HLA Recombination and Organ Allocation: Are We Missing Something?

MIDWEST TRANSPLANT NETWORK

Accurate HLA typing of potential organ donors is critical to the integrity of the UNOS renal allocation system and the reliability of the virtual crossmatch.

This case review explores the clinical implications of encountering an uncommon or ambiguous HLA typing result at the B-locus. It addresses key questions: What are the potential consequences of an atypical result? How can its accuracy be verified? And what impact might it have on organ allocation and transplant outcomes? Through the lens of a real-world donor case, this presentation highlights the importance of precise HLA characterization and the strategies used to resolve typing discrepancies.

METHODS: The MTN laboratory performs HLA typing on deceased donors using two methodologies: qPCR (QType®) and XR-rSSO (LABType™). Additional NGS sequencing was performed on this donor sample for investigational purposes using the NGSgo®-ProntoAmp assay kit.

RESULTS: SCORE™ 6 for QType analysis provided an HLA B-locus assignment of a common B*07:02 and a string of potential uncommon B*53 alleles. HLA Fusion™ for LABType analysis could not provide an immediate auto assignment for the B-locus (Figure 1).

- *Excluding one bead resulted in a Group 1 assignment of the common B*07:02 and rare B*53:01:28/53:59.
- *Excluding Exons 4 and 5 resulted in a Group 1 assignment of common B*07:02:01:01 and B*53:01:01:01.

These two analyses together gave us confidence to allocate the donor organs as HLA-B7 and HLA-B53.

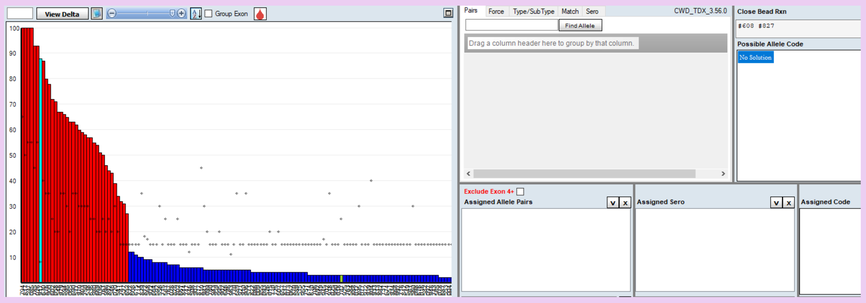

Figure 1. LABType XR kit for HLA B-locus yielded clean results, but no auto assignment.

We decided to sequence this donor sample by NGS to investigate the HLA typing for a possible recombinant allele. Upon analysis, we discovered fully phased data that demonstrated a cluster of mismatches localized at the end of the amplicon. The first half of the gene was a perfect match to the best matched reference allele: B*53:01. However, consistent mismatches appear from the middle of intron 3 through the 5' UTR (Figure 2).

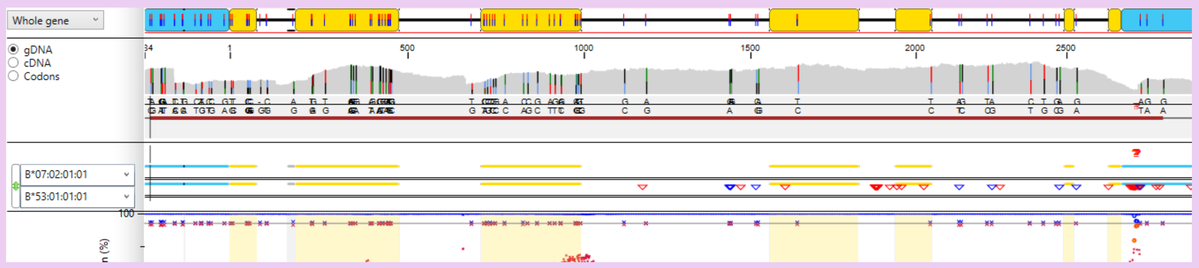

Figure 2. Initial analysis result. Distinct mismatch pattern observable through the end of the 2nd allele amplicon.

To further inspect the hypothesis of a recombinant allele, we requested the guidance of GenDx technical support.

- *Data quality values were good (i.e. QV 39), which could exclude the possibility of a sequencing artifact. This was confirmed for all mismatched positions.
- *Several reads from Exon 4 that were assigned to the reference allele HLA-B*53:01:01:01 were blasted. Interestingly, the reads with the mismatches did not match with HLA*B53:01:01:01. Instead, they matched with HLA-B*08.
- *To visualize the putative recombination, the data of the 2nd allele was manually aligned to the B*08:01 reference allele. In doing so, we were able to determine a pattern for HLA-B*08 starting in the middle of Intron 3 and continuing through the end of the amplicon (Figure 3).

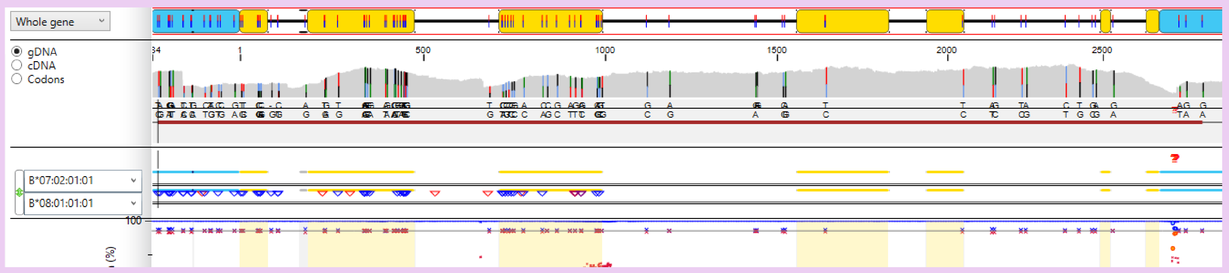

Figure 3. Blasting mismatched reads from Figure 2 led to the conclusion that there is likely a B*08 allele pattern also present in the 2nd allele. Adjusting the reference allele to B*08:01, the complimentary pattern of mismatches is obvious.

DETERMINATION: One of the deceased donor's HLA-B alleles is a B*07:02 and the other is a recombination of B*53 and B*08. The 1st half of this recombinant allele expresses B*53 and the 2nd half, after Exon 3, expresses B*08. This new B*53var allele has been submitted to GenBank, submission PV207309.

CONCLUSION: Obtaining the accurate HLA typing of donors for organ allocation can be more complex than we traditionally give it credit for. The ability to detect and confirm atypical alleles is essential for ensuring the best possible offers and outcomes for transplant recipients. This case underscores the value of utilizing multiple complementary typing methods and expert analysis when standard tools yield ambiguous results.

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