



## Original Article

# Two probable human leukocyte antigen haplotypes in association with human leukocyte antigen HLA-DRB1\*13:50:01 identified in 41 randomized unrelated Taiwanese individuals

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## ABSTRACT

**Objectives:** Here, we show two probable haplotypes associated with the human leukocyte antigen (HLA) DRB1\*13:50:01 allele. The haplotypes were observed from 41 randomized unrelated Taiwanese individuals among a population of 23,064 individuals tested. **Materials and Methods:** The samples in this study were blood samples, preserved in dipotassium ethylenediaminetetraacetic acid and/or ACD anticoagulants. The population is of donors from Tzu Chi Bone Marrow Donor Registry. Allele typing was performed using the sequence-based typing method, Sanger's sequencing. To discern the HLA-A and HLA-B alleles, exons 2 and 3 were sequenced. For DRB1 alleles, exon 2 was sequenced. Target exon sequence amplifications were done by a polymerase chain reaction and the resulting amplicons were sequenced by Bigdye Terminator Cycle Sequencing Ready Reaction kit, according to the manufacturer's protocols. **Results:** Two probable haplotypes that are associated with the DRB1\*13:50:01 were observed among the 23,064 Taiwanese randomized unrelated individuals. One of the haplotypes is observed in 39 individuals while the other in two individuals. **Conclusion:** The findings in this study may be useful in studies reinforcing the understanding and clinical application of the polymorphism of HLA genes and haplotypes.

**KEYWORDS:** DRB1\*13:50:01, Haplotypes, Human leukocyte antigen, Taiwanese

## INTRODUCTION

The genes of the human leukocyte antigen (HLA) system of the major histocompatibility complex are genes found in the short arm of chromosome number 6. These genes are characterized by a very high polymorphism tendency which allows them to activate the immune system in fighting many biological intruders. HLA holds a very significant role in the transplantation portion of precision medicine in the clinical practices, to reduce graft versus host diseases and improve graft acceptance by the host. Previous studies show associations between some HLA genes and clinical manifestations, where the gene may either show to favor or even be disadvantageous for the condition [1].

HLA genes may vary as a result of many different modification mechanisms and it is those modifications that result in the formation of the different protein product (s) that are significant in transplantation. Chen *et al.* [2] have shown the observed modification of two protein sites in the protein of DRB1\*11:01:01 for the formation of DRB1\*13:50:01.

In transplantation medicine, donor-recipient matching is focused on allele level typing and yet haplotype match may be an even closer relationship in the HLA genes [3,4].

A haplotype in HLA refers to genes that are inherited together from one parent to the offspring. Recombination of the HLA gene is a genetic modification mechanism that may result in the formation of new alleles [5], but for a haplotype to be recognized in the offspring, we see this, especially in family aggregation studies, the gene should be as it was in the parent. In this article, we show the likely HLA haplotype associated with DRB1\*13:50:01 in 41 randomized unrelated Taiwanese individuals. Up to date, the DRB1\*13:50:01 allele has been observed in two individuals, 1DM4038S1 (from Taiwan) and BY00138 (from China), according to the IPD-IMGT/HLA

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Database [6]. Here, in this retrospective study, we show the HLA typing results of 41 randomized unrelated Taiwanese individuals and propose the HLA haplotypes associated with DRB1\*13:50:01.

**MATERIALS AND METHODS**

The samples were peripheral whole blood, with dipotassium ethylenediaminetetraacetic acid and/or acid citrate dextrose (ACD) anticoagulation additives, collected from randomized unrelated Taiwanese individuals with written informed consent at the time of blood collection. All

samples were stored at -80°C until DNA extraction. Nucleic acid extraction was performed using QIAamp DNA Mini Kit-Qiagen, according to the manufacturer’s protocol. The DNA samples were stored at -20° C until use. As this is a retrospective study on reviewing and analyzing the HLA typing data of individual donors, the IRB/IRC approval is exempted.

Low-resolution amplification and sequencing of the genomic HLA genes were carried out using MEDIGEN BIOTECHNOLOGY CORP. Typing Kit, provided by TBG Biotechnology Corp. High-resolution amplification was done

**Table 1: A probable DRB1\*13:50:01 associated HLA haplotype HLA-A\*02-HLA-B\*40-HLA-DRB1\* 13:50:01 is deduced from 39 randomized unrelated Taiwanese individuals who carry A\*02, B\*40 and DRB1\*13:50:01 (underlined) in common. While a second probable DRB1\*13:50:01 associated HLA haplotype HLA-A\*11-HLA-B\*40-HLA-DRB1\* 13:50:01 (shaded) is deduced from two randomized unrelated donors 366796 and 390827 (shaded) who commonly shared A\*11, B\*40 and DRB1\*13:50:01 (shaded)**

Donor No.	HLA-A*	HLA-A*	HLA-B*	HLA-B*	HLA-DRB1*	HLA-DRB1*	Deduced probable DRB1*13:50:01 associated HLA haplotype
033544	<u>02:01</u>	-	<u>40:01</u>	46:01	09:01	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
129512	<u>02:01</u>	02:03	38:02	<u>40:01</u>	<u>13:50:01</u>	<u>14:54</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
195028	<u>02:01</u>	11:01	<u>40:01</u>	46:01	<u>13:50:01</u>	<u>14:05</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
251705	<u>02:01</u>	02:03	38:02	40:01	08:03	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
327854	<u>02:YDVJ</u>	02:ZAKP	<u>40:XX</u>	51:XX	09:01	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
337676	<u>02:XX</u>	11:XX	<u>40:JYVZ</u>	46:KCZA	09:01	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
342141	<u>02:HRTT</u>	24:JHCY	<u>40:DHHU</u>	40:MFCR	08:AAC	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
353019	<u>02:XX</u>	30:NDYD	<u>40:XX</u>	67:AC	<u>13:50:01</u>	16:02	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
355171	<u>02:RGPK</u>	02:XX	<u>40:TXZG</u>	40:TXZG	12:DUKV	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
358059	<u>02:XX</u>	24:TKKM	<u>40:UPSE</u>	56:EFXK	<u>13:50:01</u>	16:02	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
358846	<u>02:TUZN</u>	02:XX	<u>40:XFBK</u>	40:XFBK	03:TFMR	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
360054	<u>02:HJMP</u>	02:VSAT	<u>40:XX</u>	46:XX	08:03	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1*13:50:01
365183	<u>02:XX</u>	02:XX	<u>40:XX</u>	40:XX	12:02	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
<b>366796</b>	<b>11:XAZK</b>	33:XAQM	15:XX	<b>40:XX</b>	03:TFMR	<b>13:50:01</b>	<b>HLA-A*11-HLA-B*40-HLA-DRB1* 13:50:01</b>
366920	<u>02:01</u>	11:01	35:01	<u>40:01</u>	09:01	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
367885	<u>02:XX</u>	11:XX	<u>40:ZAMB</u>	40:ZAMB	08:03	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
371116	<u>02:XUCY</u>	24:XUER	15:THAD	<u>40:YBUA</u>	09:01	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
371484	<u>02:XX</u>	02:YDVK	<u>40:XX</u>	40:XX	12:DUKV	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
385965	<u>02:XX</u>	11:XX	<u>40:ABGFG</u>	40:ABGFG	09:CTZ	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
387047	<u>02:XX</u>	02:XX	<u>40:XX</u>	40:XX	08:03	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
<b>390827</b>	<b>11:01</b>	24:ABGEW	<b>40:XX</b>	56:XX	<b>13:50:01</b>	14:05	<b>HLA-A*11-HLA-B*40-HLA-DRB1* 13:50:01</b>
397763	<u>02:XX</u>	11:XX	13:XXSP	<u>40:XX</u>	<u>13:50:01</u>	15:02	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
397764	<u>02:XX</u>	02:XX	<u>40:XX</u>	51:XX	12:DUKV	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
399480	<u>02:XX</u>	11:XX	13:XXSP	<u>40:XX</u>	<u>13:50:01</u>	16:02	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
404233	<u>02:XX</u>	11:TXMM	27:KGUU	<u>40:XX</u>	12:02	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
406096	<u>02:XX</u>	02:XX	<u>40:XX</u>	54:ACHPD	<u>13:50:01</u>	14:05	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
407222	<u>02:AJTJD</u>	02:AJTJF	38:XX	<u>40:XX</u>	<u>13:50:01</u>	16:02	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
409811	<u>02:XX</u>	11:TXMM	27:KGUU	<u>40:XX</u>	12:02	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
413019	<u>02:AJTJD</u>	02:AJTJF	<u>40:AFXSK</u>	40:AFXSK	03:HMZN	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
416723	<u>02:XX</u>	02:XX	27:KGUU	<u>40:XX</u>	12:02	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
416803	<u>02:XX</u>	11:XX	13:XXSP	<u>40:ATDJN</u>	12:02	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
419548	<u>02:XX</u>	33:XX	<u>40:AHUXT</u>	58:XX	<u>13:50:01</u>	13:AHTPS	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
425603	<u>02:XX</u>	24:XX	<u>40:XX</u>	40:XX	09:CTZ	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
424775	<u>02:XX</u>	02:XX	<u>40:XX</u>	46:XX	08:03	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
430716	<u>02:XX</u>	24:XX	<u>40:AURMP</u>	40:BDMV	11:MWBN	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
435055	<u>02:XX</u>	33:XX	<u>40:XX</u>	58:XX	<u>13:50:01</u>	13:AHTPS	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
435796	<u>02:XX</u>	02:XX	<u>40:XX</u>	40:XX	12:02	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
411269	<u>02:XX</u>	02:XX	38:XX	<u>40:XX</u>	08:09	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
435072	<u>02:XX</u>	11:XX	<u>40:XX</u>	40:XX	08:03	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
442621	<u>02:XX</u>	02:XX	39:XX	<u>40:XX</u>	04:AHTPR	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01
446977	<u>02:XX</u>	02:XX	<u>40:XX</u>	46:XX	04:ASXKT	<u>13:50:01</u>	HLA-A*02-HLA-B*40-HLA-DRB1* 13:50:01

using the HLAssure SE A/B/DRB-EX Locus SBT Kit. The amplicons were then typed using sequence-based typing, Sanger sequencing. To discern the HLA-A and HLA-B loci alleles, exons 2 and 3 were sequenced and only exon 2 for HLA-DRB1. Amplicons were sequenced using the Bigdye Terminator Cycle Sequencing Ready Reaction kit, according to the manufacturer's protocol.

It was through the studying of the typing results in our database that the repeating set of genes among a few individuals was noticed. The investigation was done and the donor typing that had the DRB1\*13:50:01 was collected and analyzed to deduce a possible relationship between this DRB1 allele and alleles in HLA-A and HLA-B loci.

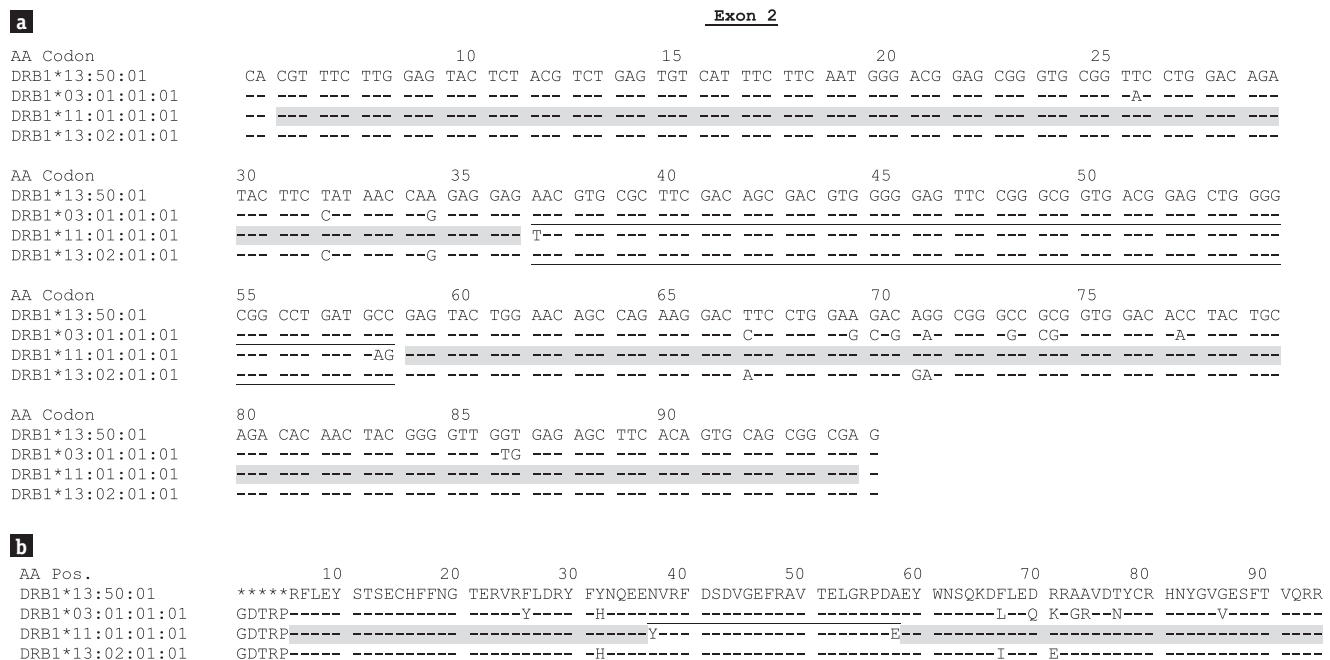
### RESULTS

The frequency of the DRB1\*13:50:01 allele in the general Taiwanese population is estimated approximately 0.18% based on the total number of 23,064 randomized unrelated Taiwanese individuals studied. This frequency is slightly higher than the 0.10% as estimated by the Allele Frequency Net Database for worldwide populations [7].

In the list of HLA typing results of individuals that have the DRB1\*13:50:01 allele [Table 1], we noticed a pattern in HLA-A and HLA-B loci where the HLA-A\*02 and HLA-B\*40 allele group presented. The constant appearance of this set of allele group HLA-A\*02, HLA-B\*40, and DRB1\*13:50:01 in these three loci, where it showed in 39 unrelated individuals

had us concluded that this is probably an HLA haplotype associated with DRB1\*13:50:01. We also observed two individuals, donors 366796 and 390827 [Table 1], who are exceptions for A\*02-B\*40-DRB1\*13:50:01 haplotype. These two have the HLA-B\*40 and HLA-DRB1\*13:50:01 alleles without HLA-A\*02 allele but have A\*11 allele in common. We postulate that they probably carry the haplotype A\*11-B\*40-DRB1\*13:50:01 alternatively.

In exon 2, the DNA sequence of DRB1\*13:50:01 bears a partial sequence identity to the sequences of DRB1\*03:01:01:01, DRB1\*11:01:01:01, and DRB1\*13:02:01:01 [Figure 1a]. Through the comparison of the sequences of DRB1\*13:50:01 and DRB1\*11:01:01:01, they have identical sequence starting from codon 6 to codon 36 and from codon 59 to codon 94. On the other hand, the sequence of DRB1\*13:50:01 shares identical sequence with that of either DRB1\*03:01:01:01 or DRB1\*13:02:01:01 from codon 37 to codon 58. This exchange of gene segments between DRB1\*03:01:01:01, DRB1\*11:01:01:01 and DRB1\*13:02:01:01 suggests a recombination event occurred where either DRB1\*03:01:01:01 or DRB1\*13:02:01:01 donated a segment of DNA sequence from codon 37 to codon 58 to the sequence of DRB1\*11:01:01:01 resulted the formation of DRB1\*13:50:01. In agreement with the DNA sequence comparison, the comparison of protein sequences between DRB1\*13:50:01 and DRB1\*03:01:01:01, DRB1\*11:01:01:01 and DRB1\*13:02:01:01 is depicted in Figure 1b. It shows that the amino acid sequence of DRB1\*13:50:01 is identical to DRB1\*11:01:01:01 from residue number 6 to residue number



**Figure 1:** (a) In exon 2 the DNA sequence of DRB1\*13:50:01 shares similarity with DRB1\*03:01:01:01, DRB1\*11:01:01:01 and DRB1\*13:02:01:01. The sequence of DRB1\*13:50:01 is identical to DRB1\*11:01:01:01 from codon 6 to codon 36 and from codon 59 to codon 94 (shaded). On the contrary, the sequence of DRB1\*13:50:01 shares identical sequence with that of DRB1\*03:01:01:01 and DRB1\*13:02:01:01 from codon 37 to codon 58 (underlined). This exchange of gene segments between DRB1\*03:01:01:01, DRB1\*11:01:01:01 and DRB1\*13:02:01:01 suggests a crossing-over event occurred during the stage of germ cell division which resulted the formation of DRB1\*13:50:01. (b) The amino acid sequence of DRB1\*13:50:01 is identical to DRB1\*11:01:01:01 from residue number 6 to residue number 36 and from residue number 59 to residue number 94 (shaded). Similarly, the amino acid sequence of DRB1\*13:50:01 is identical to either DRB1\*03:01:01:01 or DRB1\*13:02:01:01 from residue number 37 to residue number 58 (underlined). Dashes indicate nucleotide and amino acid identity with DRB1\*13:50:01

36 and from residue number 59 to residue number 94. On the other hand, the amino acid sequence of DRB1\*13:50:01 is identical to either DRB1\*03:01:01:01 or DRB1\*13:02:01:01 from residue number 37 to residue number 58.

## DISCUSSION

In this study, based on the HLA typing of 41 Taiwanese randomized unrelated individuals, we deduced two likely HLA haplotypes in association with DRB1\*13:50:01 allele, namely A\*02-B\*40-DRB1\*13:50:01 and A\*11-B\*40-DRB1\*13:50:01. The allele DRB1\*13:50:01, according to the Allele Frequency Net Database [7], was also reported in the US Asian population with a different haplotype (A\*11-B\*15:02-DRB1\*13:50:01). This observation suggests that various DRB1\*13:50:01 associated HLA haplotype may be revealed if further populations are investigated. The high-resolution variants of the most HLA-A\*02 and HLA-B\*40 alleles in the deduced haplotypes have not yet been defined here, however where the high resolution has been attained for the HLA-A and HLA-B loci, the alleles observed are HLA-A\*02:01 and HLA-B\*40:01 [Table 1]. A future in-depth study to resolve the issue may be worthwhile to continue. Comparing the findings to the information in the IPD-IMGT/HLA Database and the Allele Frequency Net Database, we were once again reminded that a bigger database is important in the course of understanding the HLA genes and HLA haplotypes.

## CONCLUSION

In this study we show the HLA typing results of 41 randomized unrelated Taiwanese individuals and propose two HLA haplotypes associated with DRB1\*13:50:01. The findings here may be useful in studies reinforcing the understanding and clinical application of the polymorphism of HLA genes and haplotypes.

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## Conflicts of interest statement

There are no conflicts of interest.

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