

Allele Dropout in NGS-Based High-Resolution HLA Typing: A Case Study Highlighting the Need for Confirmatory Testing

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CASE DETAILS

A 63 years old female diagnosed with blood malignancy and her three siblings are being evaluated for bone marrow transplantation (BMT). This case study explores an unusual finding during high-resolution HLA typing of a family patient and his three siblings. The patient underwent high resolution HLA typing using next-generation sequencing (NGS), revealing that two siblings haploidentical matched except for DP locus (Pt-DPB1*105:01/DPB1*04:02:01G, Sib-DPB1*03:01/DPB1*04:01). Other sibling was haploidentical match for the other haplotype (Table 1). This was an unexpected result, as the siblings were known to be haploidentical. Parental HLA data were unavailable. The DNA used for NGS-based typing was extracted from buccal swab.

Table 1: High resolution HLA Typing in the family with siblings and patient showed Haploidentical

Family Study	HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	HLA-DR	HLA-DR	HLA-DP	HLA-DP	HLA-DQ	HLA-DQ
Patient-NGS- Allele DropOut										105:01		
Patient- Corrected-SSO	01:01	03:01	08:01	07:02	07:01	07:02	15:01	15:01	04:02:01G	04:01:01G	06:02:01G	06:02:01G
Sib 1	23:01	03:01	44:02	07:02	07:04	07:02	04:07	15:01	03:01	04:01	03:01	06:02
Sib 2	23:01	03:01	44:02	07:02	07:04	07:02	04:07	15:01	03:01	04:01	03:01	06:02
Sib 3	01:01	02:01	08:01	44:02	07:01	05:01	15:01	13:01	04:02	02:01	06:02	06:03

Legend:
 Pt and sib Haploidentical match- 1st Haplotype
 Pt and sibs Haploidentical match 2nd Haplotype



WHAT IS ALLELE DROPOUT?

- Allele dropout refers to the phenomenon where one or more alleles at a specific HLA locus are not detected during high-resolution NGS-based HLA typing, even though they are expected to be present
- The allelic dropout is a well-known problem in PCR-based HLA typing methods
- This can lead to an incomplete or inaccurate HLA genotype.

Despite the technological advancements and benefits, allelic dropout can still occur because the NGS methods require PCR amplicon-based enrichment

METHODS & RESULTS

To resolve the discrepancy, sequence-specific oligonucleotide (SSO) Luminex based HLA Typing was performed as a confirmatory method on patient DNA. The SSO confirmed the DPB1*04:01:01G and DPB1*04:02:01G allele in patient revealing NGS results were due to an allele dropout—a phenomenon where one allele fails to amplify or sequence properly, leading to an erroneous call (Figure 1), mostly in the case of homozygous. Further, we noticed that DPB1*105:01:01 is the part of DPB1*04:02:01G, and therefore NGS-HLA results for DPB1 was technically homozygous for DPB1*04:02:01G allele, gave an indication for allele-dropout.

Figure 1: NGS and SSO based HLA typing results comparison in patient swab sample

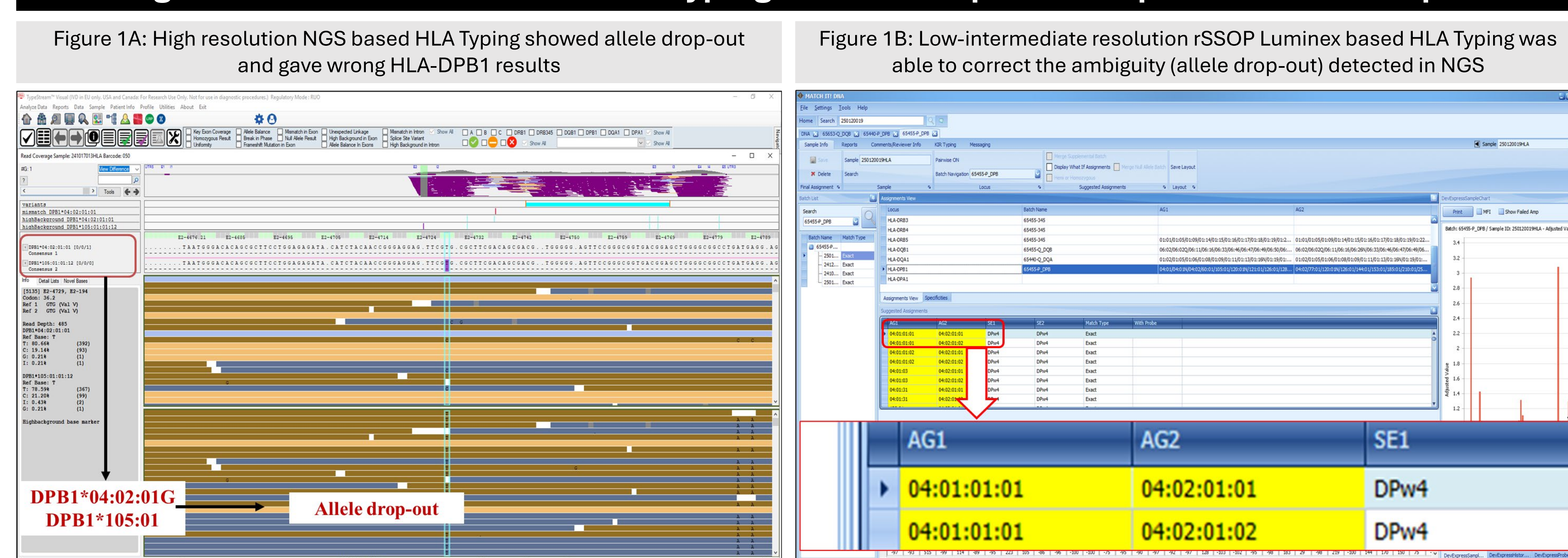
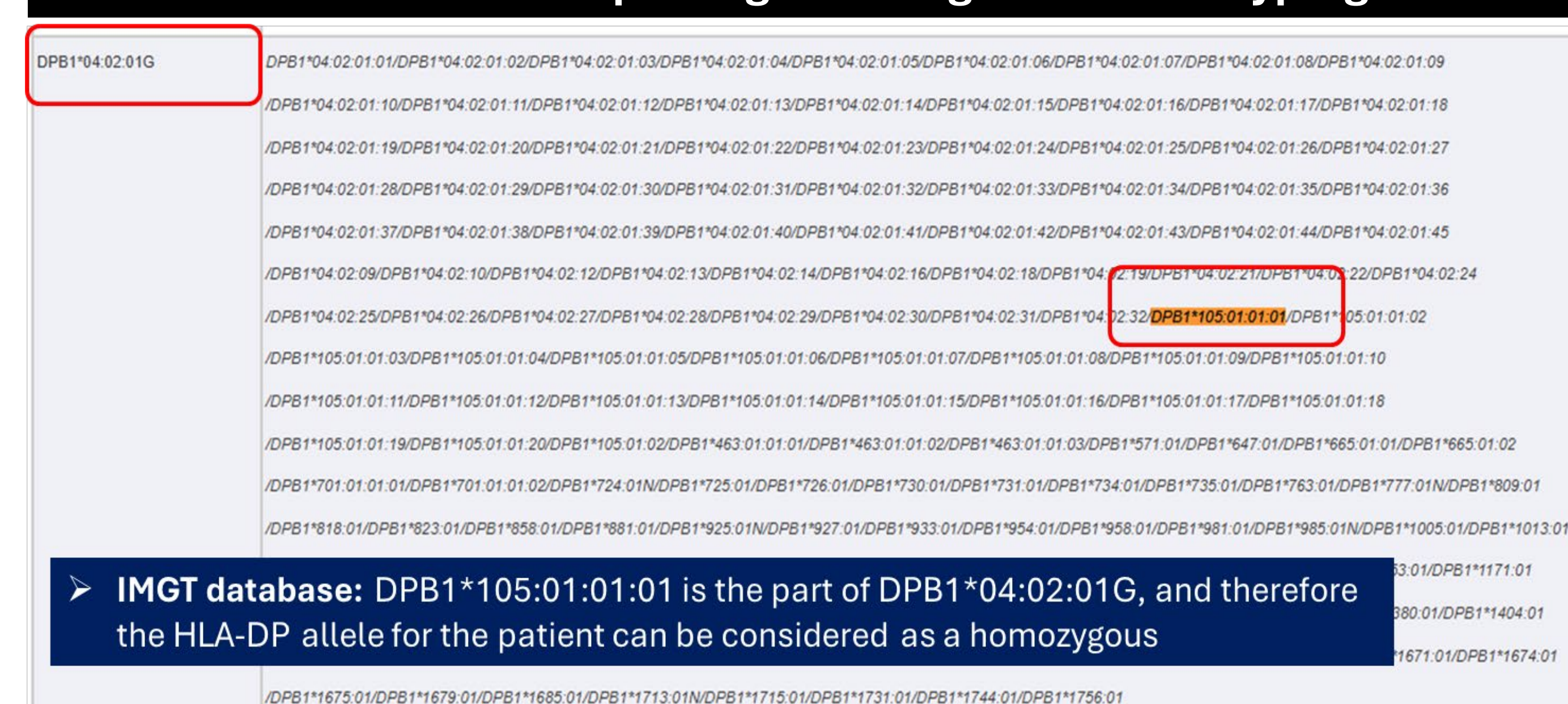


Figure 2: IMGT: http://hla.alleles.org/alleles/g_groups.html G Codes For Reporting of Ambiguous Allele Typing



CONCLUSIONS

This case highlights the potential limitations of NGS-based high-resolution HLA typing, where allele dropout can compromise the accuracy of results. Misinterpretation of HLA typing can have significant implications, especially in scenarios such as BMT, where precise donor matching is critical. Therefore, confirmatory testing using an alternative method is essential.



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