Full-Length-Characterization of more than 120 Novel HLA-DRB3/4/5 Alleles for Submission to the IPD-IMGT/HLA Database

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Introduction

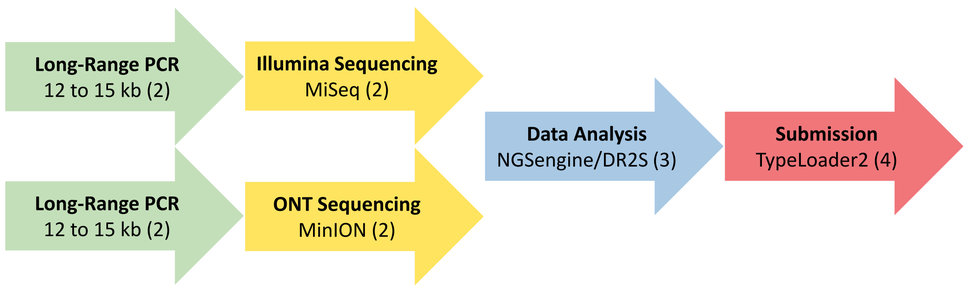
HLA-DRB3, HLA-DRB4, and HLA-DRB5 (HLA-DRB3/4/5) genes are in strong linkage disequilibrium to HLA-DRB1, yet around 10% of 10/10 matched donors for hematopoietic cell transplantation are mismatched for HLA-DRB3/4/5. Evidence suggests that HLA-DRB3/4/5 matching may reduce acute graft-versus-host disease and increase overall survival (1). To support HLA-DRB3/4/5 informed donor selection, we routinely genotype exons 2 and 3 of HLA-DRB3/4/5 for all potential stem cell donors using a high-throughput workflow. In 2024, we identified novel HLA-DRB3/4/5 alleles with a frequency of 0.08% - twice the frequency observed for HLA-DRB1 (0.04%). Consequently, we aimed to characterize novel HLA-DRB3/4/5 alleles and submit them to the IPD-IMGT/HLA Database.

Characterization of Novel Alleles

Sample selection

Novel HLA-DRB3/4/5 alleles were identified by our high-throughput genotyping workflow for potential stem cell donors. This workflow sequences exons 2 and 3 of HLA-DRB3/4/5.

Workflow for characterization of novel alleles



Primer used in long-range PCR as described by Putke et al. (2):

- HLA-DRB3 forward: GAGACTTGCCTGCTCCTCTGG
- HLA-DRB3 reverse: AGTACAGATGCACAGGAGGCC
- HLA-DRB4 forward: TGCTAGTGAACTCAGGTGCTGAC and TGCTAGTGAACTCAGATGCTGAC
- HLA-DRB4 reverse: AGTAACAACCTGGTCCTGACAAAGC
- HLA-DRB5 forward: GAGACTTGCCTGCTCCTCTGG
- HLA-DRB5 reverse: AGCTGAGGAAGCCACAAGGATG

Submissions to the IPD-IMGT/HLA Database

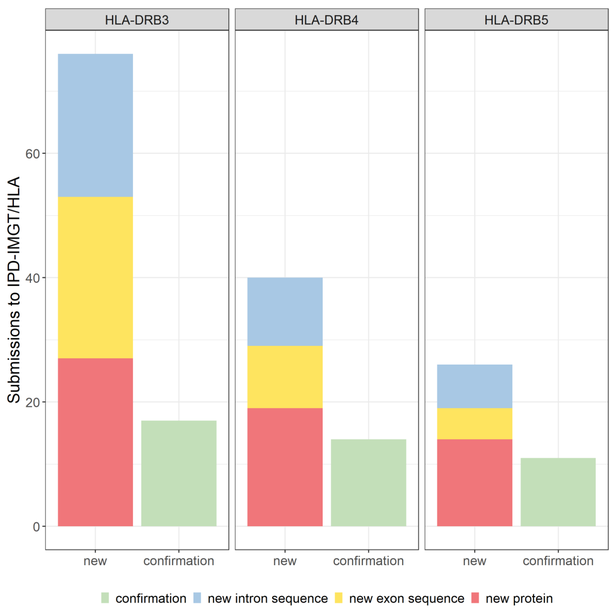


Figure 1: Submissions of HLA-DRB3/4/5 sequences to the IPD-IMGT/HLA Database

In total, 184 sequences were submitted to the IPD-IMGT/HLA Database: 93 HLA-DRB3, 54 HLA-DRB4, and 37 HLA-DRB5 sequences. Among them were 142 distinct novel alleles and 42 sequence confirmations. 60 of the novel alleles code for novel proteins (27 HLA-DRB3, 19 HLA-DRB4, and 14 HLA-DRB5) (novel null alleles included) (Figure 1). 41 novel sequences carry synonymous exon variations and the remaining 41 novel alleles harbor only intron variations.

Conclusion

We successfully characterized and submitted 142 distinct novel HLA-DRB3/4/5 alleles, among them 60 sequences coding for novel proteins, to the IPD-IMGT/HLA Database. This significantly expands the previously limited reference set, thereby reducing ambiguities and improving genotyping accuracy.

Novel HLA-DRB3/4/5 Proteins

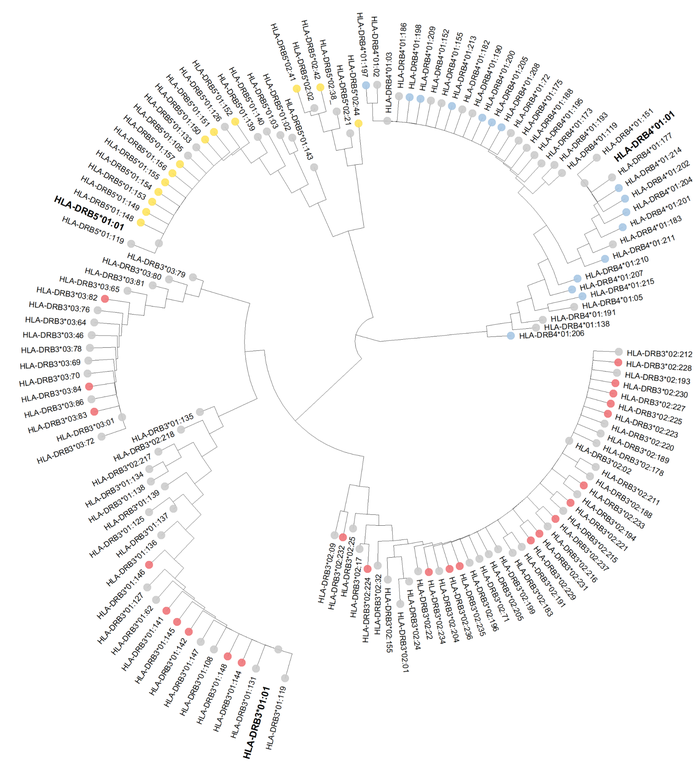


Figure 2: All HLA-DRB3/4/5 amino acid sequences with available full-gene coverage (IPD-IMGT/HLA release 3.63) are displayed as neighbor-joining tree with midpoint rooting. HLA-DRB3*01:01, HLA-DRB4*01:01 and HLA-DRB5*01:01 are highlighted in large and bold font. Colored tips highlight the novel alleles contributed by this study (red: HLA-DRB3, blue: HLA-DRB4, yellow: HLA-DRB5). Null alleles are not shown. For improved visualization, the branch lengths of the tree were square rooted before plotting the tree.

In addition to the depicted novel proteins (Figure 2), we identified and submitted 7 novel null alleles: DRB3*02:226N and DRB5*02:43N carry a frameshift or stop codon within exon 2, DRB3*01:143N, DRB3*02:222N, and DRB4*01:199N within exon 3. DRB4*01:203N and DRB4*01:212N share the non-functional splice site of exon 2 with DRB4*01:03:01:02N and consequently code for the same non-nonfunctional protein.

HLA-DRB3/4/5 Full-Gene Coverage

Historically, most known HLA-DRB3/4/5 alleles have not been described in full length (5), mostly due to their length and challenging sequence sections, e.g., homopolymers or repeats. In IPD-IMGT/HLA Database release 3.58 (before this study, Figure 3A), only 36 (HLA-DRB3), 32 (HLA-DRB4), and 11 (HLA-DRB5) alleles were described in full-length, while most alleles were only characterized in exon 2 and 3 or in exon 2 only. Interestingly, some alleles were and are still not even covered by one full exon (Figure 3, other). Since these partial alleles can lead to ambiguous or even wrong genotyping results, we should all aim to only submit full gene sequences to the IPD-IMGT/HLA Database, since suitable methods and protocols do exist today (6).

The HLA-DRB3/4/5 sequences submissions from this study substantially increased the available full-gene sequences in the IPD-IMGT/HLA Database from 5-12% (release 3.58) to 22-30% (release 3.63) (Figure 3).

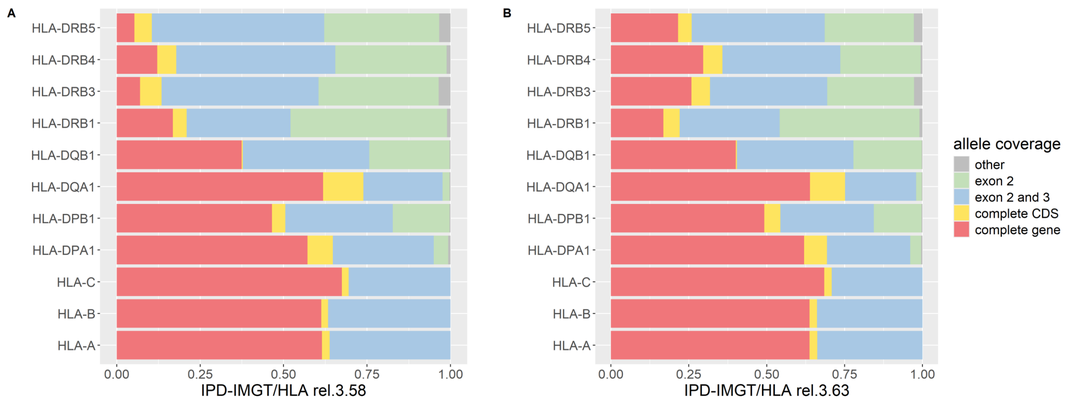


Figure 3: Proportions of allele coverage for selected HLA genes in IPD-IMGT/HLA releases 3.58 and 3.63. An allele coverage designated as “other” refers in most cases to only partially covered exons 2 or 3.

References

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