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# A Study of Genetic Polymorphisms of HLA-class I and II Genes Using Polymerase Chain Reaction

**Kyung-Ok Lee**†

*Seoul Medical Science Institute (SCL), Seoul 140-230, Korea;*
*Department of Chemistry, Kon-Kuk University*

**Abstract:** The HLA genes located in the short arm of chromosome 6 specify heterodimeric glycoproteins involved in the regulation of the immune response. Recently, in the elucidation of HLA polymorphism, serological and cellular typing methods have been replaced by DNA typing using polymerase chain reaction (PCR). The purpose of this study was to establish the HLA DNA typing methods and determine gene frequencies of HLA molecules in Koreans. PCR-SSP (sequence specific primers) and PCR-RFLP (restriction fragment length polymorphism) techniques were used for the analysis of HLA-A, -B, -C, DRB1 genes and HLA-DQA1, DQB1, DPB1 genes, respectively. The results of B-lymphoblastoid cells used for control experiment were consistent with the previous data identified in the 11th International Histocompatibility Workshop. Seventeen, 23, 16, 8, 16, 13 and 37 types of HLA-A, B, C, DQA1, DQB1, DPB1 and DRB1 alleles were found, respectively, in a total of unrelated 120 Korean individuals. The most frequent HLA alleles were A*02 (27.0%), B*40 (17.6%), Cw*01 (19.2%), DQA1*0301 (32.1%), DQB1*0303 (12.9%), DPB1*0501 (31.3%) and DRB1*1501 (9.2%) among Koreans. This study shows that DNA typing method using PCR technique is a relatively simple, fast and practical tool for the determination of the HLA-class I and II genes. Moreover, the data of HLA gene frequencies could be useful for the Korean database before clinical applications, including organ and unrelated bone marrow transplantation, anthropological study, disease association and individual identification.

**Key Words:** HLA-class I and II genes, DNA typing, Gene frequency

## INTRODUCTION

The Human Leukocyte Antigen (HLA) genes located in the short arm of chromosome 6 are mainly consist of HLA-class I (HLA-A, -B, -C) and class II (-DQA1, -DQB1, -DRB1, -DPB1) genes. HLA class I genes are highly polymorphic glycoproteins expressed on the surface of most nucleated cells and HLA class II genes are confined to the membrane-distal domain, encoded by the second exon of the respective gene[4]. These highly polymorphic HLA molecules bind to foreign or self antigenic peptides and present them to antigen-specific T and B cells in a self-restricted fashion and specify heterodimeric glycoproteins involved in the regulation of the immune response[21]. Accurate identification of their polymorphisms is a prerequisite for determination of the functional role

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†Corresponding author: 이경옥, 서울의과학연구소, 서울 용산구 동빙고동 7-14 39호 Tel: 02) 7906-500, Fax: 02) 7906-509, 517

of HLA including tissue and marrow transplantation immunity, susceptibility assessement of autoimmune diseases[7], individual identification and the development of synthetic vaccines[25]. However small genetic differences among HLA-class I and class II alleles make it difficult to do accurate HLA typing.

The tissue typing for HLA genes up to now has been by serological typing, namely the microlymphocytotoxicity assay[21]. This method requires a large panel of human alloantisera, each with single or multiple specificities for HLA antigens. Sources of reagents are limited and not generally renewable, and usually several antibodies are required to assign each antigen due to the complex patterns of serological cross reactivity between individual HLA determinants[17]. Recently, in the elucidation of HLA polymorphism, serological, cellular and biochemical typing methods have been replaced by genetic typing using polymerase chain reaction (PCR) which has many advantages[23]. Several DNA based typing methods for HLA genes - PCR-SSO (sequence specific oligonucleotde probe)[24], PCR-RFLP (restriction fragment length polymorphism)[13~16], PCR-SSP (sequence specific primers)[4,10] and sequencing based typing[18] have been introduced. In this study, HLA-class I and class II DNA typing methods were developed by using PCR techniques, PCR-RFLP and PCR-SSP. Gene frequencies of HLA-class I and class II genes in a Korean population were determined by DNA typing methods for clinical application.

## MATERIALS AND METHODS

### Study Subjects and Cell lines

One hundred twenty randomly selected healthy Korean individuals from Seoul Medical Science Institute were investigated for HLA DNA typing. For the control of experiment, homozygous control cell lines from the XIth International Histocompatibility Workshop were used.

### Synthesis of Sequence Specific Primers

Amplification primers were synthesized using a 392 DNA/RNA synthesizer and purified by oligonucleotide purification cartridge (Applied Biosystem. U.S.A.). Primers of Table 1, 2, 3 and 8 were synthesized from reference 7. The specificity of each individual primer synthesis was tested against the homozygous cell lines[11] as described in Table 9 and 10.

### Extraction and Amplification of DNA

High molecular weight DNA was isolated from proteinase-K treated peripheral blood leukocytes by phenol/chloroform extractions and ethanol precipitation[9]. For PCR-SSP reactions of HLA-A, B, C and DRB1 DNA typing, 40, 39, 26 and 31 PCR primers were synthesized by DNA/RNA synthesizer (Applied Biosystem Co., Foster city, USA). The PCR reaction mixtures containing 1 μM of each primer, 200 μM of dNTPs and 2.5 mM of $MgCl_2$ were subjected to 30 cycles for 30 sec at 94℃, 1 min at 60℃ and 30 sec at 72℃ by automated thermal cycler (GeneAmp PCR system 9600. Perkin Elmer Cetus Inc.). For the PCR-RFLP analysis of HLA-DQA1, DQB1, and DPB1 DNA typing, 5, 7 and 5 restriction enzymes were used, respectively. The restriction enzymes and their cutting sizes to specific amplified gene products, DQA1, DQB1 and DPB1, were shown in Table 5, 6 and 7, respectively. DNA without primer was used as a negative control for PCR analysis. PCR products were visualized by agarose gel in PCR-SSP assay. The restricted fragments of DNA in PCR-RFLP assay were analyzed by polyacrylamide gel elecrophoresis. Gels were examined under UV illumination and documented by photography.

## RESULTS

For the determination of accuracy of HLA DNA typing methods, B-lymphoblastoid homozygous cell lines which were distributed by 11th

**Table 1.** Primer sequences for HLA-A DNA typing

| Primers | Nucleotide sequence (5'-3') | Length |
|---|---|---|
| Coding Primers | | |
| AL#4 | GGA GTA TTG GGA CCG GAA C | 19 |
| AL#6 | ACG GAA TGT GAA GGC CCA G | 19 |
| AL#7 | AGC GAC GCC GCG AGC CA | 17 |
| AL#8 | GGC CGG AGT ATT GGG ACG A | 19 |
| AL#10 | GAT AGA GCA GGA GAG GCC T | 19 |
| AL#11 | TCA CAG ACT GAC CGA GAG AG | 20 |
| AL#12 | CCC GGC CCG GCA GTG GA | 17 |
| AL#13 | GTG GAT AGA GCA GGA GGG T | 19 |
| AL#16 | GGA CCA GGA GAC ACG GAA TA | 20 |
| AL#17 | CCG GAG TAT TGG GAC CTG C | 19 |
| AL#22 | CCA CTC CAT GAG GTA TTT CTT | 21 |
| AL#24 | CAC GCA GTT CGT GCG GTT T | 19 |
| AL#25 | TGA GGT ATT TCT ACA CCT CCA | 21 |
| AL#28 | GCG ACG CCG CGA GCC G | 16 |
| AL#30 | CGG AAT GTG AAG GCC CAC T | 19 |
| AL#31 | CGG AAT GTG AAG GCC CAG T | 19 |
| AL#32 | CCG AGT GGA CCT GGG GAC | 18 |
| AL#34 | ACT CAC AGA CTG ACC GAG C | 19 |
| AL#35 | AGG ATG GAG CCG CGG GCA | 18 |
| AL#37 | TCC TCG TCC CCA GGC TCT | 18 |
| AL#54 | GAA GGC CCA CTC ACA GAC TA | 20 |
| Non-coding primers | | |
| AL#C | ATG TAA TCC TTG CCG TCG TAA | 21 |
| AL#D | CAC TCC ACG CAC GTG CCA | 18 |
| AL#F | AGC GCA GGT CCT CGT TCA A | 19 |
| AL#G | CCG TCG TAG GCG TGC TGT | 18 |
| AL#H | CCA AGA GCG CAG GTC CTC T | 19 |
| AL#I | CTC TCT GCT GCT CCG CCG | 18 |
| AL#L | CAA GAG CGC AGG TCC TCG | 18 |
| AL#Q | CCT CCA GGT AGG CTC TCA A | 19 |
| AL#R | CCT CCA GGT AGG CTC TCT G | 18 |
| AL#V | GAG CCA CTC CAC GCA CGT | 18 |
| AL#Y | CCG CGG AGG AAG CGC CA | 17 |
| AL#Z | AGG TAT CTG CGG AGC CCG | 18 |
| AL#AE | CTC CGC CTC ATG GGC CGT | 18 |
| AL#AK | TAC TGG TGG TAC CCG CGC | 18 |
| AL#AM | CGT CGT AGG CGT CCT GCC | 18 |
| AL#AR | CTG GTA CCC GCG GAG GAG | 18 |
| AL#AW | GTG GCC CCT GGT ACC CGT | 18 |
| AL#BJ | CCG ACC CCA CGT CGC AGG CAC | 21 |
| AL#BK | GAG CCC GTC CAC GCA CTC | 18 |
| Internal control primers | | |
| 5'PIC#1 | ATG ATG TTG ACC TTT CCA GGG | 21 |
| 3'PIC | ATT CTG TAA CTT TTC ATC AGT TGC | 24 |

**Table 2.** Primer sequences for HLA-B DNA typing

| Primers | Nucleotide sequence (5'-3') | Length |
|---|---|---|
| AS#1 | GGC GCC GTG GAT AGA GCA A | 19 |
| B#1a | CCA CTC CAT GAG GTA TTT CC | 20 |
| B#4a.2 | CGC CAC GAG TCC GAG GAA | 18 |
| B#4b | ACG CCG CGA GTC CGA GAG | 18 |
| B#4c | GCC GCG AGT CCG AGG AC | 17 |
| B#4d | CGC GAG TCC GAG GAT GGC | 18 |
| B#5a.1 | GAC CGG AAC ACA CAG ATC TG | 20 |
| B#5a.2 | ACC GGG AGA CAC AGA TCT G | 19 |
| B#5b | ACC GGG AGA CAC AGA TCT C | 19 |
| B#5c | GGA GTA TTG GGA CCG GAA C | 19 |
| B#5e.2 | GAA CAT GAA GGC CTC CGC G | 19 |
| B#5g | GAC CGG AAC ACA CAG ATC TT | 20 |
| B#5'A1 | GAC GAC ACC CAG TTC GTG A | 19 |
| B#6a | TAC CGA GAG AAC CTG CGC | 18 |
| B#B14 | GAG CAG GAG GGG CCG GAA | 18 |
| B#G183 | GGC CGG AGT ATT GGG ACG | 18 |
| B#29 | GAC GAC ACG CAG TTC GTG A | 19 |
| B#30 | GAC GAC ACG CAG TTC GTG A | 19 |
| B#18 | GAC GGC ACC CAG TTC GTG A | 19 |
| B#54 | GCG GGC GCC GTG GGT G | 16 |
| AS#A | CCT CCA GGT AGG CTC TGT C | 19 |
| B#A3 | GGA GGA GGC GCC CGT CG | 17 |
| B#C2 | CCT TGC CGT CGT AGG CGG | 18 |
| B#C3 | ATC CTT GCC GTC GTA GGC T | 19 |
| B#C4 | CGT TCA GGG CGA TGT AAT CT | 20 |
| B#D1 | GCC GCG GTC CAG GAG CT | 17 |
| B#D3 | GAG CCG CCG TGT CCG CG | 17 |
| B#E2 | CGT GCC CTC CAG GTA GGT | 18 |
| B#F1 | GAG CCA CTC CAC GCA CTC | 18 |
| B#F2 | GAG CCA CTC CAC GCA CAG | 18 |
| B#F3 | CCA GGT ATC TGC GGA GCG | 18 |
| B#G1b | CCG CGC GCT CCA GCG TG | 17 |
| B#G1c | TAC CAG CGC GCT CCA GCT | 18 |
| B#H | GGG CCG CCT CCC ACT TGA | 18 |
| B#I | CGT CGC AGC CAT ACA TCC A | 19 |
| B#K | GCC ATA CAT CCT CTG GAT GA | 20 |
| B#K2 | CGT CGC AGC CAT ACA TCA C | 19 |
| B#31 | GC TCT GGT TGT AGT AGC C | 19 |
| B#32 | CGC TCT GGT TGT AGT AGC G | 19 |
| Internal control primers | | |
| 5'PIC#1 | ATG ATG TTG ACC TTT CCA GGG | 21 |
| 3'PIC#AN | ATT CTG TAA CTT TTC ATC AGT TGC | 24 |

**Table 3.** Primer sequences for HLA-C DNA typing

| Primers | Nucleotide sequence (5'-3') | Length |
|---|---|---|
| Cw#27 | CCG AGT GAA CCT GCG GAA A | 19 |
| Cw#30 | TAC TAC AAC CAG AGC GAG GA | 20 |
| Cw#31 | CAC AGA CTG ACC GAG TGA G | 19 |
| Cw#32 | AGT CCA AGA GGG GAG CCG | 18 |
| Cw#34 | CCA CTC CAT GAG GTA TTT CTC | 21 |
| Cw#130 | CCG CGG GTA TGA CCA GTC | 18 |
| Cw#136 | TCC GCG GGT ATG ACC AGT A | 19 |
| Cw#159 | TAC AAC CAG AGC GAG GCC A | 19 |
| Cw#160 | ACA ACC AGA GCG AGG CCG | 18 |
| Cw#165 | ACG ACA CGC AGT TCG TGC A | 19 |
| Cw# 35 | TCT TCT CCA GAA GGC ACC AT | 20 |
| Cw# 40 | CCT CCA GT AGG CTC TCC A | 19 |
| Cw# 41 | CAG CCC CTC GTG CTG CAT | 18 |
| Cw# 42 | CGC GCG CTG CAG CGT CTT | 18 |
| Cw# 45 | CCT CCA GGT AGG CTC TCA G | 19 |
| Cw# 126 | TGA GCC GCC GTG TCC GCA | 18 |
| Cw# 127 | GGT CGC AGC CAT ACA TCC A | 19 |
| Cw# 127.1 | GGT CGC AGC CAA ACA TCC A | 19 |
| Cw# 135.1 | AGC GTC TCC TTC CCA TTC TT | 20 |
| Cw# 143 | GCC CCA GGT CGC AGC CAA | 18 |
| Cw# 145 | GAG CCA CTC CAC GCA CTC | 18 |
| Cw# 146 | CCC TCC AGG TAG GCT CTC T | 19 |
| Cw# 147 | TCG TAG GCT AAC TGG TCA TG | 20 |
| Cw# 157 | CCG CCG TGT CCG CGG CA | 17 |
| Cw# 166 | GCG CAG GTT CCG CAG GC | 17 |
| Cw# 184 | GCC ACG GGC CGC CTC CA | 17 |
| Internal control primers | | |
| 5'C5 | TGC CAA GTG GAG CAC CCA A | 19 |
| 3'C3 | GCA TCT TGC TCT GTG CAG AT | 20 |

**Table 4.** Oligonucleotide sequences of HLA-DQA1, DQB1, DPB1 primers

| HLA | DNA sequences of primer | Use |
|---|---|---|
| DQA1 | DA1 - TGGTGTAAACTTGTACCAG | 5' |
| | DA2 - TTGGTAGCAGCCGGTAGAGTTG | 3' |
| DQB1 | DB1 - GCCTGTGCTACTTCACCAACGG | 5' |
| | DB2 - CTGCAGTGCGGAGCTCCAACTG | 3' |
| | DB3 - GGCCTGTGCTACTTCACCAACGG | 5' |
| | DB4 - CAGATCCCGCGGTACGCCACCTC | 3' |
| DPB1 | DP1 - GAAGCTTTCCCCGCAGAGAATTAC | 5' |
| | DP2 - CTGCAGTCACTCACCTCGGCGCTG | 3' |

**Table 5.** Cleavage fragments of HLA-DQA1 homozygotes

| | Dde I | Rsa I | Mbo II | Hae III | Fok I |
|---|---|---|---|---|---|
| Restriction | 2 1 1 1 1 1 | 2 1 | 2 1 1 1 | 2 1 1 | 2 1 |
| fragment | 2 2 2 1 0 0 7 | 1 8 2 | 2 4 2 0 8 | 2 9 1 7 | 2 7 5 |
| | 9 9 0 5 9 0 3 | 4 7 7 | 9 5 8 1 4 | 9 2 6 6 | 9 8 1 |
| DQA1*0101 | 0 0 0 1 0 0 1 | 0 1 1 | 1 0 0 0 0 | 0 0 1 1 | 1 0 0 |
| DQA1*0103 | 0 0 0 1 0 0 1 | 1 0 0 | 0 0 1 1 0 | 0 0 1 1 | 1 0 0 |
| DQA1*0201 | 0 1 0 0 0 1 0 | 1 0 0 | 1 0 0 0 0 | 0 1 0 0 | 0 1 1 |
| DQA1*03011 | 1 0 0 0 0 0 0 | 0 1 0 | 0 1 0 0 1 | 1 0 0 0 | 1 0 0 |
| DQA1*0401 | 0 0 1 0 1 0 0 | 0 1 1 | 1 0 0 0 0 | 0 1 0 0 | 0 1 1 |
| DQA1*0501 | 0 0 1 0 1 0 0 | 0 1 1 | 1 0 0 0 0 | 0 1 0 0 | 1 0 0 |
| DQA1*0601 | 0 0 1 0 1 0 0 | 1 0 1 | 1 0 0 0 0 | 0 1 0 0 | 0 1 1 |

*Number 1 and 0 mean cutted and non cutted sites by each restriction enzyme

**Table 6.** Cleavage fragments of HLA-DQB1 homozygotes

(A) HLA DQB1 Group I

| Enzyme | Msp I | Sac I | Sau 96I | Acy I |
|---|---|---|---|---|
| Size | 1 1 1 | 2 2 | 1 1 1 2 | 1 1 1 2 |
| | 0 3 7 | 0 3 | 0 2 5 3 | 0 1 2 3 |
| Type | 6 3 8 | 6 9 | 1 4 1 9 | 4 6 3 9 |
| DQB1*0201 | 1 1 0 | 0 1 | 0 0 0 1 | 0 0 0 1 |
| DQB1*0301 | 1 0 0 | 0 1 | 0 1 0 0 | 1 1 0 0 |
| DQB1*0302 | 0 0 1 | 0 1 | 0 1 0 0 | 0 1 1 0 |
| DQB1*03031 | 0 0 1 | 0 1 | 1 0 0 0 | 1 1 0 0 |
| DQB1*0304 | 1 0 0 | 0 1 | 0 1 0 0 | 0 1 1 0 |
| DQB1*0401 | 0 0 1 | 1 0 | 0 0 1 0 | 0 1 0 0 |
| DQB1*0402 | 0 0 1 | 0 1 | 0 0 1 0 | 0 1 0 0 |

(B) HLA DQB1 Group II

| Enzyme | ApaI | HaeII | HhaI | MspI |
|---|---|---|---|---|
| Size | 1 2 | 1 1 2 | 1 1 1 2 | 1 1 2 |
| | 7 3 | 0 3 3 | 0 2 5 0 | 0 7 3 |
| Type | 7 7 | 2 5 7 | 1 8 7 5 | 5 7 7 |
| DQB1*0501 | 1 0 | 0 0 1 | 0 0 1 0 | 1 0 0 |
| DQB1*0502 | 1 0 | 1 1 0 | 1 0 0 0 | 1 0 0 |
| DQB1*0503 | 1 0 | 0 0 1 | 0 0 1 0 | 0 1 0 |
| DQB1*0504 | 0 1 | 1 1 0 | 1 0 0 0 | 1 0 0 |
| DQB1*0601 | 0 1 | 0 0 1 | 0 0 1 0 | 0 0 1 |
| DQB1*0602 | 0 1 | 0 0 1 | 0 1 0 0 | 0 1 0 |
| DQB1*0604 | 0 1 | 0 0 1 | 0 0 0 1 | 1 0 0 |

*Number 1 and 0 mean cutted and non cutted sites by each restriction enzyme

International Histocompatibility Workshop were analyzed. The results of control cells analyzed by HLA-class I and class II DNA typing developed in this study, were consistent with the previous data[19,24]. Table 9 and 10 represented the data of control cells of HLA-class I and II genes, respectively.

**The gene frequency of HLA-class I genes**

Seventeen HLA-A alleles were found in Korean samples using HLA-A DNA typing. A*02 (27.0%), A*2401 (20.8%), A*33 (16.4%) and A*11 (11.7%) alleles were frequently found. Among A*02 allele which is consist of 17 subtypes, only five A*02 subtypes-A*0207 (13.2%), A*0206 (7.6%), A*0201 (4.6%), A-*0210 (1.0%) and A*0203 (0.6%)-were found. Nine HLA-A alleles, including A*23, A*25 A*34, A*36, A*43, A*66, A*68, A*74, A*88 were not observed in Korean samples (Table 11). By HLA-B DNA typing, 23 different HLA-B alleles were determined. Among these, frequently observed HLA-B alleles were B*40 (17.6%), B*15 (15.6%), B*51 (11.4%) and B*44 (10.0%). Whereas HLA-B*08, B*14 and B*56 were only found to 0.4%. B*18, B*41, B*42, B*45, B*47, B*49, B*50, B*57, B*67 and B*78 alleles were not observed (Table 11). Sixteen HLA-C alleles were observed by PCR-SSP DNA typing with 26 primers. Cw*01

**Table 7.** Cleavage fragments of HLA-DPB1 gene

| | EcoNI | RsaI | FokI | DdeI | SduI |
|---|---|---|---|---|---|
| Size | 2 2 2 1 1 1 | 2 2 2 1 1 1 | 2 2 1 1 | 2 1 1 | 2 2 2 1 1 1 |
| | 9 6 0 9 7 7 9 9 2 | 9 7 6 7 4 1 7 6 2 | 9 4 8 1 5 5 | 9 6 3 | 9 6 6 8 5 0 3 2 |
| Type | 4 6 2 9 4 1 5 2 8 3 | 4 5 6 5 7 9 8 9 8 | 4 0 1 3 9 4 | 4 4 0 | 4 5 0 7 3 7 4 9 |
| DPB1*0101 | 1 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 1 1 1 1 | 0 0 1 1 0 0 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*0201 | 0 0 0 0 1 0 0 1 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 1 0 0 | 0 0 1 0 0 0 1 0 |
| DPB1*0202 | 0 0 0 0 1 0 0 1 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 0 1 1 | 0 0 0 0 1 1 1 0 |
| DPB1*0301 | 0 0 0 1 0 0 1 0 0 0 | 0 0 1 0 0 0 0 0 1 | 1 0 0 0 0 0 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*0401 | 0 1 0 0 0 0 0 0 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 1 0 0 | 0 0 1 0 0 0 1 0 |
| DPB1*0402 | 0 1 0 0 0 0 0 0 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 1 0 0 | 0 0 1 0 0 0 1 0 |
| DPB1*0501 | 0 1 0 0 0 0 0 0 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 0 1 1 | 0 0 0 1 0 1 0 0 |
| DPB1*0601 | 0 0 0 1 0 0 0 1 0 1 | 0 0 1 0 0 0 0 0 1 | 0 1 0 0 0 1 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*0801 | 0 0 0 0 1 0 0 1 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 1 0 0 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*0901 | 0 0 1 0 0 0 0 1 0 0 | 1 0 0 0 0 0 0 0 0 | 0 0 1 1 0 0 | 1 0 0 | 0 1 0 0 0 0 0 1 |
| DPB1*1001 | 0 0 1 0 0 0 0 1 0 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 1 0 0 | 1 0 0 | 0 1 0 0 0 0 0 1 |
| DPB1*1101 | 0 0 0 1 0 0 1 0 0 0 | 0 0 0 0 0 1 1 1 1 | 0 1 0 0 0 1 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*1301 | 0 0 1 0 0 0 0 1 0 0 | 0 0 0 0 0 1 1 1 1 | 0 0 1 1 0 0 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*1401 | 0 0 0 1 0 0 1 0 0 0 | 1 0 0 0 0 0 0 0 0 | 1 0 0 0 0 0 | 1 0 0 | 0 1 0 0 0 0 0 1 |
| DPB1*1501 | 0 0 0 1 0 0 1 0 0 0 | 0 0 0 0 0 1 1 1 1 | 0 1 0 0 0 1 | 1 0 0 | 0 0 1 0 0 0 1 0 |
| DPB1*1601 | 0 0 0 0 1 0 0 1 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*1701 | 0 0 1 0 0 0 0 1 0 0 | 1 0 0 0 0 0 0 0 0 | 0 0 1 0 1 1 | 1 0 0 | 0 1 0 0 0 0 0 1 |
| DPB1*1801 | 1 0 0 0 0 0 0 0 0 0 | 0 0 0 0 1 1 0 0 1 | 0 0 1 0 1 1 | 1 0 0 | 0 0 1 0 0 0 1 0 |
| DPB1*1901 | 0 0 0 0 1 0 0 1 1 0 | 0 0 0 0 1 1 0 0 0 | 0 0 1 1 0 0 | 0 1 1 | 1 0 0 0 0 0 0 0 |
| DPB1*2001 | 0 0 0 1 0 0 1 0 0 0 | 0 0 1 0 0 0 0 0 1 | 0 1 0 0 0 1 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*2101 | 0 0 1 0 0 0 0 1 0 0 | 0 0 0 0 1 1 0 0 1 | 0 0 1 0 1 1 | 0 1 1 | 0 0 0 1 0 1 0 0 |
| DPB1*2201 | 0 0 0 0 1 0 0 1 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 0 1 1 | 0 0 0 1 0 1 0 0 |
| DPB1*2301 | 0 1 0 0 0 0 0 0 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 1 0 0 | 0 0 1 0 0 0 1 0 |
| DPB1*2401 | 0 1 0 0 0 0 0 0 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 0 1 1 | 0 0 1 0 0 0 1 0 |
| DPB1*2501 | 0 0 0 1 0 0 1 0 0 0 | 0 0 0 0 1 1 0 0 1 | 1 0 0 0 0 0 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*2601 | 1 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 1 1 1 1 | 0 0 1 1 0 0 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*2701 | 1 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 1 1 1 1 | 0 0 1 0 1 1 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*2901 | 0 0 0 1 0 0 0 1 0 1 | 0 0 1 0 0 0 0 0 1 | 1 0 0 0 0 0 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*3101 | 0 0 0 0 0 1 1 0 1 0 | 0 0 0 1 0 1 0 0 0 | 0 1 0 0 0 1 | 1 0 0 | 1 0 0 0 0 0 0 0 |
| DPB1*3201 | 0 0 0 0 1 0 0 1 1 0 | 0 0 0 1 0 1 0 0 0 | 0 0 1 0 1 1 | 1 0 0 | 0 0 1 0 0 0 1 0 |
| DPB1*3601 | 1 0 0 0 0 0 0 0 0 0 | 0 0 0 0 0 1 1 1 1 | 0 0 1 0 1 1 | 0 1 1 | 0 0 0 1 0 1 0 0 |

*Number 1 and 0 mean cutted and non cutted sites by each restriction enzyme

(19.2%) was the most frequent allele. HLA-Cw*14 (12.9%), Cw*07 (11.5%) and Cw*0302 (10.0%) alleles were observed more than 10%. Three HLA-C alleles, C*1203, C*13 and C*17 were not found (Table 11).

**The gene frequency of HLA-class II genes**

Seven different DQA1 alleles were deter-

**Table 8.** Oligonucleotide sequences of HLA-DRB1 primers

| Primer | Sequence |
|---|---|
| DRB 09-4 | CCC-(AC)CA-GCA-CGT-TTC-TTG-A |
| DRB 10-4 | (AC)CA-GCA-CGT-TTC-TTG-GAG-G |
| DRB 10L-1 | (AC)CA-GCA-CGT-TTC-TTG-GAG-CT |
| DRB 11D-2 | CCA-CGT-TTC-TTG-CAG-CAG-GA |
| DRB 11R-1R | CCA-CGT-TTC-TTG-GAG-CTG-CG |
| DRB 13C-2 | GTT-TCT-TGG-AGC-AGG-CTA-AGT-G |
| DRB 13G-1 | CGT-TTC-TTG-GAG-TAC-TCT-ACG-GG |
| DRB 13H-2 | CAC-GTT-TCT-TGG-AGC-AGG-TTA-AAC |
| DRB 13R-1 | CGT-TTC-CTG-TGG-CAG-CCT-AAG-A |
| DRB 13S-2 | GTT-TCT-TGG-AGC-AGG-TTA-AAC |
| DRB 14K-2 | GTT-TCC-TGT-GGC-AGG-GTA-AGT-ATA |
| DRB 26L-4 | GGA-GCG-GGT-GCG-GTT-G |
| DRB 37D-1R | AGA-CAT-CTA-TAA-CCA-AGA-GGA-GG |
| DRB 37F-2R | TCG-CTG-TCG-AAG-CGC-ACG-A |
| DRB 37L-1R | GCT-GTC-GAA-GCG-CAG-GAG |
| DRB 37N-2R | GCT-GTC-GAA-GCG-CAC-GTT |
| DRB 37S-1R | GCT-GTC-GAA-GCG-CAC-GG |
| DRB 47F-2R | TCC-GTC-ACC-GCC-CGG-A |
| DRB 58E-1R | TCA-GGC-TGT-TCC-AGT-ACT-CCT |
| DRB 57S-1R | TGT-TCC-AGT-ACT-CGG-CGC-T |
| DRB 57V-1R | CTG-TTC-CAG-GAC-TCG-GCG-A |
| DRB 67F-2R | CGC-GCC-TGT-CTT-CCA-GGA-A |
| DRB 67I-2R | CCG-CTC-GTC-TTC-CAG-GAT |
| DRB 70Q-3R | CAC-CGC-GGC-CCG-CCT-CTG |
| DRB 71A-2R | GTC-CAC-CGC-GGC-CCG-CGC |
| DRB 74E-1R | TGC-AAT-AGG-TGT-CCA-CCT-C |
| DRB 74R-1R | GTG-TCT-GCA-GTA-ATT-GTC-CAC-CC |
| DRB 74Q-2R | GTC-TGC-AGT-AAT-TGT-CCA-CCT-G |
| DRB 86G-1R | TGC-ACT-GTG-AAG-CTC-TCA-C |
| DRB 86V-1R | CTG-CAC-TGT-CAA-GCT-CTC-CA |
| DRB 81Y-1R | CTC-TCC-ACC-AAC-CCG-TAG-TTC-TA |
| DP alpha | Positive control primers |
| DPA e | GAT-CCC-CCT-GAG-GTG-ACC-GTG |
| DPA f | CTG-GGC-CCG-GGG-GTC-ATG-GCG |

mined by 5 restriction enzymes (Table 5). DQA1*0301 (32.1%) was the most frequent allele. The other DQA1 alleles occurred at frequencies of 3.7%∼14.6% and the least frequent allele was DQA1*0401 (3.7%) (Table 12).

Two groups of restriction enzymes were used for PCR-RFLP analysis for the determination of 16 DQB1 alleles (Table 6). DQB1*03032 (12.5%) was most frequently found and DQB1-*0201, *0301, *0302, *0402 and *0602 alleles

**Table 9.** HLA-class I DNA typing in 24 B-lymphoblastoid cell lines

| | | HLA-A | | HLA-B | | HLA-Cw | |
|---|---|---|---|---|---|---|---|
| WSNo[1)] | Identity | SEROLOGY[2)] | PCR-SSP[3)] | SEROLOGY | PCR-SSP | SEROLOGY | PCR-SSP |
| 9102 | ARBO | 3 | *0301 | 57 | *5701 | 6 | *06 |
| 9068 | BM9 | 2 | *0202 | 35 | *35 | 4 | *04 |
| 9033 | BM14 | 3 | *0301 | 7 | *07 | 7 | *07 |
| 9038 | BM16 | 2 | *0201 | 18 | *18 | 7 | *07 |
| 9043 | BM21 | 1 | *0102 | 41 | *4101 | – | *1701 |
| 9007 | DEM | 2 | *0202 | 57 | *5701 | 6 | *06 |
| 9075 | DKB | 24 | *2402 | 60 | *4001 | 10 | *03 |
| 9008 | D0208910 | 25 | *2501 | 18 | *18 | – | *1203 |
| 9054 | EK | 2 | *0201 | 44 | *44 | 5 | *0501 |
| 9097 | EMJ | 2 | *0202 | 60 | *4001 | 10 | *03 |
| 9055 | H0301 | 3 | *0301 | 14 | *1402 | 8 | *08 |
| 9030 | JVM | 2 | *0201 | 18 | *18 | 8 | *08 |
| 9083 | LD2B | 3 | *0301 | 7 | *07 | 7 | *07 |
| 9070 | LUYA | 2 | *0201 | 51 | *51 | – | *14 |
| 9002 | MZ070782 | 24 | *2401 | 14 | *14 | 8 | *08 |
| 9051 | PIOUT | 29 | *2902 | 44 | *44 | – | *1601 |
| 9047 | PLH | 3 | *0301 | 47 | *47 | 6 | *06 |
| 9020 | QBL | 26 | *2601 | 18 | *18 | 5 | *05 |
| 9021 | RSH | 28 30 | *6801/3001 | 42 | *4201 | – | *1701 |
| 9076 | R7526 | 2 | *0201 | 46 | *4601 | 1 | *01 |
| 9062 | WDV | 2 | *0201 | 38 | *38 | – | *1203 |
| 9015 | WT24 | 2 | *0202 | 27 | *27 | 2 | *02 |
| 9006 | WT100 | 11 | *1101 | 35 | *35 | 4 | *04 |
| 9023 | VAVY | 1 | *0101 | 8 | *0801 | 7 | *07 |

1) Workshop Number (11th International HLA workshop)
2) analyzed by serological typing25)
3) analyzed by DNA typing from this study

were observed more than 10% (Table 12). Thirty one DPB1 alleles were analyzed by using 5 restriction enzymes (Table 7). DPB1*0501 (31.3%) was most frequently found allele. DPB1*0401 (21.3%), DPB1*0201 (15.8%) and DPB1*0202 (7.9%) alleles were frequently determined. Thirty seven different DRB1 alleles were determined by PCR-SSP technique using 31 primers (Table 8). DRB1*1501 (9.2%), DRB1-*0101 (8.4%), DRB1*0701 (7.9%), DRB1*0803 (7.6%) and DRB1*0901 (7.6%) were frequently observed (Table 12).

## DISCUSSION

Recently DNA based typing methods have been sucessfully applied to the HLA class I region, including typing of the HLA-A[3)], HLA-B[5,20)] and HLA-C[4)] genes. HLA DNA typing gives a possibility of (a) precise allele characterization (b) detection of antigens not serologically detectable (blank) (c) the system does not require viable cells, and can be used to type material from cells that cannot be used

**Table 10.** HLA-class II DNA typing in 18 B-lymphoblastoid cell lines

| WSNo[1] | Cell line | DQA1[3] | DQB1[3] | DRB1[3] | DPB1[3] | DQw[2] | DR[2] | Dw[2] | DPw[2] |
|---|---|---|---|---|---|---|---|---|---|
| 9005 | HOM2 | 0101 | 0501 | 0101 | 0401 | 5 | 1 | 1 | |
| 9002 | MZ70782 | 0101 | 0501 | 0102 | 0401 | 5 | 1 | 20 | 4 |
| 9036 | SP0010 | 0102 | 0502 | 1101 | 02012 | 5 | 11 | DB2 | 2 |
| 9008 | D0208915 | 0102 | 0602 | 1501 | 02012 | 6 | 15 | 2 | 2, 4 |
| 9010 | AMAI | 0102 | 0602 | 1503 | 0402 | 6 | 15 | 2 | |
| 9017 | WT8 | 0102 | 0602 | 1501 | 0102 | 1 | 15 | 2 | 2, 4 |
| 9058 | OMW | 0103 | 0603 | 1301 | 0101 | 6 | 13 | 18 | 1 |
| 9062 | WDV | 0103 | 0603 | 1301 | 02012 | 6 | 13 | 18 | 4 |
| 9050 | MOU | 0201 | 0201 | 07 | 02012 | 2 | 7 | 17 | 2 |
| 9047 | PLH | 0201 | 0201 | 07 | 1501 | 2 | 7 | DB1 | |
| 9075 | DKB | 03 | 03032 | 0901 | 0401 | 9 | 9 | 23 | 4 |
| 9029 | WT51 | 03 | 0302 | 0401 | 02012 | 8 | 4 | 4 | 2 |
| 9068 | BM9 | 0401 | 0402 | 0801 | 02012 | 4 | 8 | 8.1 | 2 |
| 9021 | RSH | 0401 | 0402 | 0302 | 0101 | 4 | 3 | N | 1 |
| 9018 | L0081785 | 0501 | 0201 | 0301 | 0301 | 2 | 3 | 3 | 3 |
| 9019 | DUCAF | 0501 | 0201 | 0301 | 0202 | 2 | 3 | 3 | |
| 9023 | VAVY | 0501 | 0201 | 0301 | 0101 | 2 | 3 | 3 | 1 |
| 9070 | LUYA | 0601 | 0301 | 0803 | 0101 | 7 | 8 | 8.3 | 1, 4 |

[1] Workshop Number (11th International HLA workshop)
[2] analyzed by serological typing22)
[3] analyzed by DNA typing from this study

for tissue typing by conventional means (e.g, epithelial cells and cells with low or undetectable HLA expression)[6]. In this study, for the detection of all sequenced HLA-class I alleles PCR-SSP technique was used. The principle of PCR-SSP is that each group of alleles or individual allele making up a serological specificity is amplified by a primer pair matched exactly to that group. By keeping the stringent of PCR conditions, primer pairs will not amplify closely related other alleles with non specifically[12].

The HLA-A DNA typing panel was designed to cover specifically all known serologically defined antigens in 32 seperated allele or group specifications. Amplification of genomic DNA yielded HLA-A locus-specific PCR products ranging in size from 393 to 813 bp. The gene frequencies of HLA-A alleles were compared with previous reports in Koreans and Japanese. The HLA-A "blank" alleles which were 4% in serological study[1] were not found by DNA typing (Table 11). A*02 allele was most frequently found in both DNA typing and serological typing in Koreans[1]. Whereas A*24 (32.97%) was the most frequent allele in Japanese[1].

HLA-B gene which is the most polymorphic HLA gene has over 100 different alleles[5]. At present study, the HLA-B genotyping panel does not aim to achieve the level of definition which can be obtained by high-quality serology. However, the degree of resolution possible for an individual well depend on their particular genotype. The individual reactions which make up the HLA-B DNA typing panel fall into three distinct groups. Group one consisted of reactions specific for a given allele such as

**Table 11.** Gene frequencies of the HLA-A, B and C

| HLA-A Allele | Gene Freq. (%) | HLA-B Allele | Gene Freq. (%) | HLA-C Allele | Gene Freq. (%) |
|---|---|---|---|---|---|
| A*01 | 2.1 | B*07 | 2.9 | Cw*01 | 19.2 |
| A*0201 | 4.6 | B*08 | 0.4 | Cw*02 | 1.3 |
| A*0203 | 0.6 | B*13 | 5.8 | Cw*03 | 2.6 |
| A*0206 | 7.6 | B*14 | 0.4 | Cw*0302 | 10.0 |
| A*0207 | 13.2 | B*15 | 15.6 | Cw*0303 | 6.4 |
| A*0210 | 1.0 | B*27 | 2.9 | Cw*0304 | 6.4 |
| A*0301 | 0.8 | B*35 | 5.4 | Cw*04 | 5.3 |
| A*0302 | 0.4 | B*37 | 1.7 | Cw*05 | 1.3 |
| A*11 | 11.7 | B*38 | 1.7 | Cw*06 | 6.9 |
| A*24 | 20.8 | B*39 | 2.5 | Cw*07 | 11.5 |
| A*26 | 7.1 | B*40 | 17.6 | Cw*08 | 4.6 |
| A*29 | 0.4 | B*44 | 10.0 | Cw*12 | 6.4 |
| A*30 | 4.2 | B*46 | 4.2 | Cw*14 | 12.9 |
| A*31 | 7.5 | B*48 | 5.0 | Cw*15 | 1.3 |
| A*32 | 0.8 | B*51 | 11.4 | Cw*1601 | 1.3 |
| A*33 | 16.4 | B*52 | 2.1 | Cw*1602 | 2.6 |
| A*69 | 0.8 | B*53 | 0.4 | | |
| | | B*54 | 4.6 | | |
| | | B*55 | 2.1 | | |
| | | B*56 | 0.4 | | |
| | | B*59 | 0.8 | | |
| | | B*70 | 1.3 | | |
| | | B*73 | 0.8 | | |

*Total allele number: n=240

B*0801. Group two included those reactions specific for alleles of a single serological specificity such as B*44. The third group of reactions was specific for groups of alleles which had been shown to have sequence similarity (e. g. B*1501-*1507/B*1512). Typing of the HLA-B15 group was initially carried out by serology; however, some of these variants still remain difficult to define serologically due to the lack of specific sera. Low-resolution typing panel described here broadly amplifies the HLA-B15 alleles. The genotype frequencies of HLA-B in Koreans were similar with previous reports in Koreans. HLA-B15 (15.6%) was most frequently found in Koreans but only 6.2% in Japanese[1]. For more specific definition of HLA-B alleles, the sequencing based method is recommended[18].

The molecular analysis and characterization of HLA-C allelic polymorphism has been impeded by lack of serological reagents which identify individual HLA-C antigens. Serology for HLA-C is generally quick and reliable; however, approximately 20% of Caucasians and up to 50% of Japanese and 30% of Koreans have only one detectable HLA-C allele[2]. The most likely cause of serologically undefiened HLA-C allele is the lack of suitable antisera coupled with low cell surface expression[6]. Despite similar messenger RNA levels, HLA-

**Table 12.** Gene frequencies of the HLA-DQA1, DQB1, DPB1 and DRB1

| HLA Alleles | Frequency (%) | HLA Alleles | Frequency (%) | HLA Alleles | Frequency (%) |
|---|---|---|---|---|---|
| HLA-DQA1 | | HLA-DPB1 | | DRB1*0411 | 0.4 |
| DQA1*0101 | 14.6 | DPB1*0201 | 15.8 | DRB1*0701 | 7.9 |
| DQA1*0102 | 10.0 | DPB1*0202 | 7.9 | DRB1*0801 | 1.2 |
| DQA1*0103 | 11.7 | DPB1*0301 | 2.1 | DRB1*0802 | 3.7 |
| DQA1*0201 | 9.6 | DPB1*0401 | 21.3 | DRB1*0803 | 7.6 |
| DQA1*0301 | 32.1 | DPB1*0501 | 31.3 | DRB1*0804 | 0.9 |
| DQA1*0401 | 3.7 | DPB1*0901 | 1.2 | DRB1*0901 | 7.6 |
| DQA1*0501 | 12.9 | DPB1*1301 | 3.8 | DRB1*1001 | 2.1 |
| DQA1*0601 | 5.4 | DPB1*1401 | 2.1 | DRB1*1101 | 5.2 |
| | | DPB1*1601 | 0.8 | DRB1*1105 | 0.8 |
| HLA-DQB1 | | DPB1*1701 | 4.6 | DRB1*1201 | 4.6 |
| DQB1*0201 | 10.0 | DPB1*2201 | 5.8 | DRB1*1202 | 0.8 |
| DQB1*0301 | 10.4 | DPB1*2401 | 2.5 | DRB1*1301 | 1.5 |
| DQB1*0302 | 10.0 | DPB1*2701 | 0.8 | DRB1*1302 | 5.5 |
| DQB1*03031 | 0.4 | | | DRB1*1304 | 0.8 |
| DQB1*03032 | 12.5 | HLA-DRB1 | | DRB1*1305 | 0.4 |
| DQB1*0304 | 3.8 | DRB1*0101 | 8.4 | DRB1*1401 | 3.0 |
| DQB1*0401 | 5.8 | DRB1*0102 | 0.4 | DRB1*1403 | 0.4 |
| DQB1*0402 | 10.0 | DRB1*0301 | 1.2 | DRB1*1405 | 0.8 |
| DQB1*0501 | 6.7 | DRB1*0401 | 0.9 | DRB1*1406 | 0.8 |
| DQB1*0502 | 1.7 | DRB1*0402 | 0.4 | DRB1*1501 | 9.2 |
| DQB1*05031 | 2.1 | DRB1*0403 | 5.3 | DRB1*1502 | 1.6 |
| DQB1*05032 | 0.4 | DRB1*0404 | 3.8 | DRB1*1601 | 0.4 |
| DQB1*0504 | 0.4 | DRB1*0405 | 7.1 | DRB1*1602 | 0.8 |
| DQB1*0601 | 9.6 | DRB1*0406 | 2.1 | DRB1*1603 | 0.8 |
| DQB1*0602 | 10.4 | DRB1*0407 | 0.4 | DRB1*1604 | 0.4 |
| DQB1*0604 | 5.8 | | | DRB1*0409 | 0.8 |

*Total allele number: n=240

C antigens are expressed on cell surfaces at approximately 10% of the level of either HLA-A or HLA-B[8]. The frequencies of HLA-C genotypes were compared with previous reports determined by serological tying in Koreans. Only eight HLA-C alleles (C*01 to C*08) have been determined by serological typing[1]. Whereas 16 HLA-C alleles were found in Koreans by using PCR-SSP DNA typing. C*01 (19.2%) was most frequently found in Koreans but 11.8% in Japanese[1]. The gene frequency of C*07 was similar in both Koreans (17.5%) and Japanese (15.3%). HLA-C "blank" alleles were not observed by HLA-C DNA typing (Table 11).

Among several DNA typing methods, PCR-RFLP technique was used for HLA-class II genes including DQA1, DQB1 and DPB1. PCR-RFLP method which depends mainly on the cleavage sites of the amplified segment by informative restriction enzymes is relatively simple and fast and does not require the use of ra-

dioisotopes[13]. We have employed two pairs of primers to selectively amplify the DQB1 gene. DQB1 group I has DQw2, 3, 4 group specificity and DQB1 group II has DQw1 group specificity (Table 6). These group specific amplification of DQB1 gene as well as digestion with restriction enzymes could be useful for simple genotyping. Our results demonstrate that complete HLA-DQA1 and DQB1 genotyping can be unequivocally defined by the restriction enzymes selected (Table 5 and 6). The gene frequencies of DQA1 and DQB1 genes were compared with the previous report in Koreans. DQA1*0501 (14.6%) and DQB1*0201 (10.0%) were lower than previous reports in Koreans[23]. DQB1*03032 was the most frequent allele by 12.5% and 18.4% in both Koreans and Japanese[22]. By sequence analysis it has been shown that in the DPB1 locus, unlike the DRB1, DQA1 and DQB1 loci, there are very few allele specific sequences[14]. For accurate HLA-DPB1 DNA typing, five restriction enzymes - *Eco*NI, *Rsa*I, *Fok*I, *Dde*I and *Sdu*I - were used (Table 7). The gene frequencies of DPB1 were compared with Japanese and Caucacian. DPB1-*0501 was the most frequent DPB1 allele in both Koreans (31.3%) and Japanese (34.0%), but rare in Caucasian (0.8%)[22]. On the other hand, DPB1*0401 was 21.3%, 8.7% and 40.0% found in Koreans, Japanese and Caucasoid population, respectively[22]. It showed a typical difference between East and West ethnic groups.

HLA-DRB1 is the most polymorphic HLA-class II gene and applied first for DNA typing in transplantation immunity. For HLA-DRB1 genotyping, PCR-SSP method which is a powerful technique for detecting genetic variability with a high degree of resolution was used. Primers were designed and the PCR cycle profile and the compositions of the PCR reaction mixtures were adjusted to obtain highly specific and sensitive amplifications for the assignment of all phenotypically expressed DRB1 polymorphism. The gene frequencies of DRB1 gene was compared with the previous report among Koreans. DRB1*04 allele was most frequently found in both this study by PCR-SSP DNA typing and previous study in Koreans[22].

In conclusion, the HLA DNA typing is accurate and rapid method and could be useful for clinical labortory work. Moreover these data might be useful for the database studies before application to the individual identification and paternity testing among Koreans.

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=국문초록=

# 중합효소연쇄반응을 이용한 HLA-class I, II 유전자군의 유전적 다형성에 관한 연구

서울의과학 연구소 및 건국대학교 화학과

이 경 옥†

HLA 유전자군은 인간의 유전자 중에서 가장 높은 유전적 다형성을 보이며, 분석방법에 따라 판정할 수 있는 대립유전자의 수에 많은 차이가 있다. 현재까지 혈청학적 방법을 이용하여 HLA 항원형 구분을 하였으나, 최근 골수이식 등 여러 HLA 활용분야에서 HLA 유전자형 분석이 요구되고 있어, 많은 수의 HLA 유전자형을 쉽고 정확하게 구분할 수 있는 HLA DNA typing 방법이 필요한 실정이다. 본 연구에서는 HLA-A, B, C, DRB1 유전자형 구분은 PCR-SSP 방법을, HLA-DQA1, DQB1, DPB1 유전자형 구분은 PCR-RFLP 방법을 사용한 HLA DNA typing 방법으로 한국인에서 HLA 유전자형을 구분하고자 하였다. 본 방법을 이용하여 HLA 형이 규명된 B-임파아구 표준세포에서 DNA typing을 실시하였을 때, 11차 국제조직적합성학회에서 발표된 결과와 모두 일치하였다. 한국인에서 HLA-A, -B, -C 대립유전자는 17종, 23종, 16종이 확인되었으며, HLA-DQA1, -DQB1, -DPB1, -DRB1 대립유전자는 8종, 16종, 13종, 37종이 확인되었다. 한국인에서 빈도가 높은 HLA-class I 유전자는 HLA-A 유전자에서 A*02가 27.0%였으며 HLA-B 유전자에서는 B*40이 17.6%를 나타내었고 HLA-C 유전자에서는 Cw*0101이 19.2%로 가장 높은 빈도를 나타내었다. 한국인에서 가장 빈도가 높은 HLA-class II 유전자는 DQA1 유전자에서 DQA1*0301이 32.1%였고, DQB1 유전자에서는 DQB1*0303이 12.9%를 나타내었으며, DPB1 유전자에서는 DPB1*0501이 31.3%였고 DRB1 유전자에서는 DRB1*1501이 9.2%를 나타내었다. 본 연구에서 실시한 HLA DNA typing 방법은 비교적 빠른 시간내에 많은 종류의 HLA 대립유전자형을 정확하게 구분할 수 있으므로 앞으로 tissue typing 실험실에서 유용하게 활용될 수 있을 것으로 생각된다. 또한 DNA typing 방법을 이용하여 분석한 한국인의 HLA-class I, II 유전자군의 유전자형빈도는 골수이식을 비롯한 각종 이식검사, 특수 질환 관련검사나 인류유전학연구 등 HLA 유전자의 임상적 활용을 위한 자료로 사용될 수 있을 것으로 기대된다.

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†별책요청 저자