

MALDI-TOF MS for MNSs typing – high pheno/genotype concordance in 5,743 Swiss

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Background

Transfusion of alloimmunized patients and its prevention may ideally be addressed by expanded blood group antigen matching protocols. For this purpose, beside ABO and RhD, a reasonable set of considerable antigens may include RhC/c(C^w), E/e, K/k, Jk(a/b), Fy(a/b), M/N and S/s. Blood group genotyping has proven its capability in this context. However, in comparison to K/k, Jk(a/b) and Fy(a/b)¹, published performance data for MNSs genotyping are underrepresented.

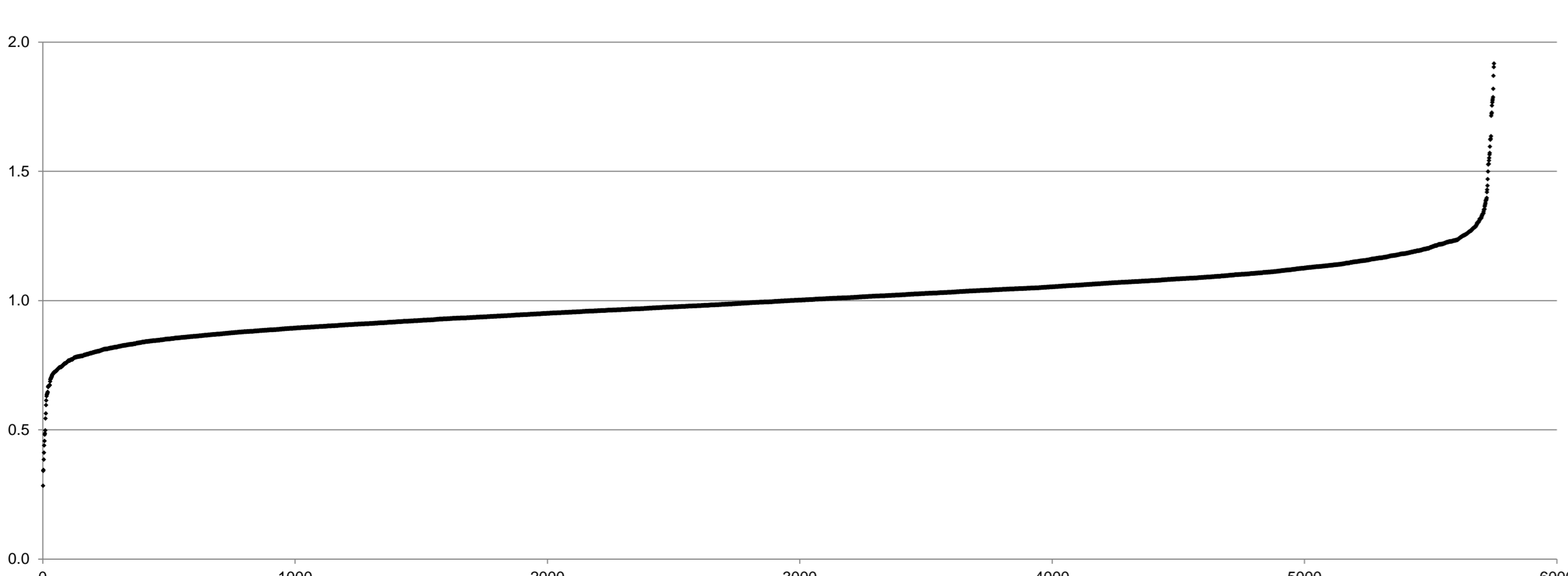
Aim of the project

Estimate performance of MALDI-TOF MS for MNSs genotyping: Resulting genotypes and existing serological values for MNSs should be compared and checked for concordance.

Results

Concordant MN phenotypes comprised into 1,711 MM (with 1 Vw), 2,807 MN (with 1 He, 4 Vw and 10 Mt(a)), and 1,208 NN (with 4 Vw and 4 Mt(a+)) all located on *GYPB* (pheno/genotype concordance rate 99,88%, 5,726 of 5,743). Four original MM, 10 MN and 3 NN phenotypes showed discrepancies in comparison to genotyping of which 4 MM (1 no follow up), 3 MN (1 no follow up), and 3 NN were due to serological mistypings. Sequencing of 7 MM genotypes with discrepant MN phenotypes revealed presence of *GYPB* hybrid genes, resulting in 1 Mur(*GYP.501*)-like, and 6 Sch(*GYP.401*)-like alleles, all known to encode N-like phenotypes, while *GYPB*02*(N) negative. Genotyping for Ss on *GYPB* delivered full concordance of pheno/genotypes for 619 SS, 2,416 Ss and 2,702 ss samples (concordance-rate 99,90%, 5,737 of 5,743). Discrepancies were due to serological mistypings (2 Ss, 1 ss) and "genotyping errors". We identified a G145A(Gly49Arg) mutation in the *GYPB*03* primer binding-site and two donors with a presumably new *GYPB*03* null-allele with a G218A(Gly73Asp) substitution (1 Ss, 2ss) (all shown in **Figure 2**).

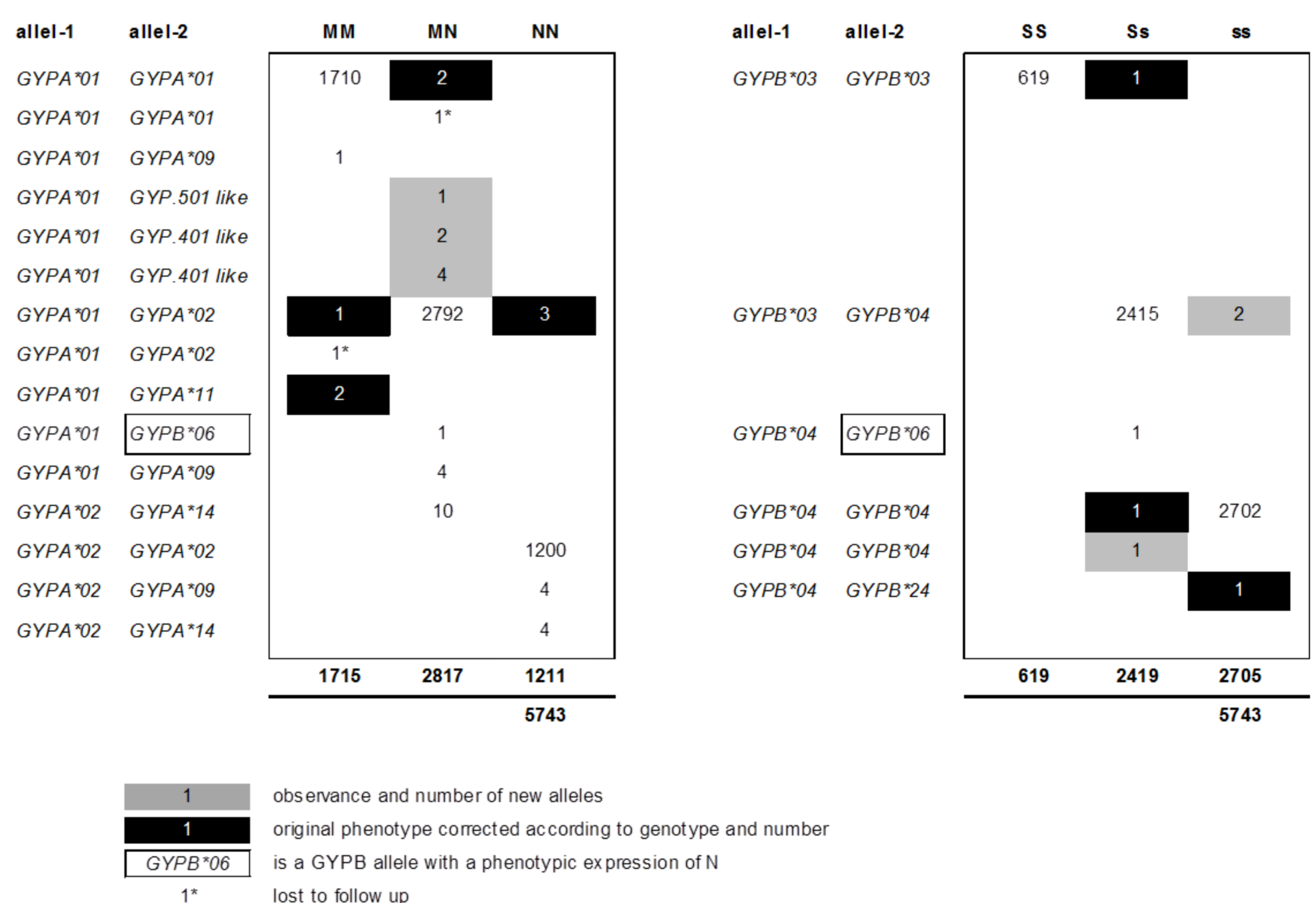
Figure 1. *GYPB:GYPE* intron 1, position 16 kbp, adjusted area under peak ratio values for all 5,743 Swiss donor samples tested are shown. Ratio values range in between 0,28 and 1,92, indicating uneven *GYPB:GYPE* gene copy numbers in a minority of samples.



Methods

Genotyping relied on MALDI-TOF MS based SNP-detection at coding nucleotides 59(C/T) and 72(T/G) for M/N and G59 for He (actually on *GYPB*), 140(C/T/A) for Vw/Hut and 230(C/T) for Mt(a+) on *GYPB*, and 143(T/C) for S/s, intron 4+5(G/T) for U+^w and intron 1+9103/+15014(C/T, C/G) zygosity typing for the "GYPB deletional U- phenotype", on *GYPB* (**Figure 1**). The approach included 10 antigens of the MNSs blood group system, encoded by 8 SNPs on 10 *GYPB* alleles, multiplexed into 1 reaction. All genotyping results were compared to existing standard-serological MNSs values of 5,743 Swiss donor samples. Generic and allele-specific PCR-SSPs and Sanger-DNA sequencing revealed genetic backgrounds in cases with confirmed pheno/genotype discordances.

Figure 2. Comparison of genotypes and phenotypes observed among 5,743 Swiss donors. Left and right panel show MN and Ss pheno- and genotyping, on *GYPB* and *GYPB*, respectively.



Summary

MNSs phenotyping errors had approximately the same frequency as "genotyping errors", which could all be explained by rarely occurring *GYPB* genetic variants, or newly discovered alleles. Consequently, "genotyping errors" may rather be interpreted as specific "indicators", than profane "errors". No repetitive genotyping was done and still, there was no evidence at all for any technical SNP-typing error judging all the 57,430 SNP-genotypes, obtained in this study. Only 57 samples, on top of the 5,743 with complete data sets, had single, or multiple SNP-genotype drop-outs, resulting in the low drop-out rate of only 0.98% (no result). MALDI-TOF MS based MNSs genotyping proved to be extremely practical, robust and accurate, and – for donors – may well be considered as a valid stand-alone method and/or valuable addition for serotyping.