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Identification of the new *HLA-B*44:02:45, DQB1*02:85, DQB1*06:210, DRB1*01:01:30* alleles by monoallelic Sanger sequencing

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Summary

Four new HLA alleles were identified using the monoallelic Sanger sequencing method: *HLA-B*44:02:45, HLA-DQB1*02:85, HLA-DQB1*06:210, HLA-DRB1*01:01:30*. A distinctive feature of the method is to implement the initial allele-specific PCR products for subsequent separate amplification of the target gene alleles. This, in turn, allows for sequencing of each allele separately and avoiding ambiguous HLA typing results observed when performing locus-specific sequencing.

The isolated sequencing of specific gene alleles is a sufficient requirement for the registration of new HLA alleles, as prescribed by the World Health Organization Nomenclature Committee for Factors of the HLA System.

Keywords

Major histocompatibility complex, novel HLA alleles, monoallelic Sanger sequencing.

Introduction

The Major Histocompatibility Complex (MHC) is among the most polymorphic genetic systems in humans. Over last decade, extensive research in HLA (Human Leukocyte Antigens) has revealed hundreds of new HLA allelles by means of intensive application of immunogenetic sequencing methods, including monoallelic Sanger-sequencing method, or, more recently, next-generation sequencing (Fig. 1).

In June 2017, the database of the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System (IPD-IMGT/HLA Database) contained information on the nucleotide sequences of 17331 different HLA alleles, of which 12631 were HLA class I, and 4700 were found for the HLA class II alleles [1-3].

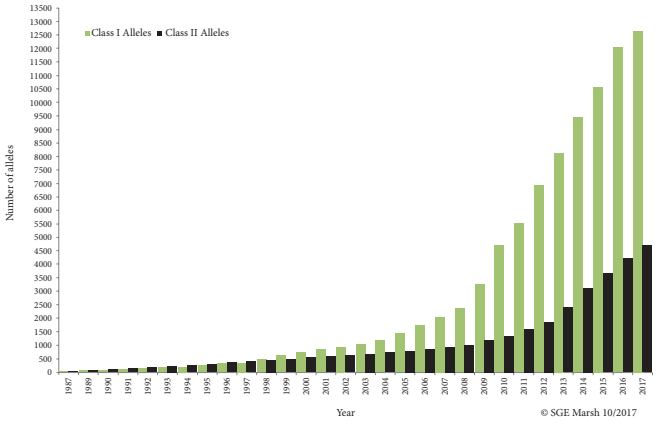

Fig. 1. A diagram showing increased numbers of HLA alleles registered between 1987 and the June 2017 (http://www.hla.alleles.org/nomenclature/)

The registration and name of new HLA alleles is carried out by the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System. The Committee developed a number of requirements ruling identification of the new HLA alleles (http://www.ebi.ac.uk/ipd/imgt/hla/subs/). In particular, the methods used to confirm new alleles should provide for separate sequencing of the alleles in the gene of interest. When performing HLA studies, appropriate primers should be used to determine the nucleotide sequence in forward and reverse directions. At the present time, sequencing of exons 2 and 3 is an obligatory requirement for identification of new HLA class I gene alleles. To verify any new alleles of HLA class II genes, one should perform the exon 2 sequencing.

The aim of this study was to evaluate the advantages of using monoallelic Sanger sequencing approach as a necessary step of identifying new HLA alleles.

Materials and methods

The test samples were obtained from potential donors of Bone Marrow Donor Registry of the I. P. Pavlov State Medical University in St. Petersburg and from a patient with acute myeloblastic leukemia who underwent HLA testing for subsequent allogeneic hematopoietic stem cell transplantation. Genomic DNA was isolated from peripheral blood leukocytes by a proteinase method using columns with a silica gel membrane and a kit PROTRANS DNA BOX reagents (Protrans, Germany). The target DNA concentration was 30 ng/μL. Quantity and quality estimation of the isolated DNA was performed with Thermo Scientific NanoDrop 2000 Spectrophotometer. The quality of isolated DNA was estimated as an optical density ratio at of 260/280 nm wavelength, with reference range of 1.6-1.8.

Immunogenetic studies were done using the method of monoallelic Sanger sequencing. Initial and control studies were performed for each sample. To perform a control typing, the DNA was isolated from the newly collected biomaterial. The Protrans reagent kits (Germany) were used as follows: PROTRANS S4 HLA-A * Cyclerstrips, PROTRANS S4 HLA-B * Cyclerstrips, PROTRANS S4 HLA-C * Cyclerstrips, PROTRANS S4 HLA-DRB1 * Cyclerstrip, PROTRANS S3 HLA-DQB1 * Cyclerstrips. To identify HLA class I alleles (HLA-A, HLA-B, HLA-C), we sequenced exons 2-3; for class II HLA alleles (HLA-DRB1, HLA-DQB1) we sequenced exon 2.

Capillary electrophoresis was performed using Applied Biosystems 3500xl genetic analyzer (USA). To specify nucleotide sequences of the target alleles, the rough laboratory data were evaluated with Sequens Pilot software, version 4.1.2 (JSI Medical Systems, Germany).

Results

The method of monoallelic Sanger sequencing is routinely applied at the tissue typing laboratory of St. Petersburg State Medical University I. P. Pavlov since 2010. A special feature of this method is carrying out allele-specific PCR at the initial stage, which usually leads to separate amplification of the analyzed gene alleles. After separate amplification, each allele may be subject to isolated sequencing.

The allele-specific sequencing allows of avoiding the so-called cis-trans ambiguities which occur when interpreting HLA typing results. About 90% of people are known to be heterozygous for each HLA gene, thus causing cis-trans ambiguities precluding the exact HLA genotyping after locus-specific sequencing [4].

Using an allele-specific sequencing approach, the novel alleles of HLA-B, HLA-DRB1, HLA-DQB1 genes have been identified in three potential bone marrow donors from the Bone Marrow Registry at the St. Petersburg State I.P. Pavlov Medical University, and in one patient from the R. Gorbacheva Memorial Research Institute for Children Oncology, Hematology and Transplantation. Later on, this patient underwent unrelated bone marrow transplantation [5-7].

HLA-B allele (B*44:02:45). A new HLA-B allele was detected in a potential bone marrow female donor (Caucasoid, living in St. Petersburg). As based on immunogenetic studies, the following HLA phenotype was determined: *HLA-A*02:01:01G, *30:01:01G, HLA-B*13:02, *44new, HLA-C*05:01:01G, *06:02:01G, HLA-DRB1*04:02:01, *10:01:01G, HLA-DQB1*03:02:01,*05:01:01G*. According to the version 3.26.0 of the IPD-IMGT/HLA database, we have revealed that the nucleotide sequence of exon 2 in the new *HLA-B *44* allele differs from the most close homologue *HLA-B*44:02:01:01* at position 243 (guanine substituted for thymine). As shown in Fig. 2, the nucleotide sequence of codon 81 GCG is changed to GCT. The substitution is synonymous since it does not lead to an amino acid change (alanine). The nucleotide sequence of the new *HLA-B*44* allele was submitted to the GenBank database being available under the accession number KY039061 (http://www.ncbi.nlm.nih.gov/Genbank/). The name of new *HLA-B*44* allele has been officially assigned as *HLA-B*44:02:45* by the WHO Nomenclature Committee for Factors of the HLA System [3].

New alleles of HLA class II genes

HLA-DQB1 allele (*DQB1*02:85*). A new HLA-DQB1 allele was identified in a potential bone marrow donor (female, Caucasoid, from Leningrad Region). Immunogenetic studies showed following results: *HLA-A*01:01:01G, *24:02:01G, HLA-B*08:01:01G, *39:01:01G, HLA-C*07:01:01G, *07:02:01G, HLA-DRB1*03:01: 01G, *04:04:01, HLA-DQB1*02new, *03:02*. According to the version 3.26.0 of the IPD-IMGT/HLA database, the exon 2 sequence of the new *HLA-DQB1*02* allele differs from the most close homologue *HLA-DQB1*02:01:01* at the position 141 (cytosine replaced by adenine). As seen from Fig. 3, codon 47 is changed from TTC to TTA, thus causing an amino acid coding change (phenylalanine to leucine). Nucleotide sequence of the new *HLA-DQB1*02* allele is available under the accession number KY014073 in the GenBank database. The new *HLA-DQB1*02* allele has been officially assigned as *HLA-DQB1*02:85* by WHO Nomenclature Committee for Factors of the HLA System [3].

HLA-DQB1 allele (*DQB1*06:210*). Another new HLA-DQB1 allele was detected in a patient with acute myeloblastic leukemia (female, Caucasoid, from Sverdlovsk Region). High-resolution HLA typing was performed to select an HLA compatible donor for allo-HSCT.

The following HLA phenotype was determined: *HLA-A*03:01,*23:01/17/69, HLA-B*44:03, *50:01, HLA-C*04:01/09N/30/82,*06:02/83, HLA-DRB1*07:01/34, *13:01/117/190, HLA-DQB1*02:02,*06new*. According to the version 3.26.0 of the IPD-IMGT/HLA database, the nucleotide sequence of exon 2 of the new *HLA-DQB1*06* allele differs from the nearest homologue *HLA-DQB1*06:03:01* at position 112 (thymine instead of guanine). The sequence of nucleotides in the codon 38 GCG is changed to TCG, thus leading to the amino acid replacement at position 38 (alanine replaced by serine, see Fig. 4). The nucleotide sequence of the new *HLA-DQB1*06* allele is available under the accession number KX988007 in the GenBank database.

The new *HLA-DQB1*06* allele has been officially assigned as *HLA-DQB1*06:210* by WHO Nomenclature Committee for Factors of the HLA System [3].

HLA-DRB1 allele (DRB1*01:01:30). A new HLA-DRB1 allele was detected in a potential bone marrow donor (male, Caucasoid, from Lipetsk). The HLA phenotype showed following distribution: *HLA-A*01:01, *24:02, HLA-B*07:02/61/161N,*57:01, HLA-C*06:02/83, *07:02/50/349, HLA-DRB1*01:01new, *17:01/34, HLA-DQB1*03:03, *05:01*. According to the version 3.26.0 of the IPD-IMGT/HLA database, the nucleotide sequence of the exon 2 of the new *HLA-DRB1*01* allele differs from the nearest homologue *HLA-DRB1*01:01:01* in position 279 (thymine is determined instead of guanine), for details see Fig. 5.

```
AA Codon                    5                  10                  15                  20                  25
B*44:02:01:01    GC TCC CAC TCC ATG AGG TAT TTC TAC ACC GCC ATG TCC CGG CCC GGC CGC GGG GAG CCC CGC TTC ATC ACC GTG
B*44:02:45       -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   30                  35                  40                  45                  50
B*44:02:01:01    GGC TAC GTG GAC GAC ACG CTG TTC GTG AGG TTC GAC AGC GAC GCC ACG AGT CCG AGG AAG GAG CCG CGG GCG CCA
B*44:02:45       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   55                  60                  65                  70                  75
B*44:02:01:01    TGG ATA GAG CAG GAG GGG CCG GAG TAT TGG GAC CGG GAG ACA CAG ATC TCC AAG ACC AAC ACA CAG ACT TAC CGA
B*44:02:45       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   80                  85                  90
B*44:02:01:01    GAG AAC CTG CGC ACC GCG CTC CGC TAC TAC AAC CAG AGC GAG GCC G
B*44:02:45       --- --- --- --- --- --T --- --- --- --- --- --- --- --- --- -
```

Figure 2. Comparison of sequence exon 2 alleles *HLA-B *44:02:01:01* and *HLA-B*44:02:45*. The picture was designed by means of IPD-IMGT/HLA Database [1]

```
AA Codon                        10                  15                  20                  25
DQB1*02:01:01     AG GAT TTC GTG TAC CAG TTT AAG GGC ATG TGC TAC TTC ACC AAC GGG ACA GAG CGC GTG CGT CTT GTG AGC AGA
DQB1*02:85        -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         30                  35                  40                  45                  50
DQB1*02:01:01     AGC ATC TAT AAC CGA GAA GAG ATC GTG CGC TTC GAC AGC GAC GTG GGG GAG TTC CGG GCG GTG ACG CTG CTG GGG
DQB1*02:85        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A --- --- --- --- --- --- ---

AA Codon         55                  60                  65                  70                  75
DQB1*02:01:01     CTG CCT GCC GCC GAG TAC TGG AAC AGC CAG AAG GAC ATC CTG GAG AGG AAA CGG GCG GCG GTG GAC AGG GTG TGC
DQB1*02:85        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         80                  85                  90
DQB1*02:01:01     AGA CAC AAC TAC CAG TTG GAG CTC CGC ACG ACC TTG CAG CGG CGA G
DQB1*02:85        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
```

Fig. 3. Comparison of sequence exon 2 alleles *HLA-DQB1*02:01:01* and *HLA-DQB1*02:85*. The picture is created by means of the IPD-IMGT/HLA Database [1]

```
AA Codon                        10                  15                  20                  25
DQB1*06:03:01     AG GAT TTC GTG TAC CAG TTT AAG GGC ATG TGC TAC TTC ACC AAC GGG ACG GAG CGC GTG CGT CTT GTA ACC AGA
DQB1*06:210       -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         30                  35                  40                  45                  50
DQB1*06:03:01     CAC ATC TAT AAC CGA GAG GAG TAC GCG CGC TTC GAC AGC GAC GTG GGG GTG TAC CGC GCG GTG ACG CCG CAG GGG
DQB1*06:210       --- --- --- --- --- --- --- --- T-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         55                  60                  65                  70                  75
DQB1*06:03:01     CGG CCT GAT GCC GAG TAC TGG AAC AGC CAG AAG GAA GTC CTG GAG GGG ACC CGG GCG GAG TTG GAC ACG GTG TGC
DQB1*06:210       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         80                  85                  90
DQB1*06:03:01     AGA CAC AAC TAC GAG GTG GCG TTC CGC GGG ATC TTG CAG AGG AGA G
DQB1*06:210       --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
```

Fig. 4. Comparison of sequence exon 2 alleles *HLA-DQB1 * 06:03:01* and *HLA-DQB1 * 06:210*. The picture is created by means of the IPD-IMGT/HLA Database [1]

```
AA Codon                        10              15              20              25
DRB1*01:01:01    CA CGT TTC TTG TGG CAG CTT AAG TTT GAA TGT CAT TTC TTC AAT GGG ACG GAG CGG GTG CGG TTG CTG GAA AGA
DRB1*01:01:30    -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         30              35              40              45              50
DRB1*01:01:01    TGC ATC TAT AAC CAA GAG GAG TCC GTG CGC TTC GAC AGC GAC GTG GGG GAG TAC CGG GCG GTG ACG GAG CTG GGG
DRB1*01:01:30    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         55              60              65              70              75
DRB1*01:01:01    CGG CCT GAT GCC GAG TAC TGG AAC AGC CAG AAG GAC CTC CTG GAG CAG AGG CGG GCC GCG GTG GAC ACC TAC TGC
DRB1*01:01:30    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon         80              85              90
DRB1*01:01:01    AGA CAC AAC TAC GGG GTT GGT GAG AGC TTC ACA GTG CAG CGG CGA G
DRB1*01:01:30    --- --- --- --- --- --- --- --- --- --- --- --- --- --T --- -
```

Fig. 5. Comparison of sequence exon 2 alleles *HLA-DRB1*01:01:01* and *HLA-DRB1*01:01:30*. The picture is created by means of the IPD-IMGT/HLA Database [1]

The sequence of nucleotides in codon 93 CGG is changed to CGT. The substitution is synonymous since a change in the amino acid (arginine) does not occur. The nucleotide sequence of the new *HLA-DRB1*01* allele is available under the accession number KY026176 in the GenBank database. The new *HLA-DRB1*01* allele has been officially assigned as *HLA-DRB1*01:01:30* by the WHO Nomenclature Committee for Factors of the HLA System [3].

Conclusion

The results of our work are in accordance with previously published data [4, 8], which demonstrate the advantage of monoallelic Sanger sequencing which provide opportunity of the separate sequencing for the initially studied gene alleles, that allowing to resolve the ambiguities when interpreting HLA typing results. Thus, we may fulfill an important requirement of the *WHO Nomenclature Committee for Factors of the HLA System* which regulates the new HLA allele identification procedure.

Conflict of interest

The authors have declared no conflicting interests.

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Идентификация новых аллелей *HLA-B*44:02:45*, *DQB1*02:85*, *DQB1*06:210*, *DRB1*01:01:30* с помощью моноаллельного секвенирования по Сэнгеру

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Резюме

С помощью метода моноаллельного секвенирования по Сэнгеру идентифицированы четыре новых HLA аллеля *HLA-B*44:02:45, HLA-DQB1*02:85, HLA-DQB1*06:210, HLA-DRB1*01:01:30*. Особенностью метода является выполнение на начальном этапе исследования аллель специфичной ПЦР, обеспечивающей последующую раздельную амплификацию аллелей анализируемого гена. Это, в свою очередь, позволяет выполнить изолированное секвенирование определенного аллеля и избежать неоднозначных результатов HLA типирования, наблюдающихся при выполнении локус-специфичного секвенирования.

Изолированное секвенирование аллелей изучаемого гена является необходимым условием регистрации новых HLA аллелей Номенклатурным Комитетом по факторам HLA системы Всемирной Организации Здравоохранения.

Ключевые слова

Главный комплекс совместимости, новые аллели HLA, моноаллельное секвенирование по Сэнгеру.