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Case report of resolving a KEL1 genotype-phenotype discrepancy with full-gene haplotype sequencing by Oxford Nanopore Technologies

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Background: Since the KEL1 antigen of the Kell blood group system is very immunogenic, it is usually determined in routine donor typing. At our blood service, KEL1 is determined by both serology and high-throughput genotyping. Genotype-phenotype discordances are normally resolved by laborious Sanger sequencing of all 19 exons without haplotype phasing capability. Here, we present an alternative protocol relying on Oxford Nanopore Technologies (ONT), which enables the generation of full-gene haplotypes.

Methods: Expression of KEL1 antigen was measured by standard serological techniques. Four variants within the *KEL* gene were part of our MALDI-TOF MS based high-throughput blood group genotyping routine, including c.578C>T determining KEL1/2 expression. Genotype-phenotype discrepancies were reassessed by commercially available PCR-SSP kits (inno-train, Germany) and extensive serological confirmation. To resolve a discrepancy in one donor, the entire *KEL* gene (~21 kb) was amplified in two long-range PCRs (fragments of 12.7 and 14.3 kb, respectively), exhibiting a large overlap (~4.4 kb) essential for haplotype phasing. Amplicons were sequenced on a Flongle flow cell (ONT) and detected exonic variants were confirmed with Sanger sequencing.

Results: We identified by routine donor typing a heterozygous *KEL*01/02* blood donor with a K-k+ phenotype. This discrepancy pointed to a potential null allele (*KEL*01N*). One hour of ONT sequencing already yielded ~450 reads covering both long-range PCR fragments, which is a high read-depth for variant calling. A heterozygous variant in the overlap sequence allowed complete gene haplotype phasing. In exon 11, we identified a missense variant c.1241C>A (Thr414Lys, rs13842342704), which was phased to the *KEL*01* allele. The variant is yet undescribed, despite the over 100 alleles collected by the ISBT, and was confirmed by Sanger sequencing. Adsorption-elution experiments for in-depth classification of true null or very weak expression are underway.

Conclusion: Using ONT sequencing, we resolved a genotype-phenotype discrepancy within short turnaround time and discovered a novel putative *KEL*01N* allele. The long reads allowed phasing detected variants to the respective *KEL*01/02* background and even constructing full-length *KEL* haplotypes. The use of a protocol and flow cell optimized for single-sample analysis kept time and expenses competitive. Overall, our approach proved very promising for resolving genotype-phenotype discrepancies.