Case example of Nanopore sequencing for resolving genotype-phenotype discrepancies in the Duffy blood group at haplotype scale: discovery of a novel null allele in a *FY*A/FY*B* heterozygous individual

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Background: The Duffy (Fy) blood group is encoded by *ACKR1*. The *FY*A*/*FY*B* alleles are defined by the SNV c.125G>A. Weak alleles are mainly caused by c.265C>T and null alleles often involve c.-67T>C in the promoter, both linked to *FY*B*. Genotyping these SNVs usually confirms serological results. However, rare unexplained phenotype-genotype discrepancies may occur. We resolve such a discrepant case using Nanopore sequencing, which, unlike Sanger sequencing, allows haplotype generation along the whole gene.

Methods: Phenotyping was performed using standard serological techniques. Genotyping of the three aforementioned SNVs in *ACKR1* was carried out with MALDI-TOF mass spectrometry, a high-throughput platform that we have been using for routine blood donor genotyping of 46 selected blood group antigens. In case of phenotype-genotype discrepancy unexplained by the routinely typed SNVs, genotyping was reconfirmed using commercial PCR-SSP kits (inno-train, Germany). To identify the genetic variation causing unexplained discrepancy in one donor, *ACKR1* including flanking regions (~2.1 kb) was amplified and sequenced as haplotype using sequencing by Oxford Nanopore Technologies (ONT). Sanger sequencing of the two *ACKR1* exons was used to confirm the results.

Results: Since 2015 more than 40,000 donors have been screened for *FY* genotype at Blood Transfusion Service Zurich. Phenotypes were available for ~33% of the donors. One heterozygous *FY*A*/*FY*B* carrier with Fy(a-b+) phenotype was identified, pointing to a *FY*01N* allele in the absence of c.265T or c.-67C. Indeed, we found a 1 bp deletion (c.655delG) accompanied by a SNV c.657C>G (rs748896745) in the second *ACKR1* exon, both discovered by ONT on reads of the *FY*A* allele. The frameshift mutation is yet undescribed and was confirmed by Sanger sequencing.

Conclusion: Nanopore sequencing proved well-suited and accurate to resolve unclear discrepancy between Duffy blood group genotype and phenotype. In particular, it provided clinical utility by directly phasing a novel frameshift mutation to the respective *FY*A*/*FY*B* allelic background. As case example, this work demonstrated that sequencing new blood group alleles as complete gene haplotypes could become the emerging standard.

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