HLA-DR TYPING OF GENOMIC DNA

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ABSTRACT: Advances in molecular biology techniques allowed for introduction of PCR- based methods for HLA typing. Due to PCR – based DNA amplification genetic identification has become available. The aim of this paper was to present the results of investigation high polymorphic HLA-DR locus by PCR-SSP method. Blood and tissues samples (muscles and brains) were obtained from 50 corpse adult and bimonthly fetus from North part of Poland. The post-mortem periods ranged from 2 to 7 days. DNA was extracted also from older – two years tissues. Genomic DNA was isolated by two methods: using phenol/chloroform extraction and "Easy Tissue DNA Prep." kit (A&A Biotechnology). HLA-DR alleles were typed using DYNAL DR " low resolution" SSP kit. PCR products were separated on agarose gel with ethidium bromide and estimated in ultraviolet light. Phenotypic and genotypic rates of blood and tissues has been analysed. In our materials 6 alleles were detected. The high frequency of HLA-DR 6 and HLA-DR 5 alleles have been found. Rare HLA-DR 1 allele was observed. We found PCR-SSP HLA typing method very useful in routine HLA-DR identification.

KEY WORDS: HLA-DR; PCR-SSP; HLA class II.

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INTRODUCTION

Resent advances in the area of molecular biology and especially introduction of polymerase chain reaction (PCR), made possible a wide application of this technique in forensic medicine [3, 5, 6]. Due to PCR-based DNA amplification, genetic determination and identification has become available. The development of PCR method has made it possible to amplify HLA-DR locus.

HLA class II (human leukocyte antigens) are polymorphic cell surface heterodimers encoded by the human major histocompatibility complex that pay a key role in the control of immune responses in transplantation immunology and in the susceptibility to autoimmune diseases. The gene encoding the HLA molecules are the most polymorphic in the human genome. The gene class II divided into HLA-DR, DQ and DP. Allele of the extreme polymorphic DR1 locus were employed in forensic medicine area for analysis of

evidence genetic material. There are a few HLA-DR allele typing methods: PCR-RFLP, PCR-SSO and PCR-SSP.

The aim of our study was to present the results of investigation high polymorphic HLA-DR locus by PCR-SSP method in human tissues [1, 2, 4].

MATERIAL AND METHODS

Samples and DNA extraction

DNA was obtained from 50 blood samples and tissues (muscles and brains) from adults corpse and bimonthly fetus from north part of Poland.

The post-mortem periods ranged from 2 to 7 days. DNA was extracted also from older – two years tissues. DNA was isolated from post-mortem muscles and brains to obtain data on the influences of autolysis and putrefaction on the stability and quantity of genomic DNA. Specimens of the muscle and the brain were collected from accident victims subjected to autopsy in the Institute of Forensic Medicine in Gdańsk. This work is continuation of previous population researches of the high polymorphic HLA-DR system in the 200 blood samples taken from unrelated persons living in northern Poland. HLA-DR alleles were typed using DYNAL DR "low resolution" SSP kit.

Genomic DNA was isolated by two methods: using phenol/chloroform extraction [7] and "Easy Tissue DNA Prep." kit (A&A Biotechnology) according to the original protocol.

A small piece of tissue (or 500 μl blood sample) was put into the TE Buffer (0,5 M/l TRIS; 0.02 M/l EDTA; 0.01 M/l NaCl, pH 9). Next proteinase K (final concentration 0.625 mg/ml, Sigma) and sodium dodecylsulfate (final concentration: 1%) were added. The solution were incubated overnight at 56°C. The proteins were extracted from DNA twice with phenol/chloroform/isoamylalcohol (25:24:1). DNA was precipitated by adding 1/10 vol. of 3 M sodium acetate (pH 5.2) and 2 vol. of absolute ethanol. The sample was stored overnight at –20°C. After drying DNA was dissolved in 100–200 μl 10 mM Tris (pH 8.5) and was stored at 4°C. DNA yield was determinated with a spectrophotometer by reading the absorbance at 260 nm or with a fluorometer.

PCR amplification

Molecular HLA typing was performed using PCR-SSP technology (Dynal-DR "low resolution"-SSP Dynal). One HLA-DRB typing procedure included 24 PCR reactions with 24 pair of primers specific for HLA alleles or groups of alleles and the internal positive control primer pair for gene fragment of human growth factor.

Typing was performed according to manufacturer's protocol with a small own modification. In brief: each PCR reaction mixture (8 μl) consisted of PCR buffer (50mM Tris-HCl, pH 8.0; 100mM NaCl; 0.1mM EDTA; 1 mM DTT, 50% glycerol; 1% TritonX-100 – Promega), 200 μM each dNTP, 25 mM $MgCl_2$, 0.4 U Taq Polymerase (Promega), genomic DNA (30–50 ng) and one of 24 Dynal's mixture of specific primers (1 μM). PCR amplifications were carried out in a thermocycler (Mastercycler Gradient).

Amplification cycles were performed in following temperatures:

– Preliminary denaturation – 94°C, 2'.
– I phase 10 cycles: denaturation – 94°C, 10"; annealing and extension – 65°C, 60".
– II phase 20 cycles: denaturation – 94°C, 10'; annealing – 61°C, 50"; extension – 72°C, 30".

Electrophoresis

PCR products were separated on 1.5% agarose gel with ethidium bromide and visualised under UV illumination. HLA-DR, PCR-SSP specificities are presented in table I (according to Dynal's interpretation). Phenotype and genotype rates of the blood and tissues has been analysed.

RESULTS AND DISCUSSION

Both of the DNA extraction methods phenol/chloroform extraction and using "Easy Tissue DNA Prep. " kit (A&A Biotechnology) gave good results. It was possible to obtain high molecular DNA from all analysed tissues. Concentration of DNA samples was determinated 300-500 ng/μl, but it was 10 times dissolved to PCR reaction. HLA-DR phenotypes were defined in the all samples of DNA.

The examples of HLA-DR phenotypes are presented on the Figure 1.

In the genomic materials basis of this study carried out on tissues taken from 50 corpse and bimonthly fetus 14 phenotypes were observed. To the most frequent observed belong 6/6 and 5/6 appearing with frequency about 25% and 18% respectively. Allelic arrangement 2/2, 2/3, 2/7, 6/7, 7/7 are rather rare. Table II shows frequencies of detected phenotypes.

In the tested tissue's material 6 alleles were observed. To the most frequent alleles in the HLA-DR system belong HLA-DR6 and HLA-DR5 (47.1%; 22.5% respectively). The most rare allele is HLA-DR1, appearing with frequency 2%.

TABLE I. HLA-DR SPECIFICITIES TYPING BY PCR-SSP METHOD

HLA DR		Size of PCR products (bp)	Amplified specificities
DR 1		200	DRB1*0101, *0102, *0104
DR 103		200	DRB1*0103
DR 2	DR 15	210	DRB1*1501–1504
	DR 16	220	DRB1*1601–1606
DR 3	DR 17	220	DRB1*03011–0304, *03012
	DR 18	180	DRB1*0301, *0302, *0303
DR 4		260	DRB1*0401–0419
DR 5	DR 11	170	DRB1*1102, *1103, *1106, *1107, *1109, *11011–1113, *11042
	DR 12	180	DRB1*1201–1203
DR 6	DR 13	200	DRB1*13011301–1304, *1305,* 1306, *1307, *1308–1310, *1311, *1312, *1313
	D 14	140	DRB1*1401, *1402, *1403, *1404, *1405, *1406, *1407, *1408, *1409, *1410, 1411*, *1412, *1414, *1415, *1416, *1417
DR 7		180	DRB1*0701
DR 8		170, 220	DRB1*0801–0811
DR 9		190	DRB1*09011, *09012
DR 10		150	DRB1*1001
DR 52		240	DRB3*0101–0301
DR 53		250	DRB4*01011–0103
DR 51		150, 170	DRB5*0101–0203

Remaining parameters characterising the usefulness of HLA-DR locus for identification studies were compared below:

– Matching probability – 0.137;
– PIC – 0.66;
– Heterozygotes – 62.7%.

Molecular PCR-SSP method allows for very rapid and highly discriminatory HLA analysis. In conclusion, we found PCR-SSP HLA technique very useful in routine HLA-DR identification, because of its simplicity procedure as well as very short typing time.

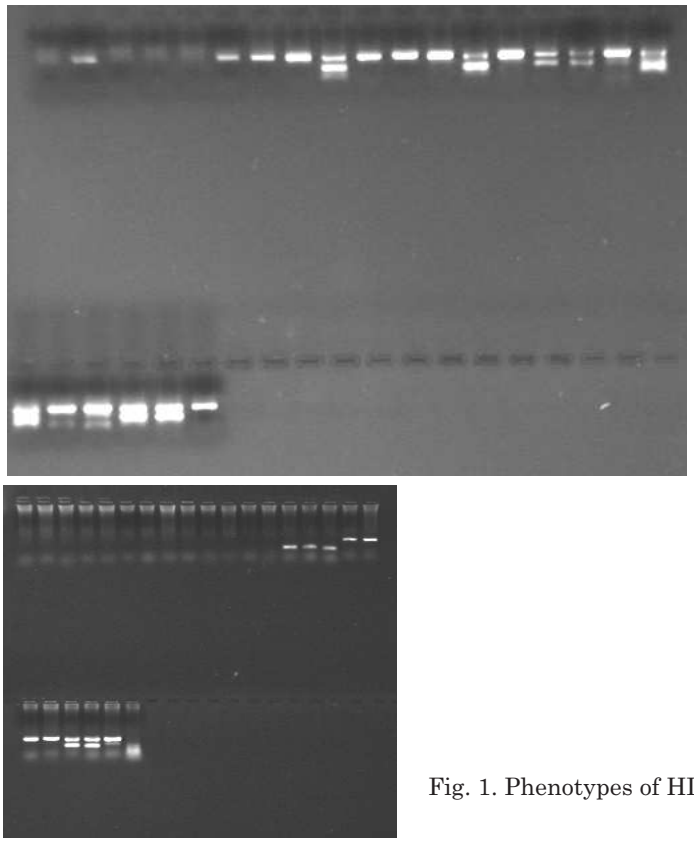

Fig. 1. Phenotypes of HLA-DR locus.

TABLE II. FREQUENCIES OF HLA-DR PHENOTYPES

Phenotype	Count (n) [%]	Frequency
1/6	2	3.920
2/2	1	1.960
2/3	1	1.960
2/6	7	13.725
2/7	1	1.960
3/5	2	3.920
3/6	3	5.882
3/7	2	3.920
5/5	4	7.843
5/6	9	17.647
5/7	4	7.843
6/6	13	25.490
6/7	1	1.920
7/7	1	1.920

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