RHD DONOR SCREENING IN SWITZERLAND: RESOLVING NOVEL ALLELES BY NANOPORE-SEQUENCING

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Background

Multiple RH1 (RhD) variants cause very weak RH1 expression and may be missed even by phenotyping methods including indirect antiglobulin test. In 2012, molecular routine screening for the presence of *RHD* was therefore implemented in Switzerland for all serologically RH1 negative first-time donors, according to the respective guidelines of the Swiss Red Cross. This screening strategy revealed previously unknown *RHD* alleles, which we resolved by Sanger and third-generation nanopore sequencing.

Methods

Here, we present results from the *RHD* screening since 2017. Screening of all RH1-negative donors was performed with the RBC-FluoGene D-Screen kit including primers for *RHD* exons 3, 5 and 10 (inno-train, DE). In case of positivity, Rhesus genotypes/phenotypes were reassessed by commercially available SSP-PCR kits as well as standard and extended serological techniques. Sanger-sequencing and newest long-read sequencing technology of Oxford Nanopore Technologies (ONT) were applied to resolve unknown *RHD* alleles. For the latter, the entire coding region of *RHD* (~57 kb, exon 1 to 10) was amplified in six overlapping long-range PCRs with known *RHD*-specific primers. The PCR-products (~10 kb) were sequenced on MinION flow cells.

Results

More than 10,000 serologically RH1 negative samples have been screened at the Blood Transfusion Service Zurich in the last 5 years. Overall, 0.57% (n= 58) were genetically positive for at least one of the three typed *RHD* exons. Strikingly, our combined sequencing strategy elucidated three novel *RHD* alleles. All were caused by frameshift mutations and serologically defined as null-alleles, also by adsorption/elution techniques, when applicable: one sample had a small duplication in exon 3 (c.395_396dup, p.K133Gfs*10), one sample had a single basepair deletion in exon 2 (c.245delT, p.F82Sfs*17) and the third donor carried an allele with a 4-bp deletion (c.1199_1202del, p.K400Ifs*48) in exon 9 in addition to the DAU-specific SNV 1136C>T.

Conclusion

Molecular *RHD* screening of RH1 negative donors represents an efficient strategy to detect RH1 variants of very low expression, hence reducing the potential risk of alloimmunization in patients. Here we describe three previously unknown *RHD* variants all defined as null alleles based on genetic and phenotypic data. Remarkably, confirmation of all novel alleles by our *RHD* long-read sequencing strategy provides evidence that ONT is a reliable and emerging tool for routine diagnostics.