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# Rapid Prenatal Diagnosis of Numerical Aberrations of Chromosome 21 and 18 by PCR-STR Method

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## ABSTRACT

*In this study we reported the results for the first time of applying Polymerase Chain Reaction-Short Tandem Repeats (PCR-STR) method in the field of detection of aneuploidies for chromosomes 21 and 18 in Croatians. The aims of the study were: (I) validation of the diagnostic informativeness of 6 STR loci (D18S51, D18S858, D18S535, D21S1435, D21S1411, and D21S1414) in sample of 205 unrelated healthy individuals; (II) evaluation of diagnostic power of the PCR-STR method for those 6 microsatellites; (III) establishment protocol for use STRs as routine method for rapid prenatal detection of trisomy 21 and 18. DNA samples were amplified by fluorescence-based PCR reaction, subjected to electrophoresis in automated laser fluorescence DNA sequencer (ALFexpress). Results of our study were: (I) all 6 tested loci are informative (68–85% of heterozygous individuals); (II) comparison between PCR-STR method and conventional cytogenetics did not revealed any false positive or false negative results; (III) in prenatal screening of 105 samples of uncultured amniotic fluid 6 (5.7%) samples with chromosomal abnormalities were identified.*

**Key words:** aneuploidies, PCR, prenatal diagnosis, STR, Croatia

## Introduction

Prenatal diagnosis of chromosomal aneuploidies is routinely accomplished by standard cytogenetic techniques. Prenatal diagnosis of trisomy 21 and 18 is usually performed using *in vitro* cultures of amniocytes or chorionic cellular cells, followed by the analysis of metaphases stained by conventional procedures<sup>1,2</sup>. The main disadvantage of these procedures is that definitive results can be obtained only after 2–3 weeks of *in vitro* cultures which delays the time of diagnosis and requires high costs.

During 1978–2001, a total of 17 004 routine prenatal cytogenetic diagnosis have been performed in our Department and 314 (2.00%) aneuploidies were detected. Trisomy 21 was detected in 181 (1.06%) cases, 54 (0.32%) cases had trisomy 18 and 11 (0.07%) cases of aneuploidy was detected as trisomy 13<sup>3</sup>.

An alternative rapid method which is suitable for diagnosing trisomies, fluorescent polymerase chain reaction using polymorphic short tandem repeats (STRs) have been proposed<sup>4–7</sup>. Namely, the human genome con-

tains many repeated DNA sequences, which can be widely dispersed or in short tandem arrays. STRs are short sequences of DNA, usually 2–5 base pairs in length that are repeated numerous times.

Aims of this study were: (I) to validate the diagnostic informativeness of 6 STR loci (D18S51, D18S858, D18S535, D21S1435, D21S1411, and D21S1414) in a sample of 205 unrelated healthy individuals; (II) to evaluate diagnostic power of the PCR-STR method for those 6 microsatellite loci; (III) to establish protocol for usage of PCR-STR as routine method for rapid prenatal detection of trisomy 21 and 18.

## Materials and Methods

Genomic DNA, from 205 unrelated healthy individuals for population study, was isolated from peripheral blood leukocytes by NucleoSpin Blood isolation kit<sup>8</sup>.

Six hundred and thirty-four amniotic fluid samples were previously tested by conventional cytogenetic analysis. In this group, twenty-two trisomic samples (trisomy 21 or trisomy 18) were also analysed by PCR-STR method. DNA of these samples was isolated from cultured cells by the same isolation kit.

One hundred and five amniotic fluid samples were tested blind using PCR-STR method. Samples were collected from pregnant women who underwent amniocentesis for prenatal chromosome analysis between gestational weeks 16 and 20. The referral criteria were maternal age (38 years), positive biochemical screening for Down syndrome and abnormal fetal ultrasonographic scan. DNA from 5 ml uncultured amniotic fluid was also extracted using NucleoSpin Blood isolation kit.

Amplification of samples was performed in a total volume of 11.5 µl in Mastercycler gradient thermocycler (Eppendorf-Germany). The primers' sequences were as follows: for D18S51 5'gagccatgttcatgccactg, 3'caaaccgactaccagcaac; for D18S858 5'agctggagaggatagcatt, 3'tgcattgcatgaaagttaga; for D18S535 5'tcatgtgacaaa-gccacac, 3'agacagaatatagatgagaatgca; for D21S1435 5'ccctctcaattgttctacc, 3'gcaagagatttcagtccat; for D21S1411 5'atgatgaatgcatagatggatg, 3'ccagccctctaaatcatcc; and for D21S1414 5'aaattagtgtctggcaccagta, 3'caattcccaagtgaattgctctc. The PCR amplification conditions were the following: for D18S51, D18S535 and D21S1435: 95°C-30"; 53°C-1'; 72°C-30" (35x); for D18S858, D21S1411, and for D21S1414: 94°C-30"; 55°C-1'15"; 72°C-15" (27x).

After amplification, the PCR products (1.5 µl) were mixed with 3 µl of loading buffer and 1 µl of each of two internal size markers. On each gel we also included one external, commercial sizer. Electrophoresis was performed using a 6% standard denaturing polyacrylamide gel in an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia Biotech, Uppsala, Sweden). The amplification products were analysed and their relative fluorescent intensities calculated using Fragment Manager software (Pharmacia Biotech). The patterns obtained by the analysis were the following: for the normal subjects two peaks with 1:1 ratio or one single peak; for trisomic cases three peaks with ratio 1:1:1 or two peaks with 2:1 ratio or single peak in the case of homozygosity. Polymorphism information content (PIC) value was obtained as suggested by Hearne<sup>9</sup>.

## Results and Discussion

Population study (N=205) confirmed that all six STR loci are informative in the Croatian population, which is also the case for the other populations so far reported<sup>10-13</sup>.

The observed frequencies of heterozygotes are in concordance with the expected frequencies for all tested loci, PIC value was also calculated and revealed that these systems have a high PIC value (Table1). With respect to the observed heterozygosity and PIC value they can be used in detection of aneuploidies. However, this and previous studies also showed that STR loci have a relatively

**TABLE 1**  
STATISTICAL PARAMETERS FOR STR MARKERS D18S51, D18S535, D18S858, D21S1435, D21S1411 AND D21S1414 OBTAINED FROM POPULATION STUDY (N=205)

Marker	Ob.H	Ex.H	PIC
D18S51	0.81	0.90	0.91
D18S535	0.74	0.77	0.78
D18S858	0.68	0.83	0.82
D21S1435	0.76	0.96	0.77
D21S1411	0.85	0.99	0.89
D21S1414	0.76	0.93	0.93

Ob.H – observed heterozygosity, Ex.H – expected heterozygosity, PIC – polymorphism information content

high instability of their alleles and a high level of mutation in comparison with other genetic markers<sup>14</sup>. For that reason, the occurrence of the intermediate alleles (differ by 2bp instead of 4bp) is frequent and it can lead to problems in the case of distinguishing heterozygosity and homozygosity. Therefore we used 3 STR loci for each chromosome.

Among 634 samples of amniotic fluid successfully processed, trisomies 21 were diagnosed by standard cytogenetic methods in 19 cases while trisomy 18 was observed in 3 cases. For each amniotic fluid sample with trisomy, we also performed amnio-PCR analysis by amplifying STR markers on chromosome 21 and chromosome 18, respectively. The results of testing the samples with trisomy 21 using three STR loci (D21S1435, D21S1411, and D21S1414) are shown in Table 2. In diagnosis of Down's syndrome the most informative were D21S1414 and D21S1435 loci, while at the same time D21S1411 showed homozygosity in three cases. Diagnosis of Edward's syndrome was always successful with D18S51 and D18S535 loci, while D18S858 locus was not informative in one case. Comparison study between conventional cytogenetic analysis and PCR-STR method in a sample of 22 amniotic fluids did not reveal false positive or false negative results.

**TABLE 2**  
RESULTS OF TRISOMIC SAMPLES TESTED FOR TRISOMY 18 AND TRISOMY 21 PARALLEL BY PCR-STR METHOD AND CLASSICAL CYTOGENETIC

Samples (N=22)	STR locus	Triallelic pattern (No)	Diallelic pattern (No)	Monoallelic pattern (No)
Trisomy 18 (N=3)	D18S51	2	1	0
	D18S535	1	2	0
	D18S858	1	1	1
Trisomy 21 (N=19)	D21S1435	11	8	0
	D21S1411	8	8	3
	D21S1414	14	5	0

No – number of occurrence

**TABLE 3**  
DATA FOR 6 SAMPLES WITH AUTOSOMAL ANOMALIES IN SCREENING OF 105 AMNIOTIC FLUIDS

Trisomy 18 (n=1)		Trisomy 21 (n=4)					Triploidy (n=1)	
Locus	Sample	Locus	Sample				Locus	Sample
	1		2	3	4	5		6
D18S51	1:1:1	D18S51	1:1	NI	1:1	1:1	D18S51	1:1:1
D18S535	2:1	D18S535	1:1	1:1	1:1	1:1	D18S535	1:1:1
D18S858	2:1	D18S858	NI	1:1	1:1	NI	D18S858	1:1:1
D21S1435	1:1	D21S1435	1:1:1	2:1	1:1:1	NI	D21S1435	1:1:1
D21S1411	1:1	D21S1411	2:1	1:1:1	1:1:1	NI	D21S1411	1:1:1
D21S1414	1:1	D21S1414	2:1	1:1:1	1:1:1	1:1:1	D21S1414	1:1:1

1 – normal monoallelic, 1:1 – normal diallelic, 2:1 – trisomic diallelic, 1:1:1 – trisomic triallelic, NI – not informative

We performed prenatal screening of 105 amniotic fluid samples taken from women who matched one of the three criteria listed in Material and Methods. Among these samples, 6 (5.7%) autosomal anomalies were identified by PCR-STR method (Table 3). All anomalies were also subsequently verified by conventional karyotyping. Trisomy 21 was diagnosed in four cases; in one case we detected trisomy 18, while the last anomaly was shown to be triploidy. There were also no false positive or false negative results in comparison to classical cytogenetics. However, from our experience PCR-STR cannot completely replace conventional karyotyping due to the possible chromosomal abnormalities other than most common trisomies. On the other hand, conventional karyotyping ideally takes 3–4 weeks but due to the limited resources available for this procedure in Croatia, it can last up to 5 weeks. Use of PCR-STR method as a screening procedure can give results within 3 days and therefore reduce parental anxiety but also can point out which samples should be given priority in standard procedure, namely, those cases where the PCR-STR analysis has discovered an aneuploidy. This is necessary because our protocol does not follow the policy of terminating the pregnancy on the base of the PCR-STR analysis results without waiting for the conventional cytogenetic confirmation as is the case in some other centres.<sup>15</sup> In this aspect, our protocol is similar to protocols suggested by Andonova et al.<sup>16</sup> We would also like to emphasize that compared to the other fast reporting technique for analysis of amniotic fluid – interphase fluorescence in situ hybridisation, PCR-STR method is less labour intensive.

At the same time, it is important to mention that PCR-STR technique can not always detect mosaics, translocations and deletion or duplication syndromes which is possible to detect by conventional cytogenetic analysis. The use of a larger panel of STR markers which cover wider regions of chromosome as well as those located on the other key chromosomes will improve the detection rate of PCR-STR technique in future.

Furthermore, one of the advantages of this method is also the possibility of determination of maternal or paternal origin of chromosomal aneuploidies<sup>17</sup>. During our

study we had one family with three children, two of them had trisomy 21. Molecular analysis showed that one child inherited supernumerary chromosome 21 from mother, while second child with Down's syndrome inherited supernumerary chromosome 21 from father.

This investigation also included one case of mosaicism involving chromosome 18. When tested by D18S535 this sample showed unexpected triallelic pattern (all peaks were not equal – 3.5:1:3.5), while the rest of two STR loci for chromosome 18 showed two allelic pattern also with unusual ratio of two peaks. This result is in good concordance with data from Ogilvie et al. (2005) who reported that PCR-STR can identify mosaicism at level greater than 15%<sup>18</sup>. In our sample, the percentage of mosaicism was 20%. When we compared this method to the chromosome analysis and karyotyping on cultured amniocytes, it proved to be simpler and less expensive which is in concordance with data from several other groups of authors.

Namely, the present study confirmed the results of other, much larger studies demonstrating the usefulness of PCR-STR method for detection of common autosomal aneuploidies (Down's and Edwards' syndrome). Nicolini summarized the results from many authors with different views of what might be the role of PCR-STR technique in prenatal diagnostics<sup>19</sup>. Almost all of them characterised this method as being an economic and rapid technique. More recently, Ogilvie (2003), Leung et al. (2003) and Leung et al. (2004) suggested that the advantages of PCR method clearly outweigh the disadvantages and their suggestion was the replacement of conventional prenatal cytogenetic analysis with PCR-STR method after positive serum screening for Down's syndrome<sup>20–22</sup>. Mann et al. suggested that PCR-STR technique could be also used as a stand-alone test in cases of advanced maternal age<sup>23</sup>. In conclusion, this study demonstrated that trisomy 21 and 18 can consistently be identified by PCR-STR. Method is fast, sensitive and it is possible prenatal identification of the two most frequent trisomies within 3 days after collecting samples. Our set of STR loci showed to be sufficient for diagnosis of Down's and Edward's syndrome.

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## BRZA PRENATALNA DIJAGNOSTIKA ANUPLOIDIJA 21 I 18 POMOĆU METODE PCR-STR

### SAŽETAK

U ovom istraživanju su po prvi puta prikazani rezultati analize trisomija kromosoma 21 i 18 primjenom metode PCR-STR (lančana reakcija polimerazom – kratka uzastopna ponavljanja) u hrvatskoj populaciji. Ciljevi ovog rada bili su: (I) Istražiti informativnost 6 lokusa STR (D18S51, D18S858, D18S535, D21S1435, D21S1411 i D21S1414) na uzorku od 205 zdravih nesrodnih osoba; (II) Ocijeniti dijagnostičku vrijednost metode PCR-STR za navedene mikrosatelitske lokuse; (III) Razviti protokol za brzo otkrivanje trisomija 21 i 18 primjenom metode PCR-STR kao rutinske metode u prenatalnoj dijagnostici. Uzorci DNA amplificirani su metodom PCR, dužina alela lokusa STR određena je elektroforezom u automatskom laser sekvenceru (ALFexpress). Rezultati istraživanja su: (I) Svih 6 lokusa STR su informativni (68–85% osoba su bili heterozigoti); (II) Usporedba metode PCR-STR s metodom klasične citogenetike nije pokazala ni lažno pozitivne, kao ni lažno negativne rezultate; (III) Prenatalnim pretraživanjem 105 uzoraka nekultiviranih plodovih voda otkrivena je promjena u broju kromosoma kod 6 (5,7%) uzoraka.