Two Prevalent *GYPB* Deletions are Causative of the MNSs Blood Group U Negativity in Black Africans

Christoph Gassner[1], Gregory A. Denomme[2], Claudia Portmann[1], Stefan Meyer[1], Nadine Trost[1], Christof Jungbauer[3], Burkhard Just[4], Jill Storry[5], Michael Forster[6]*, Andre Franke[6]*, Beat M. Frey[7]*

1 Blood Transf. Service Zurich, SRC, Molecular Diagnostics and R&D, Swiss Red Cross, Zürich-Schlieren, Switzerland
2 Blood Research Institute, BloodCenter of Wisconsin, Medical College of Wisconsin, Milwaukee, Wisconsin, USA.
3 Austrian Red Cross, Blood Service for Vienna, Lower Austria and Burgenland, Vienna, Austria.
4 German Red Cross Blood Donation Service West, Hagen, Germany.
5 Hematology & Transfusion Medicine, Lund University, Department of Laboratory Medicine, Lund, Sweden
6 Institute of Clinical Molecular Biology, Christian Albrechts University of Kiel, Kiel, Germany.
7 Blood Transfusion Service Zurich, SRC, Head Office, Zürich-Schlieren, Switzerland.
* Contributed equally. Conflict of interest: patent pending.

Background

Antigen U has originally been described in 1953 and was characterized as a high-frequency-antigen, absent in 1.2% of African Americans (Wiener, JAMA, 1953). In 1954 the association with the MNS blood group system and concurrent phenotype S, s negativity became evident (Greenwalt, PNAS, 1954), and later postulated to be caused by a homozygous deletion of *GYPB* (Huang, Blood, 1987). Until now however, lack of exact molecular definition of the causative deletions prohibited real (better “true”?) genotyping, e.g. unequivocal interrogation of both parental *GYPB* negative haplotypes.

Aims

The study aimed for an exact molecular definition of *GYPB* deletions, causative of recessive negativity in phenotype S-s-U- Black Africans. Added to classical MNS genotyping, positive detection of the respective deletions should enable for real (better “true”?) *GYPB* genotyping.

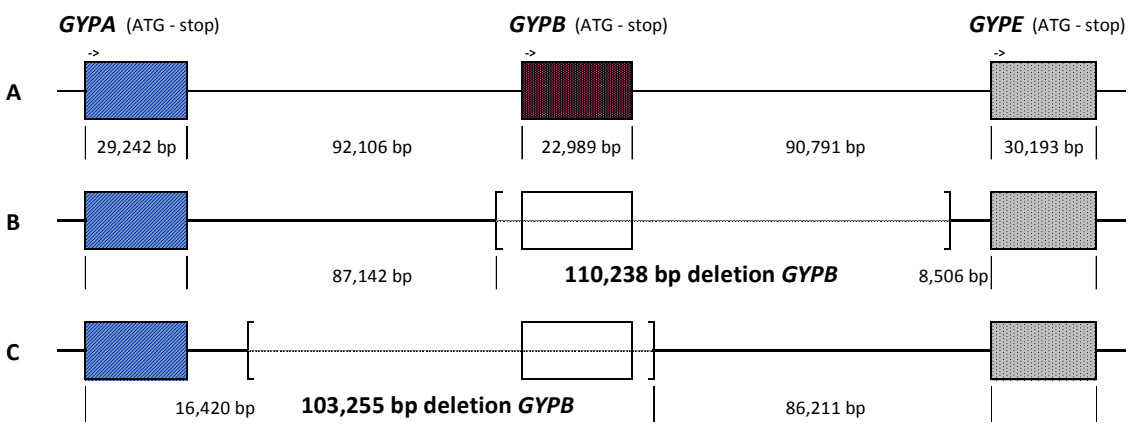

Figure 1: Schematic representation of *GYPB* deletions observed in individuals of S-s-U- phenotype. *GYPA*, *GYPB* and *GYPE* displayed to scale and given in blue, red and gray colour, respectively. Wildtype *GYP* locus (A), *GYPB* deletion of “110 kb” (B) and *GYPB* deletion of “103 kb” (C).

Methods

Bioinformatical analysis of 1000 human genome (hgh) data revealed several hits for two distinct, ~100 kb, and one hit each for a ~32 kb and ~18 kb *GYPB* deletion. Hits were predominantly identified among Black Africans. Sanger sequences of analytical gap-PCRs bridging these deletions in predefined S-s-U- samples revealed their exact molecular positions and were used to device specific diagnostic PCRs using sequence specific priming (PCR-SSP). Genotyping was performed in 24 samples of known S-s-U- phenotype and concomitant negativity for both public alleles *GYPB*03* (S) and *GYPB*04* (s), plus the hgh samples of the Coriell Human Genetic Cell Repository, showing the ~32 and ~18 kb deletions.

Results

One 110.24 kb deletion stretched from 4.96 kb 5’ of the start codon of *GYPB* until 8.51 kb 5’ of the start codon of *GYPE*. The other 103.26 kb deletion started 16.42 kb 3’ of the stop codon of *GYPA* and ended 4.58 kb 3’ of the *GYPB* stop codon. Both deletions encompassed the whole *GYPB* gene and involved highly paralogous intergenic sequences of the *GYP*-locus, suggesting unequal crossing-over as causal molecular origin for this variation (**Figure 1**). Of 23 S-s-U- samples, 13 genotyped *GYPB*05N(del110kb)* homozygous, 6 *GYPB*05N(del110kb)/ (del103kb)* heterozygous, one *GYPB*05N(del103kb)* homozygous and three were heterozygous for *GYPB*05N(del110kb)* and a as yet undefined, second parental *GYPB*-deletion. The 32 kb deletion was *GYPB*05N(del110kb)/(del103kb)* heterozygous thereby overlapping for 32 kb with complete negativity for *GYPB* sequences. The suggested 18 kb deletion was only observed in the original Coriell sample. Of 48 haplotypes with a presumptive *GYPB* deletion analyzed in total, 35 (72.9%) were *GYPB*05N(del110kb)*, 9 (18.8%) were *GYPB*05N(del103kb)*, and 3 (6.3%) remained unresolved. Overall haplotype-frequency was estimated to be 11.0%, considering above mentioned 1.2% S-s-U- phenotype frequency in Black Africans.

Summary

This study describes real blood group MNS genotyping for individuals with an involvement of a S-s-U- causative haplotype. Now, the presented positive genotyping of two causal *GYPB* deletions (**Figure 2**), performed simultaneously with classical genotyping for S, s and the rudimentary expressed Uvar alleles, allows for unequivocal results and correct phenotype predictions of all genotypes involved. Taking above mentioned 1.2% S-s-U- phenotype frequency as an example, heterozygous involvement of such *GYPB* deletions may be expected in about 19.5% of all Black Americans. Recent reports on host-Malaria interaction are further supportive of our independent findings (Leffler, Science, 2017).

References. Wiener AS, et al 1953. Fatal hemolytic transf. rctn. caused by sensitization to a new blood factor U: case. J Am Med Assoc. 1953 Dec 19;153(16):1444-6. **Greenwalt TJ, et al 1954**. An Allele of the S(s) Blood Group Genes. PNAS U S A. 1954 Dec;40(12):1126-9. **Huang CH et al 1987**. Delta glycophorin (gp B) gene deletion in two individuals homozygous for the S--s--U-- bg phenotype. Blood. 1987 Dec;70(6):1830-5. **Leffler EM, et al 2017**. Resistance to malaria through structural variation of red blood cell invasion receptors. Science. 2017 Jun 16;356(6343).

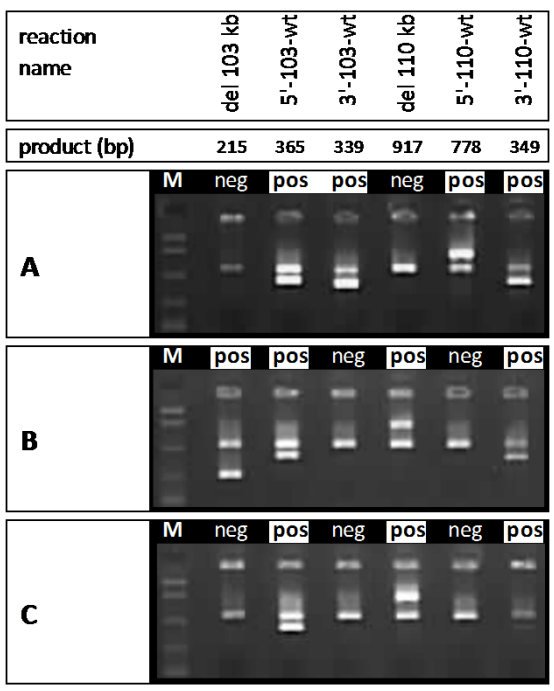

Figure 2: Genotyping for *GYPB*05N* haplotypes of the “del103kb” and the “del110kb” type using PCR-SSP.

Panel A wildtype sample with two regular *GYPB* genes. Phenotype SS, Ss, or ss.

Panel B, compound heterozygous sample with genotype *GYPB*05N*(del103kb/del110kb). Phenotype S-s-U-.

Panel C, homozygous sample genotype *GYPB*05N*(del110kb). Phenotype S-s-U-.