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Original Article

Recognition of the deduced probable HLA haplotypes associated with HLA low incidence alleles B*13:50 (A*11:02-B*13:50-DRB1*07:01) and B*51:39 (A*02-B*51:39-DRB1*15; and A*11-B*51:39-DRB1*15) in Taiwanese unrelated hematopoietic stem cell donors

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ABSTRACT

Objectives: HLA-B*13:50 and -B*51:39 are two low incidence alleles in the HLA-B locus. The objective of this study is to report the deduced probable human leukocyte antigen (HLA) haplotypes in association with HLA-B*13:50 and -B*51:39 in Taiwanese unrelated bone marrow hematopoietic stem cell donors.
Materials and Methods: A sequence-based typing method was used to confirm the two low incidence alleles observed. Polymerase chain reaction was performed to amplify exons 2 and 3 in the HLA-A and HLA-B loci and exon 2 in the HLA-DRB1 locus with group-specific primer sets. Amplicons were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit in both directions according to the manufacturer's protocols.
Results: The DNA sequence of B*13:50 is identical to B*13:01:01 in exons 2 and 3, except for a one nucleotide substitution at residue 482 (A→T), which results in a one amino acid replacement at position 137 (aspartic acid→valine). We deduced the probable HLA haplotype in association with B*13:50 in Taiwanese as A*11:02-B*13:50-DRB1*07:01. The DNA sequence of B*51:39 is identical to B*51:01:03 in exons 2 and 3 except for two nucleotide exchanges at residue 226 (A→G) and residue 228 (A→G), which result in a one amino acid substitution at position 52 (isoleucine→valine).The probable HLA haplotypes associated with B*51:39 in Taiwanese may be deduced as A*02-B*51:39-DRB1*15 and A*11-B*51:39-DRB1*15.
Conclusion: Information on the deduced HLA haplotypes in association with the low incidence B*13:50 and B*51:39 alleles that we report here is valuable for HLA testing laboratories for reference purposes and for stem cell transplantation donor search coordinators, to determine the likelihood of finding compatible donors in unrelated bone marrow donor registries for patients carrying these two uncommon HLA alleles.

1. Introduction

The continuing discovery of new human leukocyte antigen (HLA) alleles and recognition HLA low incidence alleles has enriched our understanding of the complexity of the HLA system. The major histocompatibility complex (MHC) in humans consists of several loci of genes located on the short arm of chromosome 6 at 6p21.3. These loci are classified into Class I, II, and III of the MHC. The genes of HLA alleles are located in the MHC Class I and II regions. The HLA genes are characterized by their extreme allelic polymorphism and their variations and diversity among different ethnic groups and racial populations. HLA molecules have been definitely defined as transplant antigens with strong relevance in tissue transplantation, and their molecule similarity between donors and recipients is being considered as a prediction factor for graft survival and graft versus host disease. It is imperative to

Conflict of interest: none.

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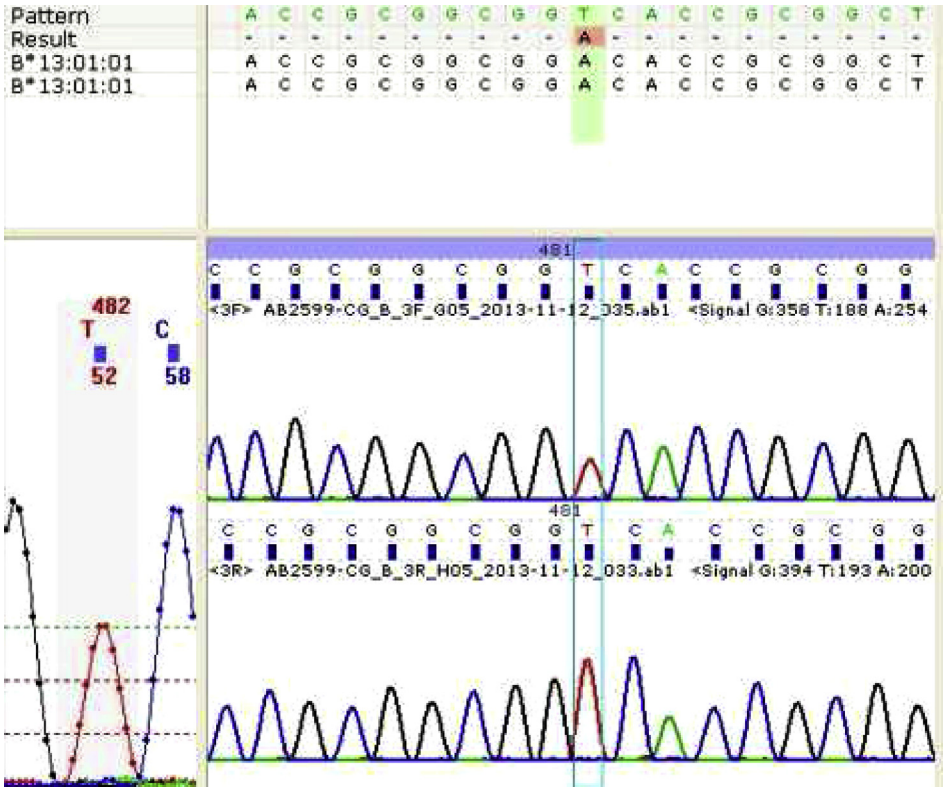
precisely characterize any new and low incidence alleles encountered during routine HLA typing procedures. To facilitate successful and comprehensive unrelated bone marrow donor searches for patients in need of hematopoietic stem cell transplantation, persistent effort is needed to resolve unidentified, ambiguous or low incidence alleles in order to offer better services for HLA matching and donor selection.

HLA-B*13:50 and HLA-B*51:39 were first reported to the International ImMunoGeneTics/HLA (IMGT/HLA) database in 2011 and 2013 (Cell ID HC4927 and Cell ID HC27439) and 2005 (Cell ID HC13952), respectively [1]. Here, we report the deduced probable HLA haplotypes in association with B*13:50 and B*51:39. We further postulate that there are two plausible HLA haplotypes in association with B*51:39 in Taiwanese and that the haplotypes associated with B*13:50 and B*51:39 are restricted to Asians.

2. Materials and methods

Peripheral whole blood samples from unrelated bone marrow stem cell donors with Taiwanese ethnicity were collected in acid citrate dextrose anticoagulant. Formal written consents were signed by the donors before blood collection. The acid citrate dextrose whole blood samples were stored at −80°C until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The DNA material was subjected to HLA genotyping for



B

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cDNA                 10         20         30         40         50         60         70         80         90        100
 B*13:01:01  ATGCGGGTCA CGGCGCCCCG AACCCTCCTC CTGCTGCTCT GGGGGGCAGT GGCCCTGACC GAGACCTGGG CCG|GCTCCCA CTCCATGAGG TATTTCTACA
 B*13:50     ********** ********** ********** ********** ********** ********** ********** ***|------- ---------- ----------

 cDNA                110        120        130        140        150        160        170        180        190        200
 B*13:01:01  CCGCCATGTC CCGGCCCGGC CGCGGGGAGC CCCGCTTCAT CACCGTGGGC TACGTGGACG ACACCCAGTT CGTGAGGTTC GACAGCGACG CCACGAGTCC
 B*13:50     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                210        220        230        240        250        260        270        280        290        300
 B*13:01:01  GAGGATGGCG CCCCGGGCGC CATGGATAGA GCAGGAGGGG CCGGAGTATT GGGACCGGGA GACACAGATC TCCAAGACCA ACACACAGAC TTACCGAGAG
 B*13:50     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                310        320        330        340        350        360        370        380        390        400
 B*13:01:01  AACCTGCGCA CCGCGCTCCG CTACTACAAC CAGAGCGAGG CCG|GGTCTCA CATCATCCAG AGGATGTATG GCTGCGACCT GGGGCCGGAC GGGCGCCTCC
 B*13:50     ---------- ---------- ---------- ---------- ---|------- ---------- ---------- ---------- ---------- ----------

 cDNA                410        420        430        440        450        460        470        480        490        500
 B*13:01:01  TCCGCGGGCA TAACCAGTTA GCCTACGACG GCAAGGATTA CATCGCCCTG AACGAGGACC TGAGCTCCTG GACCGCGGCG GACACCGCGG CTCAGATCAC
 B*13:50     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -T-------- ----------

 cDNA                510        520        530        540        550        560        570        580        590        600
 B*13:01:01  CCAGCTCAAG TGGGAGGCGG CCCGTGTGGC GGAGCAGCTG AGAGCCTACC TGGAGGGCGA GTGCGTGGAG TGGCTCCGCA GATACCTGGA GAACGGGAAG
 B*13:50     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                610        620        630        640        650        660        670        680        690        700
 B*13:01:01  GAGACGCTGC AGCGCGCGG|A CCCCCCAAAG ACACACGTGA CCCACCACCC CATCTCTGAC CATGAGGCCA CCCTGAGGTG CTGGGCCCTG GGCTTCTACC
 B*13:50     ---------- ---------|- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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Fig. 1. (A) The raw sequence data (forward and reverse strains) show that at residue 482, the nucleotide A of B*13:01:01 is replaced by the T (in red) of B*13:50; (B) the DNA sequence of B*13:50 is identical to B*13:01:01 in exons 2 and 3, except for a one nucleotide substitution at residue 482 (A→T) (shaded).