NANOPORE SEQUENCING FINDS NOVEL REGULATORY *ABO* VARIANT CAUSING MIXED-FIELD AGGLUTINATION IN AB DONOR

GA Thun[1], M Gueuning[1], S Sigurdardottir[2], E Meyer[2], E Gourri[1,2], L Schneider[2], C Engström[3], J Sigle[4], BM Frey[1,2,3], S Meyer[2*], MP Mattle-Greminger[1*]

[1]Department of Research and Development, [2]Department of Molecular Diagnostics and Cytometry, [3]Department of Immunohematology, Blood Transfusion Service Zurich, Schlieren, Switzerland
[4]Blood Transfusion Centre Aargau-Solothurn (SRC), Aarau, Switzerland

* equal contribution

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Background

- Mixed-field agglutination in ABO phenotyping (e.g. A_3/B_3) often linked to variants in *ABO* exon 7 and regulatory regions[1-3]
- Very limited knowledge on genetic diversity in regulatory regions because rarely sequenced
- Haplotype-resolved long-read sequencing with great potential to explain cryptic ABO phenotypes
- Proof of concept: nanopore sequencing to resolve a case of mixed-field agglutination in ABO forward typing

Methods

- ABO serology, including anti-A1 and anti-H specific agglutination (Fig. 1a)
- *ABO* genotyping using PCR-SSP
- Flow cytometry: A-, B-, and H-antigen expression (Fig. 1a)
- Exclusion of chimerism using digital PCR
- Nanopore sequencing of entire *ABO* gene
  → 2 overlapping long-range PCR products (~13 kb each)
- Variant confirmation by Sanger sequencing
- Germline vs. somatic origin discriminated by analyzing donor's mother and brother

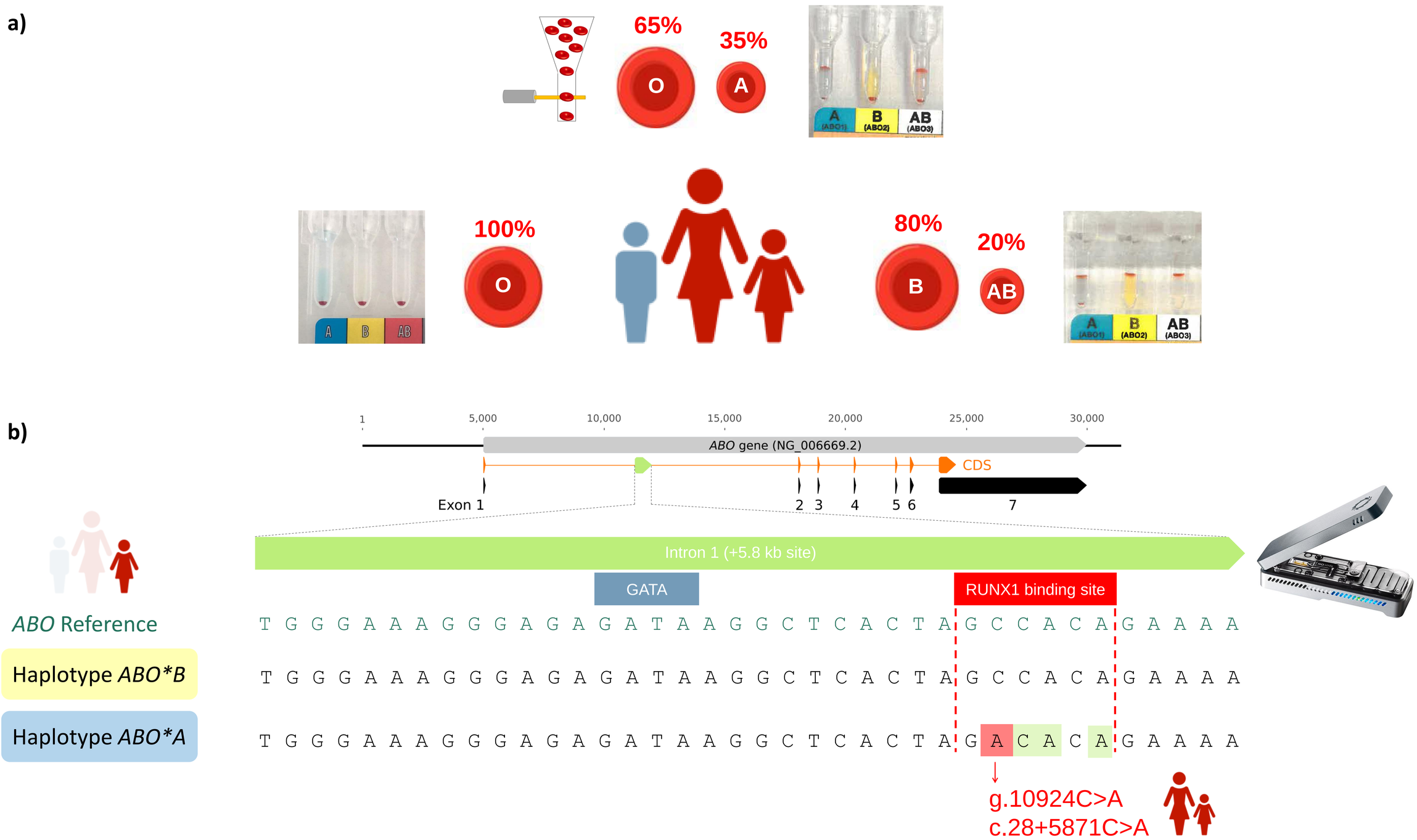

Fig 1. (a) Serological and flow cytometry results for donor, her mother and brother. **(b)** Alignment of *ABO* reference (NG_006669.2) and nanopore-based haplotype sequences showing novel variant in RUNX1 binding site in intron 1 (+5.8 kb site) on *ABO*A* allele of donor. Same SNV was found on the mother's *ABO*A* allele. Sequence positions of previously reported RUNX1 variants linked to anti-A or anti-B mixed-field agglutination are highlighted in light green[2, 3].

Results

- Mixed-field reaction with anti-A in donor genotyped as AB (Fig. 1a)
- Agglutination with anti-H was weak and absent with anti-A1
- Nanopore sequencing: novel variant (g.10924C>A) on the *ABO*A* allele in a binding motif for the transcription factor Runt-related transcription factor 1 (RUNX1) (Fig. 1b)
- Germline SNV:
  Mother (genotype: *ABO*A* | *ABO*O.01*; serology: mixed-field agglutination)
  - ➢ SNV in RUNX1 binding site of *ABO*A* allele (Fig. 1b)

  Brother (genotype: *ABO*O.01* | *ABO*O.01;* serology: O)
  - ➢ No SNV in RUNX1 binding site

Conclusions

- Discovery of a unknown SNV in RUNX1 binding motif causing an A_3 phenotype
- Extends current knowledge of four other variants[2,3] affecting this motif, all leading to A_3/B_3 or A_m/B_m phenotypes
- Nanopore sequencing of long-range PCRs allowed haplotype generation of the entire *ABO* gene
  → This simplifies the crucial assessment of known and unknown regulatory regions in cases of complex antigen expression

References

1. ABO blood group alleles v1.1 21-OCT-2017 ISBT
2. Ying et al. (2018). Vox Sanguinis, 113(6), 594-600
3. Hult et al. (2020). Vox Sanguinis, 115(Suppl. 1), 15, 3A-S04-03

Abstract