MNS antigen Mg exclusively appears as 68C>A mutant of *GYPA*02* (N) within the Zurich area of Switzerland

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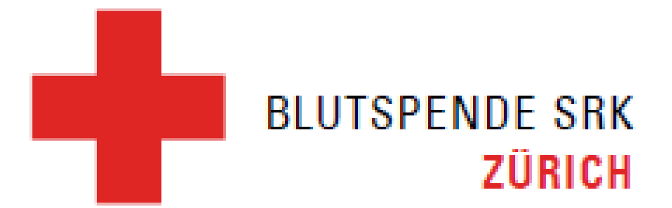

Background

The human MNSs blood group system is encoded by the genes *GYPA* and *GYPB* and is considered as second in complexity to Rh. Mg, encoded by *GYPA*11*, is a low-frequency antigen located on *GYPA*. It has repeatedly been described to have a *GYP(A-B-A)* hybrid structure with a C>A substitution at coding nucleotide (cdnt) 68. Dating back to the 1960ies, Mg had been reported to be found on both *GYPA* alleles, i.e. M and N, in both cases with virtually undetectable expression of M, or N. (**Figure 1**).

exons (*GYPA*)		Exon 1, 37 bp												Exon 2, 99 bp														
GypA nascent aa count		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
GypA cleaved aa count																					1	2	3	4	5	6	7	8
M	*GYPA*01*	M	Y	G	K	I	I	F	V	L	L	L	S	A	I	V	S	I	S	A	S	S	T	T	G	V	A	M
N	*GYPA*02*	M	Y	G	K	I	I	F	V	L	L	L	S	E	I	V	S	I	S	A	L	S	T	T	E	V	A	M
Mg (on N)	*GYPA*11*	M	Y	G	K	I	I	F	V	L	L	L	S	E	I	V	S	I	S	A	L	S	T	N	E	V	A	M
S	*GYPB*03*	M	Y	G	K	I	I	F	V	L	L	L	S	E	I	V	S	I	S	A	L	S	T	T	E	V	A	M
s	*GYPB*04*	M	Y	G	K	I	I	F	V	L	L	L	S	E	I	V	S	I	S	A	L	S	T	T	E	V	A	M
		Leader Sequence (cleaved from nascent peptide)																										

Figure 1: Amino-terminal **peptide sequences** of GypA (blue boxed cells including M/N amino-acid (aa) exchanges) and GypB (S/s aa exchange not comprised in figure), first 27 aa of nascent, 8 aa of mature peptides. Of note, identity of GypB (red boxes) to N of GypA. Predicted peptide sequence of Mg, e.g. specific p.Thr23Asn substitution (black cell), located on N of GypA.

Aims

Mg is very rare, with higher incidences only reported for Swiss and Sicilians, reaching up to one Mg positive individual among 600. A number of seven available *GYPA*11* positive cases prompted us to (re)investigate Mg and its molecular background in detail.

Methods

MALDI-TOF MS based blood group MNSs genotyping interrogated cdnt 59C>T of *GYPA* for MN, and cdnt 143T>C of *GYPB* for Ss phenotype predictions. [1,2] All genotyping results were compared to MNSs phenotypes, obtained by standard-serological methods. All *GYPA*11* positive samples were identified by original discrepancy of genotype versus existent phenotype, and repetition of genotyping using a commercially available PCR-SSP based method, including testing for *GYPA*11* (inno-train GmbH, Kronberg i.T., Germany). All *GYPA*11* positive samples and two individuals each of MMSS, MMss, NNSS, and NNss phenotypes were sequenced for *GYPA* from intron 1 (102 bp), across exon 2 and intron 2 (335 bp).

Results

MALDI-TOF MS based MN genotyping of 11.240 blood donors of the Zurich area in Switzerland delivered seven cases with M+N- serology, but a preliminary *GYPA*01/02* (MN) genotype. All genotype repetitions delivered final *GYPA*01/11* heterozygous results. Alignments of the investigated sequence did not show any *GYPB* specific nucleotides on *GYPA*11* and exactly corresponded to the *GYPA*02* (N) allele, beside its specific cdnt 68C>A point mutation (**Figure 2**). *GYPA*11* allele frequency was calculated to be 0.136%. Consequently, the expected overall frequency of Mg positive individuals is one among 368 in the Zurich area of Switzerland.

			intron 1								exon 2					intron 2							
			-153	-152	-137	-118	-83	-67	-66	-50	38	59	68	71	72	41	42	54	60	149	155	156	167
reference	*GYPA* 01*	*M*	A	T	C	C	-	C	A	C	C	C	C	G	T	C	A	T	-	C	T	G	T
in house	*GYPA* 01*	*M*	A	T	C	C	-	C	A	C	C	C	C	G	T	C	A	T	-	N	N	N	N
in house	*GYPA* 02*	*N*	A	T	C	C	-	C	A	C	C	T	C	A	G	C	A	T	-	C	T	G	T
in house	*GYPA* 11*	**Mg**	A	T	C	C	-	C	A	C	C	T	A	A	G	C	A	T	-	C	T	G	T
in house	*GYPA* 11*	**Mg**	A	T	C	C	-	C	A	C	C	T	A	A	G	C	A	T	-	C	T	G	T
reference	*GYPB* 04*	*s*	G	C	G	T	A	T	G	T	A	T	C	A		G	A	C	T	T	C	A	A
in house	*GYPB* 03*	*S*	G	C	G	T	A	T	G	T	A	T	C	A		G	A	C	T	T	C	A	A
in house	*GYPB* 04*	*s*	G	C	G	T	A	T	G	N	A	T	C	A		G	A	C	T	T	C	A	A
reference	*GYPE* n.a.*	*n.a.*	A	C	G	C	-	T	G	C	G	C	C	G		C	G	C	T	C	C	A	T
in house	*GYPE* n.a.*	*n.a.*	A	C	G	N	-	T	G	C	N	C	C	G		C	G	C	T	C	C	A	T
in house	*GYPE* n.a.*	*n.a.*	A	C	-	N	-	T	G	C	A	C	C	G		C	G	C	T	N	C	A	T

Figure 2: Polymorphisms in between *GYPA, B,* and *E* exon 2 and adjacent intronic **nucleotide sequences**, in blue, red and grey, respectively. Each *GYP* gene is represented by one publically retrieved (NCBI) and two in house reference sequences (from MMSS and NNss phenotype homozygotes). Lines *GYPA*11* (Mg) display sequences derived from the "serologically un-expressed *GYPA*02* allele" of two samples, later confirmed in all seven discrepant samples. Mg-specific mutation 68C>A (black). There is no evidence for Mg arising from a *GYP(A-B-A)* hybrid.

Summary

Molecular analysis of seven *GYPA*11* (Mg) positive individuals did not deliver any evidences for Mg being encoded by an M-allele. Controversially to reports of Mg, supposedly having a *GYP(A-B-A)* hybrid structure, results rather suggested presence of a simple point mutation instead. *GYP*11*, common in Switzerland, seems to be a derivative of *GYPA*02* (N) with a simple 1 bp substitution at cdnt 68C>A.

1: Meyer S, Vollmert C, Trost N, et al. MNSs genotyping by MALDI-TOF MS shows high concordance with serology, allows gene copy number testing and reveals new St(a) alleles. Br J Haematol. 2016 Aug;174(4):624-36.

2: Meyer S, Trost N, Frey BM, Gassner C. Parallel donor genotyping for 46 selected blood group and 4 human platelet antigens using high-throughput MALDI-TOF mass spectrometry. Methods Mol Biol. 2015;1310:51-70.

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