Letter to the Editor

Description of the first case of c.137-8C>T *GYPB* mutation not associated to the *GYPB(P2)* allele

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Dear Sir,

The MNS gene complex (*GYP*) is located on the long arm of chromosome 4 (4q31.21) and consists of three closely linked genes: *GYPA* encoding for glycophorin A which carries M and N antigens, *GYPB* encoding for glycophorin B which carries S, s and U, *GYPE* that is not responsible for any MNS antigen specificity[1,2]. A few dozens of Single Nucleotide Polymorphisms (SNPs) in *GYPA* and *GPYB*, generating variant antigens in the MNS system, are reported in the literature[3,4]. Two alleles of the *GYPB* gene are responsible for the S–,s–, U^{var} phenotype, the so-called *P2* and *NY* alleles. Importantly, all *GYPB(P2)* and *GYPB(NY)* alleles analysed so far carry the *GYPB*S*-specific SNP. In 2013 Arnaud *et al.* described a *GYPB* mutation that interfered in genotyping the *GYPB*S* allele with the BioArray HEA v1.2 BeadChip™ kit (Werfen, Milan, Italy): the discovery of two patients genotyped as heterozygous for *GYPB(P2)* and homozygous for *GYPB*s* with that kit, led to the identification of a novel c.137-8C>T mutation –associated with the *P2* allele– in their DNA[5].

Our extended serological typing profiles include the characterization for ABO, C, E, c, e, K, k, Kpa, Kpb, Fya, Fyb, Jka, Jkb, M, N, S, s, Lua, Lub, P1, Xga, Lea, Leb, using a fully automated gel test method (DG Gel) supplied by Grifols (Barcelona, Spain).

For genotyping, we adopted the microarray-based BioArray HEA v1.2 BeadChip kit supplied by Werfen that involves characterization for C, E, c, e, V, VS, K, k, Kpa, Kpb, Jsa, Jsb, Fya, Fyb, Fyb-weak, Fy-null, Jka, Jkb, M, N, S, s, U-var, U-neg, Lua, Lub, Dia, Dib, Doa, Dob, Hy, Joa, Coa, Cob, Sc1, Sc2, LWa, LWb. U^{var} *P2* and *NY* alleles are detected by the BioArray HEA v1.2 BeadChip kit with the use of specific probes for c.270+5G>T and c.230C>T mutations, respectively.

Donor indicated as MS, Caucasian male, age 38, A ccDEE kk was selected for extended serotyping and genotyping because of his RhCE haplotype. Extended serotyping gave us the following results: Fya+b+, Jka+b+, Kpa-b+, Lea–b+, Lua–b+, M+N+S+s+, P1+. The S antigen was positive, but with a moderate reactivity (+---). Predicted phenotype based on genotyping was as follows: C–c+, E+e–, K–k+, Kpa–b+, Jsa–b+, Fya+b+, Jka+b+, M+, N+ S (not determined), s+, U+, Lua–b+, Dia–b+, Coa+b–, Doa+b–, Hy+, Joa+, LWa+b–, Sc1+2–.

In order to better characterize the *GYPB*S* allele, a genomic DNA aliquot from donor MS was sent to a reference laboratory for DNA bidirectional sequencing. As shown in **Figure 1**, genomic sequencing confirmed the presence of the *GYPB*S* and *GYPB*s* alleles, thus predicting an S+s+ phenotype. However, a single heterozygous c.137-8C>T point mutation was also observed. This polymorphism, recorded in the databases as rs183176514,

Arrived: 24 April 2024
Accepted: 3 May 2024
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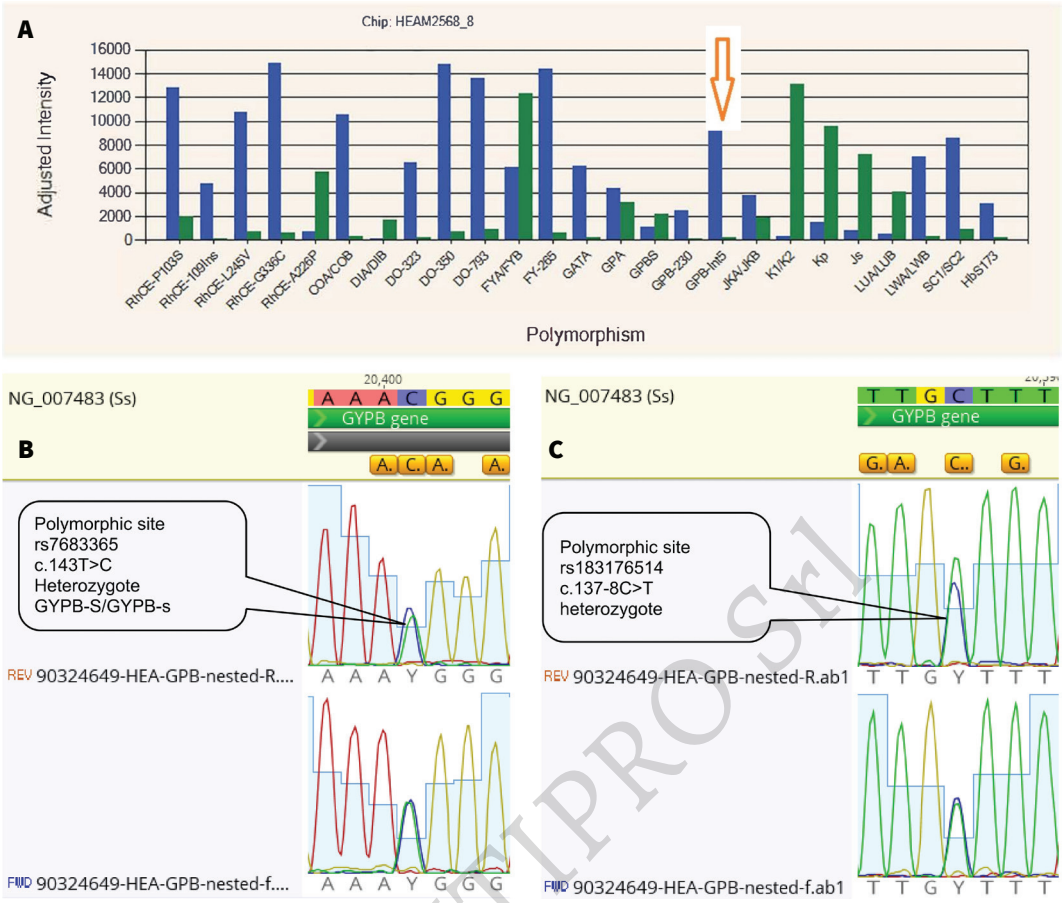


Figure 1 - Genotyping results
(**A**) shows the genotyping performed using the Werfen BioArray HEA v1.2 BeadChip kit the arrow indicates the absence of the *GYPB(P2)* allele. (**B** and **C**) in the sequences: red: adenine (A), blue: cytosine (C), yellow: guanine (G), green: thymine (T), green and blue: pyrimidine (Y).

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is in the HEA BeadChip™ kit probe binding region, and affects the detection of the S antigen resulting in the indeterminate calls repeatedly obtained in our molecular analyses. Both sequencing analysis and HEA BeadChip™ results (**Figure 1A**, orange arrow) showed no evidence for the c.270+5G/T polymorphism, which is characteristic of the *GYPB(P2)* allele

The c.137-8C>T *GYPB* mutation is known to affect the detection of the S antigen (*GYPB* c.143T) by the BioArray HEA v1.2 BeadChip kit, resulting in the indeterminate calls we have observed. In previous reports this mutation was associated to the c.270+5G/T polymorphism, which is characteristic of *GYPB(P2)* allele[5].

To the best of our knowledge this is the first description of a case where this gene alteration is not associated with the presence of a *P2* allele. Based on our serological data, this mutation causes a weak expression of the S antigen when it's not associated to other *GYPB* polymorphisms.

DISCLOSURE OF CONFLICTS OF INTEREST

TM is a Werfen employee, the other Authors declare no conflicts of interest.

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