DIVERSITY AND FREQUENCY OF HLA-DRB1*15:03—DRB5* HAPLOTYPES IN A LARGE COHORT: THE CASE OF THE ABSENT HLA-DRB5 REVISITED

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Introduction

The HLA Class II region contains nine DRB genes: DRB1, -B3, -B4, and -B5 express functional gene products, whereas -B2, -B6, -B7, -B8, and -B9 are pseudogenes. It has long been established that antigens of the broad HLA-DR2 serotype, encoded by HLA-DRB1*15 and HLA-DRB1*16, are in strong linkage disequilibrium with serotype HLA-DR51, encoded by the HLA-DRB5 gene. In this study we assessed the frequency and diversity of two functional DRB genes, DRB1 and DRB5; in particular, HLA-DRB1*15:03 with absence of the associated HLA-DRB5*01:01 allele. We aimed to determine the frequency of DRB1*15:03+DRB5*01:01- in a large cohort and to define any putative full or partial haplotypes that may show this genotype pattern.

Methods and Materials

Our cohort consisted of **6,268** specimens (blood or buccal swab) of SOT patients and donors processed from Oct 2021 through March 2024. Specimens were extracted on either the ThermoFisher KingFisher Flex or Promega Maxwell RSC, using either the MagMax DNA Multi-Sample Ultra 2.0 kit or the Maxwell RSC Buffy Coat DNA kit, respectively.

HLA genotyping was performed by NGS using either ThermoFisher Scientific AllType or CareDx AlloSeq Tx17. Specimens were typed at 11 loci (HLA-A, B, C, DRB1, DRB3/4/5, DQA1, DQB1, DPA1, DPB1). Absence of DRB5 for HLA-DRB1*15:03+ heterozygotes was confirmed using ThermoFisher Scientific LABType SSO DRB3,4,5. Confirmation of single copy DRB5 for select HLA-DRB1*15:XX, DRB1*15:03 specimens was established by performing copy number assessment using CareDx Assign CopyNumber RUO tool.

DRB Genotype	Numbers	Percentage
DRB1*15:03+	554/6,268	8.8% of cohort
DRB1*15:03+ DRB5*01:01-	**48/554**	**8.7% of DRB1*15:03+**
DRB1*15:03+ DRB5*01:01+	506/554	91.3% of DRB1*15:03+
DRB1*15:03+, DRB1*15:XX+, DRB5*01:01+	47/506	9.3% of DRB1*15:03+
DRB1*15:03+, DRB1*15:XX DRB5*01:01+ fitting haplotypes of Table 2	14/47	*

Table 1. DRB1*15:03 frequency in cohort of 6,268 SOT patients and donors.
***Possibly 14+48/554 = 11.2% of DRB1*15:03+ haplotypes of cohort absent DRB5**.

Putative HLA Haplotypes	A*	B*	C*	DRB1*	DRB5*	DQB1*	DPB1*
Haplotype 1 (19/48)	01:02 (7/19)	**49:01**	**07:01**	**15:03**	**ABSENT**	**06:02**	18:01 (13/19)
	02:02 (4/19)						
Haplotype 2 (12/48)	74:11 (9/12)	**15:03**	**02:10**	**15:03**	**ABSENT**	**06:02**	18:01 (9/12)
Haplotype 3 (4/48)	68:02 (3/4)	**51:01**	**16:01**	**15:03**	**ABSENT**	**06:02**	02:01 (3/4)

Table 2. Putative haplotypes of HLA-DRB1*15:03+DRB5-

Results

In this cohort, **554** individuals were identified as HLA-DRB1*15:03+, of which, **48 (8.7%)** individuals did not have the associated DRB5*01:01 allele present (Table 1). 35/48 DRB1*15:03+DRB5- individuals displayed 3 distinct haplotype patterns (Table 2). 506 (91.3%) of DRB1*15:03+ individuals typed as DRB5*01:01+. However, 47/506 DRB1*15:03+DRB5*01:01+ were heterozygous DRB1*15:XX+, DRB1*15:03+, and limitations of the assays did not allow us to determine if individuals were homozygous DRB5*01:01 or hemizygous. 14/47 of DRB1*15:XX+, DRB1*15:03+ individuals fit haplotype patterns (Figure 1 and Table 2), so the frequency of DRB1*15:03+DRB5*01:01- could be as high as 11.2% (Table 1).

Full and partial putative haplotypes associated with this phenomenon were characterized. 13/48 instances of HLA-DRB1*15:03+DRB5*01:01- did not fit any discernable haplotype pattern, though A*02:02 (5/13), B*53:01 (5/13), and DPB1*18:01 (6/13) retained higher frequency in these instances. HLA-A and HLA-DPB1 are more prone to crossover events due to genetic distance, which explains their sporadic absence from putative haplotypes. However, certain HLA-A, B, DRB1 genotypes were predictive of DRB5-. Individuals typing as HLA-A*01:02, B*49:01, DRB1*15:03 and HLA-A*74:11, B*15:03, DRB1*15:03 were 100% predictive to be absent of DRB5*01:01 (Table 3).

A*	B*	C*	DRB1*	DRB345*	DQA1*	DQB1*	DPA1*	DPB1*
02:02:01G	49:01	07:01	15:03	5*01:01	01:02	06:02	01:03	01:01
68:02:01G	53:01	04:01	15:03	5*01:01	01:02	06:02	02:02	18:01
A2, A68	B49, B53 Bw4, Bw4	Cw4, Cw7	DR15, DR15	DR51		DQ6, DQ6		

Figure 1. DRB1*15:XX+, DRB1*15:03+, DRB5*01:01+ fitting haplotype 1 outlined in Table 2. DRB5 copy number needed to determine DRB5 zygosity.

Haplotype	A*	B*	DRB1*	DRB5*
Haplotype 1	**01:02**	**49:01**	**15:03**	**ABSENT**
Haplotype 2	**74:11**	**15:03**	**15:03**	**ABSENT**

Table 3. Haplotypes predictive of absent DRB5

Conclusions

To our knowledge, this is the largest cohort investigating the diversity and frequency of the HLA-DRB1*15:03 apparent haplotypes. Our results show a considerable proportion of DRB1*15:03+ individuals lack DRB5. This occurrence should no longer be considered rare. The results of this study are useful for unrelated donor searches, transplantation, anthropological and disease association studies.

The frequency of DRB1*15:03+DRB5- genotypes in our cohort was unexpected and could lead to a broader discussion about the value in knowing the HLA-DRB3/4/5 genotype of all HSCT and SOT recipients and donors. HLA-DR51 has immunogenic epitopes that can induce antibody. Therefore, full knowledge of mismatches within HLA-DRB1*15:03 genotypes can be crucial. Also, mismatches at lower expression HLA loci, such as DRB3/4/5, can increase mortality of recipients. However, while it is required that HLA-DRB3/4/5 be typed for SOT donors, it is not required for SOT recipients, or HSCT recipients or donors. A standardized approach to typing HLA-DRB3/4/5 for all transplant recipients and donors would be advantageous.