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Original Article

Deduced probable human leukocyte antigen haplotypes associated with HLA-A*11:256Q and HLA-A*02:621 identified by case analyses of Taiwanese individuals

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Abstract

Objective: HLA-A*11:256Q and HLA-A*02:621 are two low-frequency HLA-A alleles. The aim here is to report the ethnicity of A*11:256Q and A*02:621 and associated human leukocyte antigen (HLA) haplotypes among Taiwanese individuals. **Materials and Methods:** HLA data from randomized Taiwanese registered in the Tzu Chi Stem Cells Centre and China Shanghai Tissuebank Diagnostics were analyzed. HLA typing of the donors was carried out using a sequence-based typing method to confirm the two low-incidence alleles. Polymerase chain reaction was performed to amplify exons 2 and 3 of the HLA-A and HLA-B loci and exon 2 of the HLA-DRB1 locus using group-specific primer sets. The amplicons were sequenced in both directions using BigDye Terminator Cycle Sequencing Ready Reaction kits and the manufacturer's protocols. Exon 1 and exons 4-8 of the A*11:256Q allele were also sequenced and analyzed. **Results:** The Taiwanese ethnicity for both A*11:256Q and A*02:621 alleles was confirmed in this study. Further, the DNA sequence of A*11:256Q was confirmed to be identical to A*11:02:01from exon 1 to exon 8 except for the residues from 409 to 417 where a segment of nine nucleotides (TACCGGCAG) is deleted in A*11:256Q. The HLA haplotype associated with A*11:256Q was deduced as A*11:256Q-B*27-DRB1*12. In exons 2 and 3, the DNA sequence of A*02:621 is identical to A*02:01:01:01 except at residue 169 where T of A*02:01:01:01 is replaced by C in A*02:621 (at codon 33; TTC->CTC). The HLA haplotype in association with A*02:621 was deduced as A*02:621-B*15:18-DRB1*12:02. **Conclusion:** The information on the ethnicity of the A*11:256Q and A*02:621 alleles and the deduced probable HLA haplotypes associated with the two low-incidence alleles reported here are valuable to HLA testing laboratories for reference purposes. In addition, they can be used by stem cell transplantation donor search coordinators to aid in finding compatible donors in unrelated bone marrow donor registries when a patient carries these uncommon HLA alleles.

Keywords: *A*02:621, A*11:256Q, Human leukocyte antigen, Sequence-based typing, Taiwanese*

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Introduction

Transplantation of allogeneic hematopoietic stem cells has been employed as a curative therapy for hematological malignancies and other hematological or immune disorders. Human leukocyte antigen (HLA) molecules have been definitely defined as transplant antigens and have a strong relevance to tissue or organ transplantation. The molecular similarity of these genes between transplant donors and recipients is considered a predictive factor for graft survival and graft versus host disease; this is because they can elicit immune responses either by recognition of polymorphic fragments of foreign HLA molecules or through the presentation of variable peptides [1,2]. The genes encoding the HLA alleles are located in the major histocompatibility complex Class I and II regions. HLA genes are characterized by their extreme allelic polymorphism as well as their variation and diversity across different ethnic groups [3].

Determination of HLA haplotypes is essential when matching between donor and recipient for unrelated stem cell

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transplantation since this increases the likelihood of matching at other loci within the HLA region compared to merely matching at the individual allele level. Determination of HLA haplotypes may be accomplished by HLA typing of genetically related family members [4] and by prediction based on tissue typing in large populations [5-7]. Alternatively, it can be achieved by deduction using the typing results from donors with allelic homozygosities in the HLA-A, HLA-B, and HLA-DR loci [8]. In family studies, segregation of HLA individual alleles provides evidence of allelic linkage [4]. In population studies, determination of haplotypes involves noting whether alleles at the other two loci are consistently present, and in such cases, a family study is not performed. Instead, most available haplotype data are derived from studies of a population of unrelated individuals in whom the putative haplotype is defined by statistical association analysis [6,7].

The nucleotide sequences of HLA-A*11:256Q and HLA-A*02:621 were first identified in two Taiwanese individuals and submitted to GenBank (accession numbers Kx810861 and LT223710, respectively) and the IMGT/HLA Database in October 2016 (submission numbers HWS10026903 and HWS10026194, respectively) [3,9,10]. However, neither HLA haplotype in association with A*11:256Q and A*02:621 nor their ethnicity was definitely suggested. Here, we confirm the Taiwanese ethnicity of A*11:256Q and A*02:621 and report their deduced most probable HLA-associated haplotypes based on HLA typing of unrelated Taiwanese individuals bearing the A*11:256Q and A*02:621 alleles.

Materials and methods

Peripheral whole blood samples from Taiwanese individuals and mainland Chinese individuals in the Tzu Chi Stem Cells Centre and China Shanghai Tissuebank Diagnostics were collected in acid citrate dextrose (ACD) anticoagulant. Formal written consents were given by the donors before blood collection. The ACD whole blood samples were stored at −80°C until use. Peripheral blood genomic DNA was extracted from a total of 5081 Tzu Chi Stem Cells Centre donors and 33839 China Shanghai Tissuebank Diagnostics donors using QIAamp DNA Blood Mini kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The genomic DNA obtained was subjected to HLA genotyping for the HLA-A, HLA-B, and HLA-DRB1 loci using commercial polymerase chain reaction-sequencing based typing kits (TBG, Medigen Biotechnology, Taipei, Taiwan). The amplicon was then sequenced in both directions using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions [11-14]. Exon 1 and exons 4–8 of the A*11:256Q allele were also sequenced and analyzed.

Determination of the deduced probable A*11:256Q and A*02:621-associated HLA haplotypes in this study was carried out by looking at the commonly shared HLA-A, HLA-B, and HLA-DRB1 typing of the donors carrying A*11:256Q and A*02:621 in this study and the donors with A*11:256Q and A*02:621 reported previously [3,9,10].

Results

In this study, we confirmed the Taiwanese ethnicity of the A*11:256Q and A*02:621 alleles. The DNA sequence of A*11:256Q is identical to A*11:02:01 from exon 1 to exon 8 except for the residues from 409 to 417 where a segment of nine nucleotides (TACCGGCAG) is deleted in A*11:256Q [Figure 1a]. The nucleotide deletion generates deletion of three amino acids at the residues 113 (Y; tyrosine), 114 (R; arginine), and 115 (Q; glutamine) in A*11:256Q [Figure 1b] [9]. The World Health Organization Committee for Factors of the HLA System has added a "Q" to the allele name to indicate that expression is questionable because the cell carrying A*11:256Q is not available to determine the impact on expression of the nucleotide deletion [9].

In a total of 5081 randomized Taiwanese individuals tested, two individuals with A*11:256Q were recognized which makes the frequency of A*11:256Q in the Taiwanese population approximately 0.039%. However, in a total of 33839 mainland Chinese individuals tested, no individual with A*11:256Q was recognized.

We also confirmed that in exons 2 and 3, the DNA sequence of A*02:621 is identical to A*02:01:01:01 except at residue 169 where T of A*02:01:01:01 is substituted by C in A*02:621 (at codon 33; TTC->CTC) [Figure 2a]. The nucleotide substitution causes a one amino acid exchange at codon 33 where phenylalanine (F) of A*02:01:01:01 is altered to leucine (L) in A*02:621 [Figure 2b] [10]. In a total of 5081 randomized Taiwanese individuals tested, two individuals with A*02:621 were detected which makes the frequency of A*02:261 in the Taiwanese population approximately 0.039%. As with A*11:256Q, no individual with A*02:621 was found to carry the allele in 33839 mainland Chinese individuals studied. The frequency variation of A*11:256Q and A*02:621 between Taiwanese and mainland Chinese populations is statistically significant ($P = 0.017$, Fisher's exact test).

The extended HLA typing of the donor with A*11:256Q in this study is A*02:01, A11*256Q, B*27, B*40, DRB1*11, and DRB1*12:02. Together with the typing (A*11, A*11:256Q, B*27, B*46, DRB1*09, and DRB1*12) of the previous donor with A*11:256Q reported to the IMGT/HLA Database [3,9], the most probable HLA haplotype in association with A*11:256Q may be deduced as A*11:256Q-B*27-DRB1*12 [Table 1].

The extended HLA typing of the donor with A*02:621 is A*02:621, A*03:01, B*15:18, B*35:03, DRB1*12:01, and DRB1*12:02. Together with the typing (A*02:621, A*24:02, B*15:18, B*51:02, DRB1*08:03, and DRB1*12:02) of the previous donor with A*02:621 reported to the IMGT/HLA Database [3,10], the most probable HLA haplotype in association with A*02:621 may be deduced as A*02:621-B*15:18-DR B1*12:02 [Table 1].

Discussion

In this study, we show HLA-A*11:256Q and HLA-A*02:621 are two rare HLA-A locus alleles in the Taiwanese population. The frequency of each allele is about 0.039% as estimated in 5081 Taiwanese individuals studied. Most likely, both alleles

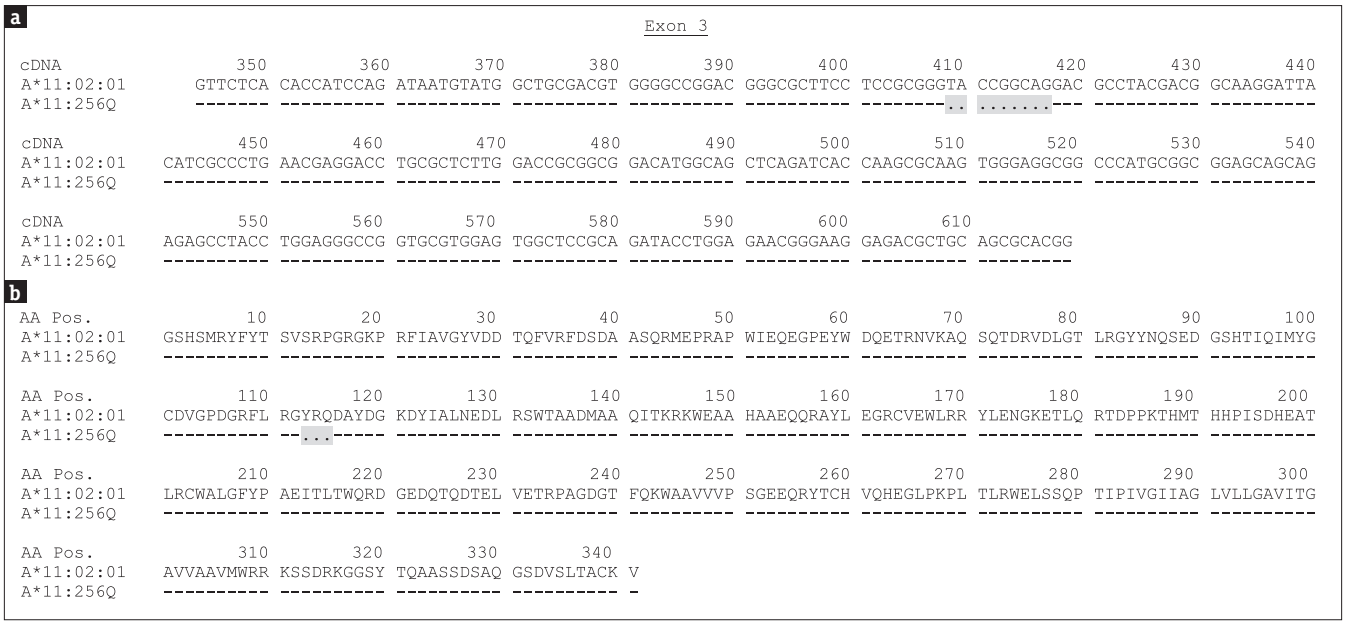

Figure 1: (a) The DNA sequence of A*11:256Q is identical to A*11:02:01 from exons 1 to 8 except for the residues from 409 to 417 of exon 3 (only exon 3 is shown here) where a segment of nine nucleotides (TACCGGCAG) is deleted in A*11:256Q (shaded). (b) The nucleotide deletion introduces deletion of three amino acids at the residues 113 (Y; tyrosine), 114 (R; arginine), and 115 (Q; glutamine) in A*11:256Q (shaded). Dashes indicate nucleotide or amino acid identity with A*11:02:01

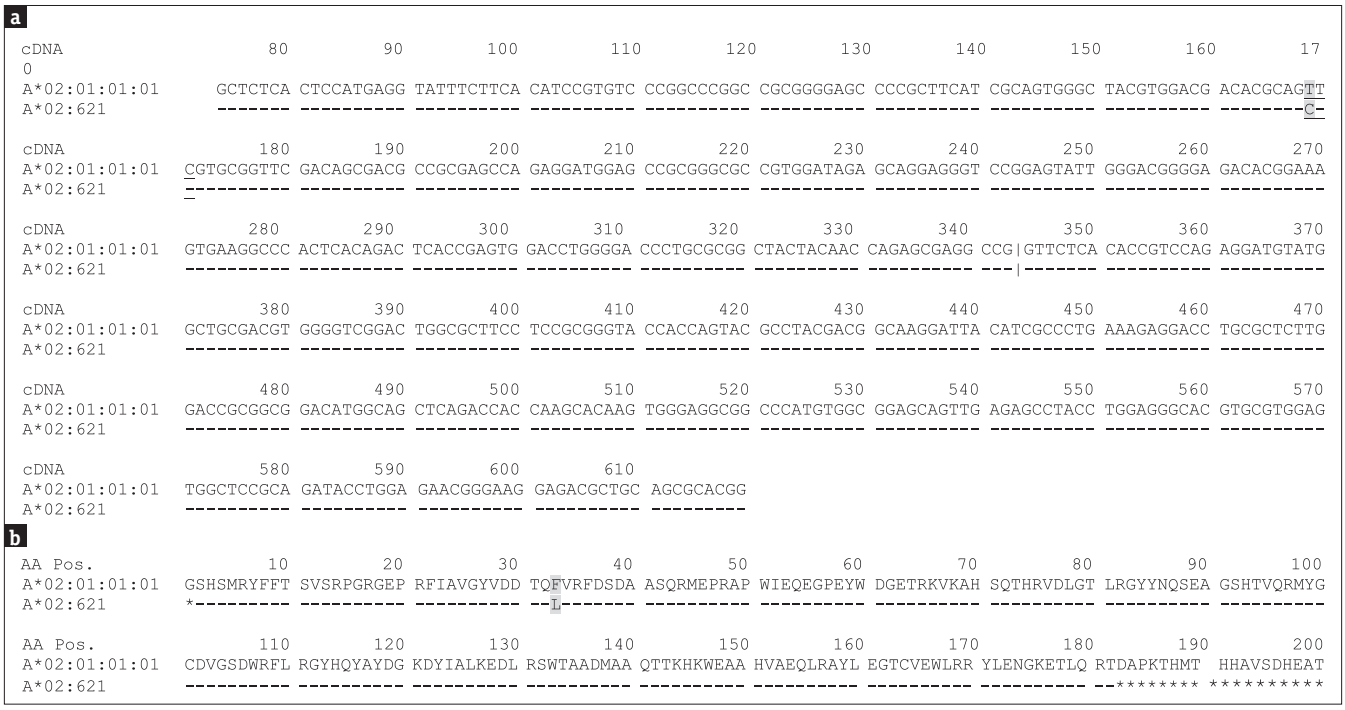

Figure 2: (a) In exon 2 and exon 3, the DNA sequence of A*02:621 is identical to A*02:01:01:01 except for residue 169 (at codon 33; underlined) where T of A*02:01:01:01 is replaced by C in A*02:621 (shaded). Exons 2 and 3 are separated by pipes (|) between nucleotides 343 and 344. (b) The nucleotide substitution leads to a one amino acid change at codon 33 where phenylalanine (F) of A*02:01:01:01 is changed to leucine (L) in A*02:621 (shaded). Dashes indicate nucleotide or amino acid identity with A*02:01:01:01

are restricted to the Taiwanese population since none of the 33839 mainland Chinese individuals tested had A*11:256Q and A*02:621, and furthermore, the Allele Frequency Net Database (http://www.allelefrequencies.net/) does not have any record of A*11:256Q and A*02:621 reported so far.

Information on the ethnicity of A*11:256Q or A*02:621 and associated HLA haplotypes may be employed in anthropological investigations. In addition, search coordinators working at unrelated bone marrow donor registries can use this information in the allocation of appropriate unrelated bone marrow hematopoietic stem cell donors to patients with A*11:256Q or A*02:621 who are in need of a transplant.

Based on the commonly shared HLA-A, HLA-B, and HLA-DRB1 allele typing between the donors

Table 1: The deduced probable human leukocyte antigen haplotypes in association with A11*256Q and A*02:621 (underlined). In a total of 5081 randomized unrelated Taiwanese individuals tested, two individuals with A*11:256Q and two individuals with A*02:621 were detected, which makes the incidence of A*11:256Q and A*02:621 about 0.039% in the Taiwanese population

Donor ID	HLA-A, HLA-B, HLA-DRB1 typing of donors	Deduced probable HLA haplotype
Donor 1	A*02:01, A11*256Q, B*27, B*40, DRB1*11, DRB1*12:02	A*02:256Q-B*27-DRB1*12
Donor 2	A*11, A*11:256Q, B*27, B*46, DRB1*09, DRB1*12	A*02:256Q-B*27-DRB1*12
Donor 3	A*02:621, A*03:01, B*15:18, B*35:03, DRB1*12:01, DRB1*12:02	A*02:621-B*15:18-DRB1*12:02
Donor 4	A*02:621, A*24:02, B*15:18, B*51:02, DRB1*08:03, DRB1*12:02	A*02:621-B*15:18-DRB1*12:02

HLA: Human leukocyte antigen

carrying A*11:256Q and A*02:621 and the donors with A*11:256Q and A*02:621 reported to the IMGT/HLA Database previously [3,9,10], we deduced that the two most probable A*11:256Q- and A*02:621-associated HLA haplotypes are A*11:256Q-B*27-DRB1*12 and A*02:621-B*15:18-DRB1*12:02. If our assumption that A*11:256Q and A*02:621 are restricted to the Taiwanese population is correct, the haplotypes A*11:256Q-B*27-DRB1*12 and A*02:621-B*15:18-DRB1*12:02 may very well be restricted to the Taiwanese population as well.

The most direct and classic method to determine HLA haplotypes is through family studies if suitable test material from a number of key family members is available. Alternatively, a population study may be employed if a significant number of unrelated donors are available [5-7]. However, the haplotypes deduced through a population investigation are considered to be either likely or most probable.

The number of known HLA alleles is increasing dramatically due to recent developments in DNA-based molecular typing technology [3]. The vast HLA diversity across ethnic groups is both unique and important. Facilitating an appropriate HLA-match for a given unrelated bone marrow stem cell donor allows for successful stem cell transplantation and relies on the accuracy of HLA typing. It also depends on having the spirit and strength to resolve the unknown, ambiguous, and low-incidence genes that still are present in the HLA system.

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Declaration of patient's consent

The authors certify that all patients provided appropriate patient consent forms. In the form, all patients gave consent for their images and other clinical information to be reported in the journal. All patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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