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 by extended phenotyping methods
- In 2012, molecular routine screening for the presence of *RHD* was therefore implemented in Switzerland for all serologically RH1 negative first-time donors
- Here, we present results from the last five years of *RHD* screening in Zurich

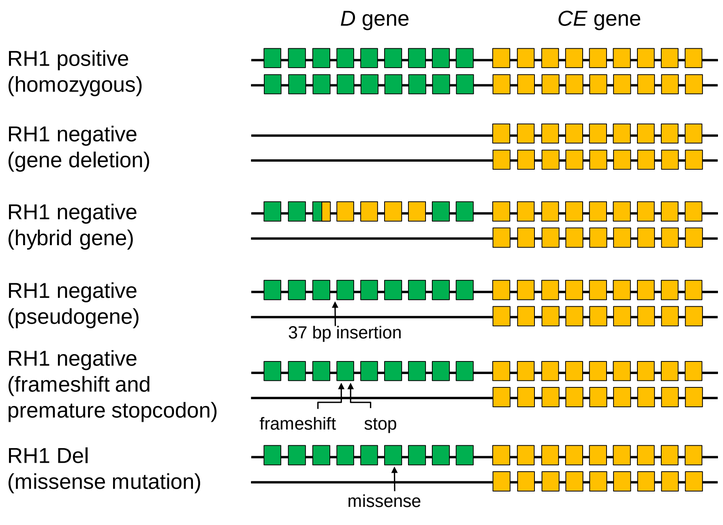

Fig. 1: Schematic *RHD/RHCE* gene locus showing the 10 exons and common RH1 negative or very weak variants

Methods

- Screening was performed with the RBC-FluoGene D-Screen kit (*RHD* exons 3, 5 and 10; inno-train Diagnostik)
- In case of *RHD* positivity, genotypes/phenotypes were reassessed by SSP-PCR kits and serological techniques
- Classical Sanger-sequencing as well as third-generation long-read sequencing technology of Oxford Nanopore Technologies (ONT, Fig. 2) were applied to resolve unknown *RHD* alleles
- For the latter, *RHD* coding region was amplified in six overlapping long-range PCR-fragments (~10 kb; Tounsi *et al.*, 2018) and sequenced on MinION flow cells (Fig. 2, 3)

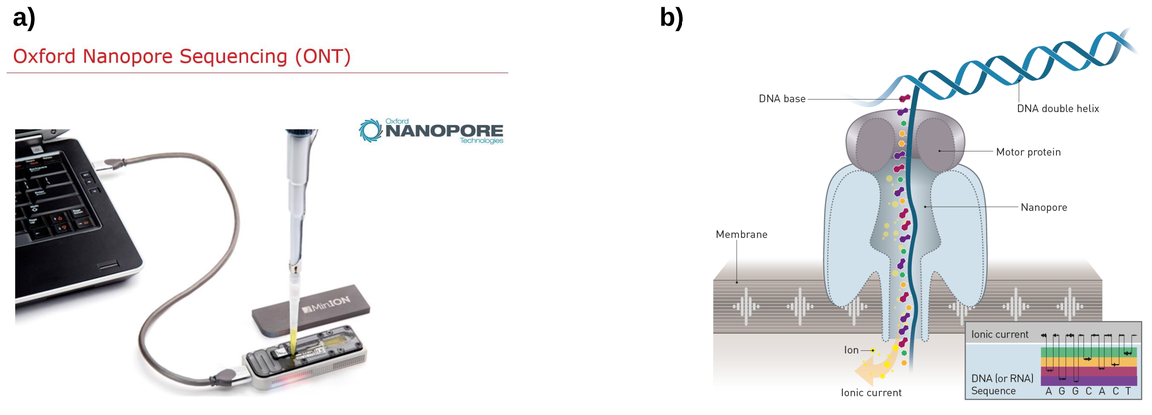

Fig. 2: (a) View of pocket size MinION device and **(b)** structural composition of nanopore sequencing of single-stranded DNA

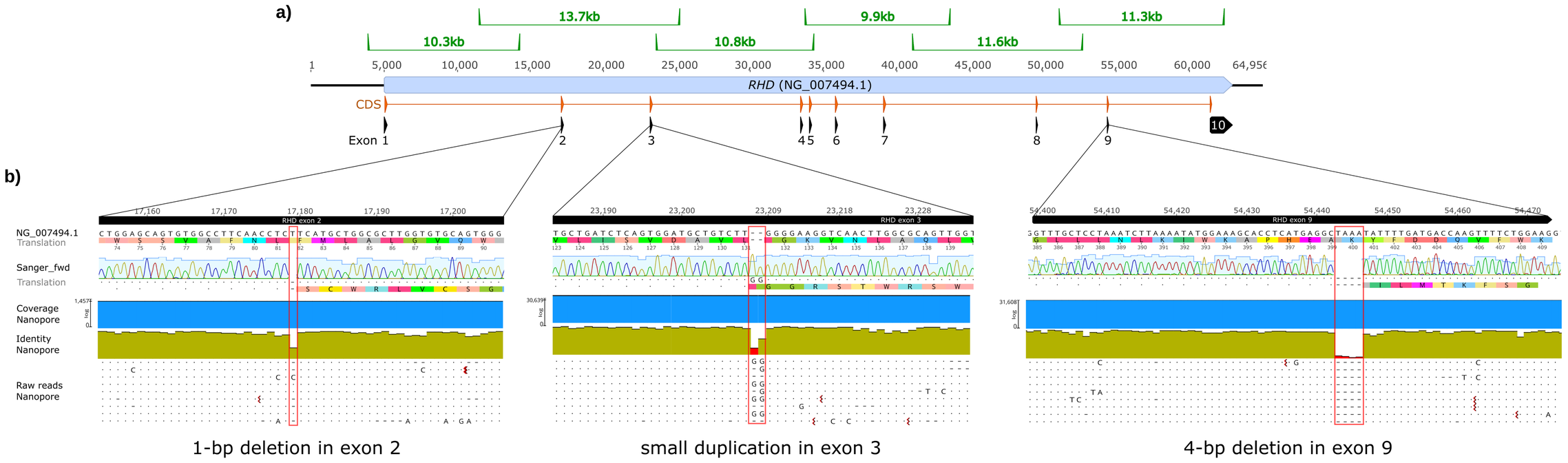

Fig. 3: (**a)** *RHD* gene showing exons (black), coding DNA sequence (CDS, orange), and coordinates of six long-range PCR-fragments (green) (**b)** Zoom on the novel *RHD* alleles with reference sequence, results of Sanger-sequencing as well as mapping results of long-read sequencing technology of ONT.

Results

- >10,000 serologically RH1 negative samples screened at the Blood Transfusion Service Zurich in the last 5 years
- 0.57% (n= 58) were genetically positive for at least one of the three typed *RHD* exons; 46% (n= 27) of these donors carried an *RHD* allele resulting in reclassification as serologically RH1 positive
- Sequencing of unknown *RHD* alleles elucidated three novel alleles (Fig. 3):
  - 1-bp deletion in exon 2 (c.245delT, p.F82Sfs*17)
  - small duplication in exon 3 (c.395_396dup, p.K133Gfs*10)
  - 4-bp deletion in exon 9 (c.1199_1202del, p.K400Ifs*48) + DAU-specific SNV 1136C>T
- All novel alleles were serologically defined as null-alleles, also by adsorption/ elution techniques, when applicable

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
- Three previously unknown *RHD*-null variants were discovered by long-read sequencing technology
- Nanopore sequencing is becoming a reliable and emerging tool for routine diagnostics

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References

Tounsi WA, Madgett TE, Avent ND. Complete RHD next-generation sequencing: establishment of reference RHD alleles. Blood Adv. 2018;2(20):2713-2723

Abstract