HLA-B*51:11N REVEALED BY NGS IN CONFIRMATORY DECEASED DONOR HLA TYPING

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CASE DESCRIPTION

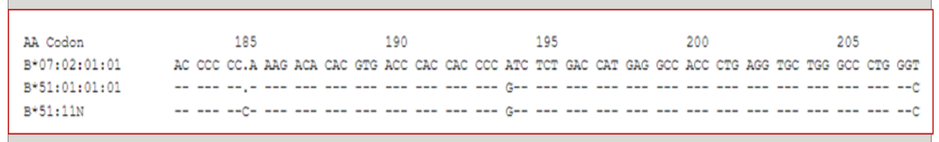
A deceased liver donor was re-typed by NGS at the Barnes-Jewish Hospital (BJH) HLA lab after the organ was transplanted, as the local standard of care to verify the deceased donor HLA typing and to facilitate donor-specific antibody (DSA) analysis at the allelic level. The donor was initially typed at a local OPO lab by real-time PCR using both LinkSeq and QTYPE assays. The HLA type reported at the time of organ allocation was A2, A3; B7 (Bw6), B51 (Bw4); Cw7, Cw7; DRB1*04:02, DRB1*11. The NGS typing at BJH was performed using the AllType 11-loci assay on the Ion Chef/Ion S5/TypeStream Visual platform, which showed a discrepant HLA-B genotype: B*07:02 (Bw6) and B*51:11N. All health metrics of the NGS typing were satisfactory.

Based on reference sequences from the IPD-IMGT/HLA database, B*51:11N differs from B*51:01:01:01 by an extra C inserted after a homopolymer of 6C in exon 4 (**Figure 1**). We attempted to verify the NGS result using the SSO LABType CWD B-locus typing assay. Two probes (Bead#777 and Bead#629) can potentially distinguish the two alleles with expected hybridization patterns shown in **Figure 2** (top). However, both beads were positive, so neither allele seems to fit exactly. Bead#777 fell in a region where the reference sequence is missing for B*51:11N in the IPD-IMGT/HLA database (**Figure 2**, bottom). After excluding Bead#777 from the analysis, B*51:11N was assigned by Fusion. Although no positive QC data was available for Bead#629 from the vendor, the hybridization signal for the 7C homopolymer region was 51, significantly higher than the cutoff of 15.

RESULTS

Interestingly, B*51:11N was included in the string of B alleles on the LinkSeq but excluded in the string of the QTYPE results. Based on the above analyses, we reported the donor HLA-B genotype as B*07:02 (Bw6) and B*51:11N. The lab also reported the discrepant result back to UNOS through TEIDI.

Figure 1. Alignment of HLA-B exon 4 DNA sequences (top) and protein sequences (bottom) from codon 181 to 200 of three alleles B*07:02:01:01:01, B51:01:01:01:01, and B*51:11N. The extra C after a homopolymer of 6C causes a frameshift and introduces premature stop at codon 196.



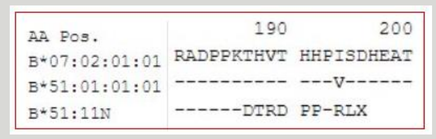
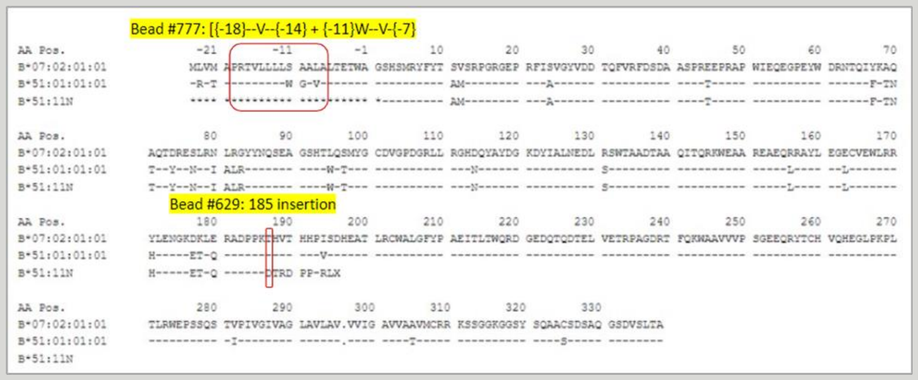


Figure 2. Top: different reaction patterns for the null allele B*51:11N versus B*51:01:01:01 in Fusion. Bottom: the recognition sites of the two probes shown on the aligned amino acid sequences of B*07:02:01:01, B*51:01:01:01:01, and B*51:11N.

	Bead #777	Bead #629
B*51:11N	Negative	Positive
B*51:01:01:01	Positive	Negative
Sample Reaction	*Positive*	*Positive (deemed as "false positive" in Fusion)*



CONCLUSIONS

Deceased donor HLA typing is often conducted using real-time PCR, which allows for low-to-intermediate resolution results and quick turnaround times. However, this approach can occasionally miss rare null alleles, potentially affecting organ allocation and the interpretation of post-transplant donor-specific antibodies (DSAs). These discrepancies are challenging to identify due to the inherent limitations of the PCR method. Therefore, confirmatory typing using a more precise technique, such as next-generation sequencing (NGS)[1, 2], would be advantageous in these cases to ensure accuracy and improve transplant outcomes.

REFERENCES

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