For all of these children analysis of six STR loci on chromosome 21 was performed in order to determine the effectiveness of molecular genetic method in the detection of Down syndrome. In our study of 73 samples, molecular confirmation of trisomy of chromosome 21 was achieved in all children with a standard type of Down syndrome. We did not obtain electrophoregram in case of two samples which would confirm the cytogenetic diagnosis of Down syndrome. Both samples were from children with the mosaic type of Down syndrome (Figure 2.). For the remaining five samples, with the mosaic type of Down syn-

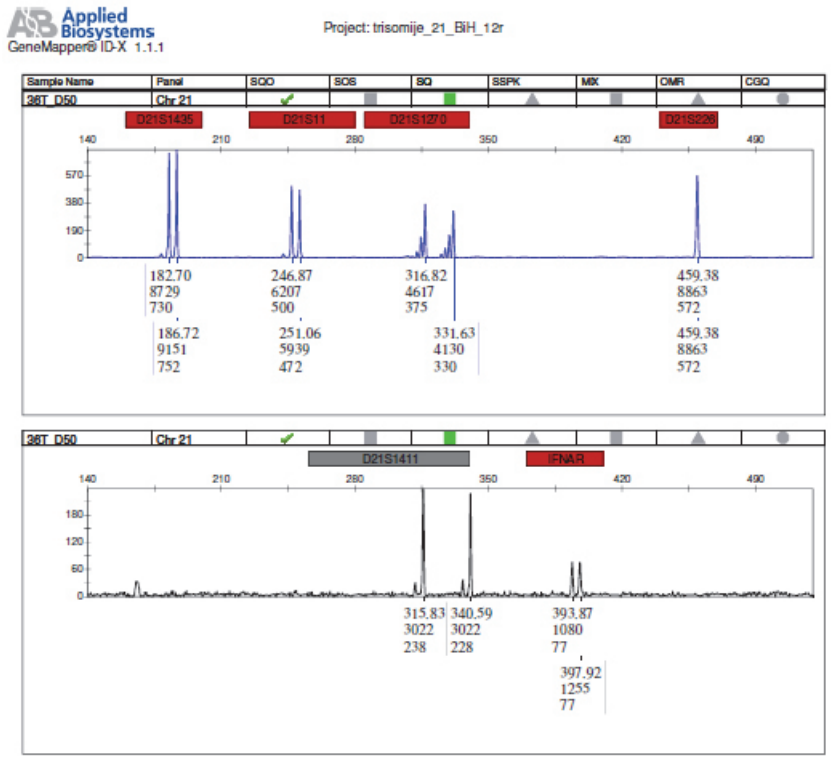

FIGURE 2. Electrophoregram of a child with mosaic type, which did not confirm the cytogenetic diagnosis of DS.

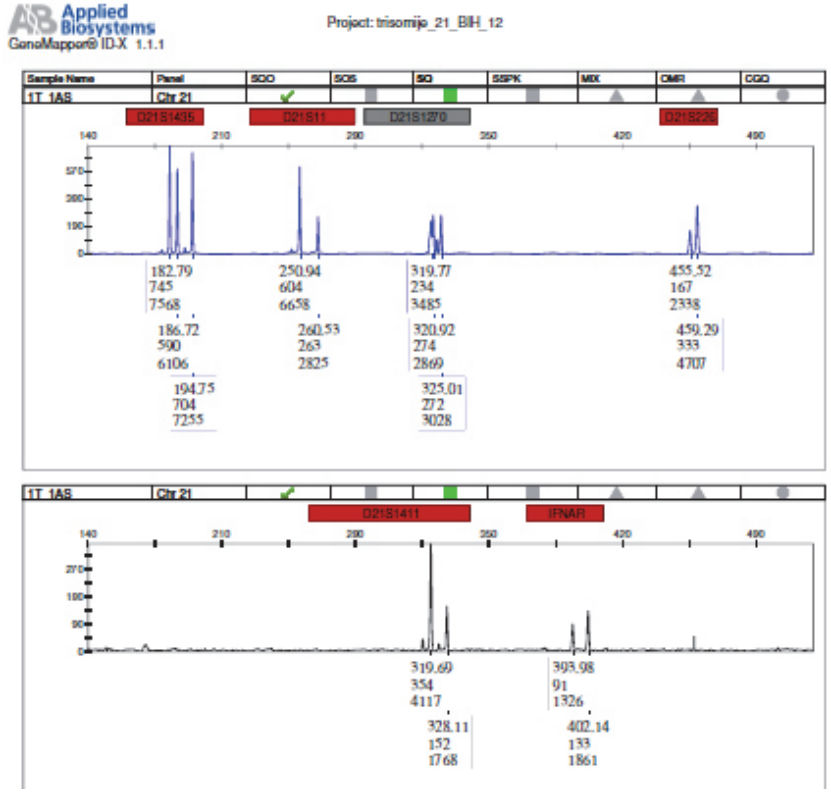

FIGURE 2. Electropherogram of a child with mosaic type of DS.

drome and five samples with translocation form of Down syndrome trisomy of chromosome 21 was confirmed with the molecular method (Figure 3.). Based on Table 2 it can be seen that the markers D21S1435, D21S11, D21S1270, D21S1411 and IFNAR are proven as a good polymorphic markers in the detection of chromosome 21 trisomy, because almost equal number of samples had three or two alleles (with the peaks in the approximate ratio of 2:1) at the above-mentioned loci. In contrast to them, the marker D21S226 was not informative for 19 samples because it had only one allele; however by the analysis of the remaining five STR markers we obtained informative results and confirmation of trisomy 21. Value of the peaks with two alleles was 2.65 - 1.14 for D21S1435; 2.53 - 1.38 for D21S11; 3.05 - 1.06 for D21S1270; 3.35 - 1.46 for D21S226; 2.49 - 1.52 for D21S1411 and 2.65 - 1.40 for IFNAR. Markers with the peak range less than 1.5 were not taken in the analysis [8, 9, 10]. In the nine samples, per one marker was identified, while one sample had two markers with a peak range less than 1.5. However, on the basis of the other five markers in all samples the trisomy was confirmed.

DISCUSSION

The expected evidence of the trisomy is the presence of three alleles with the peak ratio 1:1:1 and two alleles in an approximate peak ratio of 2:1 [11]. For more reliable diagnosis it is necessary to use at least three different STR markers located on the same chromosome [10]. One of the reasons for the use of a large number of STR markers for each chromosome lies in the fact that sometimes some of the markers are not informative because of homozygosity of parents or in cases where the parents have the same alleles [12, 13]. However, an excessive number of markers could lead to false positive results in the diagnosis. For

TABLE 2. Polymorphism and ratio of peak areas in trisomy 21 samples

STR marker	Triallelic samples (n)	Diallelic samples (n)	Monoallelic samples (n)	Ratio of peak areas in diallelic samples		
				Range	Mean	SD
D21S1435	25	38	7	2.65 - 1.14	1.81	0.32
D21S11	33	34	3	2.53 - 1.38	1.92	0.24
D21S1270	44	24	2	3.05 - 1.06	1.97	0.54
D21S226	5	46	19	3.35 - 1.46	2.05	0.35
D21S1411	36	31	3	2.49 - 1.52	2.08	0.24
IFNAR	28	40	2	2.65 - 1.40	1.99	0.28

these reasons, in this study 6 markers were used. According to Rahil et al. detection of two alleles, with the peaks in a 2:1 ratio, requires special caution especially for dinucleotide markers. Also, the size of the peaks can vary from one to another allele and also a small amplified PCR fragments can appear [14, 15]. One of the often present products are starters, small peaks of several bases. Height of the starter for standard, tetranucleotide repeated sequences is usually below 15% of the height of the corresponding peak in the standard amplification conditions. Forming of a starter depends on the length of the markers, sequence of STRs and the speed of DNA polymerase action [16]. This study used tetranucleotide markers, so there was no problems in obtaining readings from the two alleles, which peaks are in a 2:1 ratio (except in three samples at two markers that had almost equal peaks). It has been shown for all six markers to be suitable for the identification of trisomy 21, regardless the type of DS. In the 73 buccal swab samples, for 71 of them the trisomy 21 was molecularly confirmed. In the case of two children with mosaic type molecular analysis did not confirm the presence of the DS. One child had 22 % and another 8% of cells with trisomy 21. Other samples, in which the electrophoresis confirmed Down syndrome, had a higher percentage of mitosis in respect to normal karyotype. In the study conducted by Yoon et al. [17] on 23 samples with trisomy 21, only one sample had mosaicism of DS. For this sample the researchers obtained negative result by using quantitative fluorescent polymerase chain reactions (QF PCR) multiplex. This was explained by the small number of the cells with an excess of chromosomes 21. They concluded that the larger number of samples is needed to determine the degree of QF PCR test reliability, when it comes to the samples with a low percentage of mosaicism [17, 18]. However, cases of mosaicism which are analyzed to date using the PCR-STR is definitely insufficient to make a final conclusion of the necessary number of the cells and the method sensitivity [3]. In recent studies, analysis of STR markers proved to be fast, simple and extremely sensitive method (only 50 ng of genomic DNA is needed) for detecting trisomy and requires less sample for analysis in relation to cytogenetic methods [19]. STR loci most commonly used in molecular diagnostics detect most frequent trisomies (13, 18, 21, X and Y). The majority of structural and numerical changes occur on these chromosomes, but also changes occur on other chromosomes, for which additional STR markers, which would increase the time of analysis, as well as the cost of chemicals, are needed. Thus, the lack of this type of analysis is strict specificity of applied analysis to only certain (targeted) type of aberrations. Although cytogenetic analysis cannot detect microdeletions and microduplications on chromosomes and the analysis itself takes longer than the analysis of STR markers, it is still the gold standard in diagnosis. Therefore it should be used together with the molecular analyzes, which could be recognized as preliminary test for trisomy as well as confirmation of cytogenetic analysis [20].

CONCLUSION

Based on the results, the application of the STR markers in the identification of DS presents a simple, fast and inexpensive molecular technique. However, with the application of molecular method itself, there is a reduced chance for detection of mosaicism. Therefore, in the initial stage it may provide auxiliary, but also strong additional confirmation to standard cytogenetic assays. Combined use of these methods would significantly contribute to improving the quality of work of cytogenetic laboratories in the diagnosis of chromosomopathy.

DECLARATION OF INTEREST

The authors declare no conflict of interest

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