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Effect of major histocompatibility complex haplotype matching by *C4* and *MICA* genotyping on acute graft versus host disease in unrelated hematopoietic stem cell transplantation

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We explored whether matching of human leukocyte antigen (*HLA*) haplotypes between the recipient and donor of hematopoietic stem cell transplantation (HSCT) predicted by *C4* and *MICA* typing is associated with the incidence of acute graft versus host disease (aGVHD). DNA preparations collected from a total of 81 recipient and donor pairs were used for PCR-based *C4* subtyping and/or *MICA* sequence-based typing. Incidences of aGVHD were compared according to *C4* and *MICA* matching. The six most common *MICA* alleles were *MICA*008:01*, **010:01*, **002:01*, **004*, **009:01/049*, and **012:01*. Among the 59 unrelated pairs, *HLA* alleles were matched in 34 (57.6%). *C4* subtypes were identical between the recipient and donor in 28 (82.4%) *HLA*-matched unrelated pairs, while *MICA* genotypes were matched in all *HLA*-matched unrelated pairs. In the 22 *HLA*-matched related pairs, all recipients showed identical *C4* subtypes with their respective donors. In multivariate analysis, *C4* mismatch was a significant risk factor associated with the development of aGVHD in unrelated HSCT (hazard ratio = 3.24, $P = 0.006$). PCR-based *C4* subtyping is a simple method for assessing the genetic identity of the *HLA* region between a recipient and unrelated donor. This test would be also useful for prediction of aGVHD in HSCT.

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1. Introduction

The combination of human leukocyte antigen (*HLA*) allele types for different loci on one of the chromosome pairs is defined as the 'haplotype'. *HLA* genes are located in a relatively small domain (~3600 kb) on chromosome 6p; thus, one of the two *HLA* haplotypes from each parent is inherited by the progeny under most circumstances.

Matching of genes that belong to the human major histocompatibility complex (MHC) including *HLA* loci between the donor and recipient for hematopoietic stem cell transplantation (HSCT) is one of the most important factors for patient prognosis [1]. However, many other genetic factors are known to be related to the outcomes of HSCT [2–9]. Therefore, a hematopoietic stem cell donor should be more genetically identical to a patient to improve clinical outcomes after HSCT. In the current guidelines from the National Marrow Donor Program, genotyping and matching for at least four *HLA* loci (*HLA-A*, *-B*, *-C*, and *-DRB1*) are recommended [10].

HLA genotypes in a certain ethnic group or population are known to be relatively conserved along repeated generations

Abbreviations: HSCT, hematopoietic stem cell transplantation; SBT, sequence-based typing; HSC, hematopoietic stem cell; aGVHD, acute graft versus host disease; SNP, single nucleotide polymorphism; HR, hazard ratio; OS, overall survival; DFS, disease free survival; CI, confidence interval.

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[11]. However, irrespective of *HLA* genotype, genes known to be related to immunological functions can be inherited heterogeneously because there is a chance of recombination between the pair of the same chromosome during meiosis [12]. Moreover, it is currently not practical to perform high-resolution sequence-based typing (SBT) for most of the relevant or suspicious genes, including *HLA* loci other than *HLA-A*, *-B*, *-C*, and *-DRB1*. Therefore, a proxy marker on chromosome 6p, which can represent extended haplotypes of genes other than *HLA*, can be useful to predict the degree of genetic concordance between the recipient and unrelated donor to estimate the risk for developing graft-versus-host disease (GVHD) and other adverse events after HSCT.

In most cases when the *HLA* genotypes of the donor and the recipient are identical, a closely related donor for HSCT would have identical genotypes for not only *HLA* genes but also other genes located on chromosome 6p. However, *HLA* zero-mismatched HSCT does not always guarantee matching of both *HLA* haplotypes and other relevant genes on chromosome 6p between the recipient and unrelated donor when only *HLA* loci such as *HLA-A*, *-B*, *-C*, and *-DRB1* are considered for matching. In these contexts, genotyping and matching of some immunoregulatory genes on chromosome 6p between the hematopoietic stem cell (HSC) donor and the recipient can be helpful to ensure more genetically identical transplantation, particularly when the donor is unrelated to the recipient [13,14]. However, only few reports have explored the effect of both haplotypes matching on the clinical outcomes of unrelated HSCT [15–18].

GVHD remains one of the significant adverse events after HSCT. There have been many studies on the genetic polymorphisms associated with the risk of developing GVHD [2,7]. However, proxy markers for the identification of genetic identity between HSC donor and recipient pairs and for prediction of the probability developing GVHD have not been well-assessed. The *C4* gene belongs to the MHC class III (gamma block of MHC), which is located between the MHC class I and II domains, and is a possible marker for identifying the degree of genetic identity between a recipient and a donor. The *MICA* gene is located on the proximal region of the MHC beta block (chromosome 6p 31.37–31.38) and can also be used as a proxy marker.

In this study, we explored whether matching of both *HLA* haplotypes for ensuring more genetically identical HSCTs between the recipient and unrelated donor can be assessed by determining matching of proxy genes, such as *C4* and *MICA*. We also evaluated whether matching of these proxy markers between the unrelated HSCT donor and recipient are related to the incidence of acute GVHD (aGVHD).

2. Materials and methods

2.1. Subjects

During 2008 and 2013, a total of 162 DNA preparations were collected at Severance Hospital from 22 recipients and related donors, as well as 59 unrelated recipient/donor pairs, and were stored at −70 °C for later use. DNA from whole blood samples was extracted using a QuickGene-Mini 80 nucleic acid isolation instrument with the QuickGene DNA whole blood kit S (Kurabo Industries Ltd., Osaka, Japan). Only samples with a sufficient quantity and those collected from HSC donors and their respective recipients suffering from acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), aplastic anemia, and other hematologic diseases excluding chronic myelogenous leukemia (CML) were included in this study. All donors and recipients included in this study were Korean. This study was approved by the Institutional Review Board of Severance Hospital.

2.2. Review of medical records

Medical records including sex and age of the patients, clinical and genetic characteristics of the disease, symptoms and clinical grades of GVHD, and other clinicopathologic conditions were reviewed. Diagnosis of aGVHD was referred to the clinicians including the hemato-oncologist, ophthalmologist, and dermatologist or to the results of skin or mouth mucosa biopsies, when the first symptom of GVHD developed within 100 days after HSCT.

2.3. C4 subtyping

We performed sequence specific primer-PCR-based *C4A* (Rodgers blood group antigen) and *C4B* (Chido blood group antigen) subtyping as described previously [19]. Isotype-specific *C4A*/*C4B* primers were as follows: A-5′, GCATG CTCCT GTCTA ACACT GGAC; A-3′, AGGAC CCCTG TCCAG TGTTA GAC; B-5′, TGCTC CTATG TATCA CTGGA GAGA; B-3′, AGGAC CTCTC TCCAG TGATA CAT. In addition, sequence-specific Rg/Ch primers were as follows: Ch-5, TGCGG CTTGG TTGTC ACGGG A; Ch5, TGCGG CTTGG TTGTC ACGGG G; Rg1, AGGTT GTTGT GGGCA ACACC GA; Ch1, AGGTT GTTGT GGGCA ACACC CC; Rg3, AGCCT CCATC TCAAA GGCAA A; Ch6, AGCCT CCATC TCAAA GGCAA G. Primers for the 780-bp fragment from the *C4* gene (7b, TGAGG GGACC AGCTG GAAGA GTC; 8, CAAGC GCCGC CACCT GTGCC CTA) were used for all PCR reactions as an internal control. Primer combinations for *C4A* subtyping included A-5′/Ch-5, A-5′/Ch5, A-3′/Rg1, A-3′/Ch1, Rg1/Rg3, and Rg1/Ch6, and those for *C4B* were B-5′/Ch5, B-5′/Ch-5, B-3′/Ch1, B-3′/Rg1, Ch1/Ch6, and Ch1/Rg3. PCR was performed with a mixture of 30–50-ng genomic DNA, 10 pmol of each control primer and respective forward and reverse primers, and PCR premix (BioSewoom Inc., Seoul, Korea) with a final volume of 20 μL using the C1000™ Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). PCR products were separated on a 1% agarose gel stained with ethidium bromide, and any results without amplification of both the internal control fragment and allele-specific product were repeated. Detailed procedures including cycle conditions were based on previous studies [19,20]. Results for a HSCT recipient and respective donor were compared, and the pair was defined to have identical *C4* subtypes when the banding patterns for PCR products were identical between the recipient and donor (Fig. 1).

2.4. MICA genotyping

PCR primers were designed to amplify the *MICA* gene from exon 2 to exon 5. Detailed procedures including cycle conditions and primer sequences can be found in the previous study [21]. Purified PCR products were sequenced with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using *MICA* gene-specific primers. The reaction products were purified and analyzed using an ABI 3730xl DNA analyzer (Applied Biosystems). The sequence data were compared with previously reported sequences for *MICA* alleles using the *HLA* Analysis program (Biosewoom Inc., Seoul, Korea). All homozygous results for *MICA* genotyping were tested for the presence of a null allele using sequence-specific PCR primers, as described previously [22].

2.5. Single nucleotide polymorphism (SNP) microarray

Selected samples collected from the recipient and unrelated donor pairs were used to determine the SNP genotypes of various genes that belong to and are outside of the human MHC domain. The SNP microarray experiments were performed using Infinium HumanExome BeadChip kits (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. This method is designed for genotyping of more than 250,000 SNPs in the human

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