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FROM HIGH-THROUGHPUT GENOTYPING TO NANOPORE SEQUENCING: RESOLVING GENOTYPE/PHENOTYPE DISCREPANCIES OF THE KIDD BLOOD GROUP SYSTEM REVEALED NOVEL NULL ALLELES AND A LARGE DELETION

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Background: Since 2015, MALDI-TOF mass spectrometry (MS) is implemented as routine high-throughput single nucleotide variant (SNV) genotyping platform for blood donors at the Blood Transfusion Service Zurich. So far, more than 36,000 donors have been genotyped for 46 selected blood group antigens, including the clinically relevant antigens of the Kidd system (Jk, SLC14A1). We assessed genotype/phenotype concor-dance of Kidd-typing and resolved discrepancies by Sanger and third-gen-eration Nanopore sequencing.

Methods: High-throughput genotyping of JK relied on MALDI-TOF MS based SNV detection at c.838G>A (JK*A/B), and the null alleles JK*01N.03(c.582C>G) and JK*02N.01/.02 (c.342-1G>A/C). Phenotyping was per-formed using standard serological techniques. Genotype/phenotype dis-crepancies were reassessed by commercially available PCR-SSP kits. To resolve discrepancies, coding exons of SLC14A1 were Sanger-sequenced. Moreover, we took advantage of the long-read sequencing technology of Oxford Nanopore Technologies (ONT) to identify potential large deletions and assign allele haplotypes with respect to causing SNVs. For this, the entire coding region of SLC14A1 (~24 kb, exon 3 to 10) was amplified in two overlapping long-range PCRs (~13 kb each).

Results: Kidd phenotypes were available for ~12,000 donors (33%). We identified genotype/phenotype discrepancies in 17 donors (0.142%), seven caused by JK*01N.03 (n=4) and JK*02N.01 (n=3), respectively. All further discrepancies were resolved by our combined sequencing strategy.

Conclusion: MALDI-TOF MS based genotyping of the major JK alleles reached 99.86% concordance to phenotyping. Sequencing of discrepant samples uncovered two new null alleles caused by single SNVs and a novel JK*A linked ~5 kb deletion allele which became recognizable only by long-read sequencing. Facing the complexity of certain blood group systems, ONT provides a reliable tool to overcome challenges in diagnos-tics with respect to hybrid genes, deletions, and duplications.

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