Molecular Blood Group Screening in Donors from Arabian Countries and Iran Using High-Throughput MALDI-TOF Mass Spectrometry and PCR-SSP

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Keywords

Arabian donors · Blood groups · Donor screening · PCR · Genotyping · MALDI-TOF MS · Molecular blood group typing

Abstract

Background and Aims: Only little is known about blood groups other than ABO blood groups and Rhesus factors in Arabian countries and Iran. During the last years, increased migration to Central Europe has put a focus on the question how to guarantee blood supply for patients from these countries, particularly because hemoglobinopathies with the need of regular blood support are more frequent in patients from that region. Therefore, blood group allele frequencies should be determined in individuals from Arabian countries and Iran by molecular typing and compared to a German rare donor panel. **Methods:** 1,111 samples including 800 individuals from Syria, 147 from Iran, 123 from the Arabian Peninsula, and 41 from Northern African countries were included in a MALDI-TOF MS assay to detect polymorphisms coding for Kk, Fy(a/b), Fy_{null}, C_w, Jk(a/b), Jo(a+/-), Lu(a/b), Lu(8/14), Ss, Do(a/b), Co(a/b), In(a/b), Js(a/b), Kp(a/b), and variant alleles *RHCE**c.697C>G and *RHCE**c.733C>G. Yt(a/b), S-s-U-, Vel_{null}, Co_{null}, and *RHCE**c.667G>T were tested by PCR-SSP. **Results:** Of the Arabian donors, 2% were homozy-

gous for the *FY**02.01N allele (Fy_{null}), and 15.7% carried the heterozygous mutation. However, 0.8% of the German donors also carried 1 copy of the allele. 3.6% of all and 29.3% of Northern African donors were heterozygous for the *RHCE**c.733C>G substitution, 0.4% of the Syrian probands were heterozygous for *DO**01/*DO**01.-05, a genotype that was lacking in German donors. Whereas the *KEL**02.06 allele coding for the Js(a) phenotype was missing in Germans; 0.8% of the Syrian donors carried 1 copy of this allele. 1.8% of the Syrian but only 0.3% of the German donors were negative for *YT**01. One donor from Northern Africa homozygously carried the *GYPB**270+5g>t mutation, inducing the S-s-U+^w phenotype, and in 2 German donors a *GYPB**c.161G>A exchange, which induces the Mit+ phenotype, caused a *GYPB**03 allele dropout in the MALDI assay. The overall failure rate of the Arabian panel was 0.4%. **Conclusions:** Some blood group alleles that are largely lacking in Europeans but had been described in African individuals are present in Arabian populations at a somewhat lower frequency. In single cases, it could be challenging to provide immunized Arabian patients with compatible blood.

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Introduction

The main goal in transfusion medicine is to provide compatible blood products for each patient. Blood supply can be a challenging if the patient has got immunized to high-frequency blood group alloantigens (HFA) [1, 2]. Special national and international rare donor panels and the ISBT working party on rare donors support transfusion specialists to identify donors with rare blood groups [3]. Particularly, if a patient with an alloantibody to HFA belongs to another ethnicity than the major donor population it can be cumbersome to find a compatible donor. During the last years, a higher number of individuals migrated from Arabian countries like Syria, Afghanistan, Iraq, the Maghreb states, and other African countries to Europe. Since 2015, more than 3 million people arrived at the European Union [4], and about 1.68 million refugees applied for asylum in Germany, most of them originating from Syria [5, 6]. The impact of migration on patient-centered care was reported in a longitudinal study over 4 decades [7], and an increase in hemoglobinopathies like thalassemia and sickle cell disease was observed in Europe [8]. Unfortunately, only little is known about frequencies of blood groups other than ABO and Rhesus factors in Arabian countries. Available ABO and Rhesus phenotyping data demonstrate different frequencies even in geographically adjacent regions, which might be explained by the presence of rather closed populations without admixture [9–12]. For example, a French-Afghan study that applied multiplex PCR and SnapShot typing to different blood group systems in Afghan and Pakistani populations could demonstrate slightly varying allele frequencies for most examined blood groups [13]. An Israeli study showed that the Fy_{null} phenotype on red blood cells, which is caused by the $FY^{*}-67t>c$ promoter mutation, varies between 0% in Ashkenazi and Sephard Jews and 10% in Muslim Arabs [14]. In some sub-Saharan regions, the Fy_{null} phenotype predominates since deficiency in the Fy antigen prevents red blood cells and their precursors from being infected by *Plasmodium vivax*, an elicitor of Malaria tertiana [15, 16]. Single divergent data were reported on the Kell blood groups K and Js^a [10, 13, 14, 17, 18].

Although single reports on blood group antigen frequencies exist, no comprehensive overview from Syria is available. Thus, we aimed to determine blood group frequencies other than ABO, Rhesus D, C, c, E, or e predominantly in donors from Syria and other Arabian countries. Extensive phenotyping is cumbersome due to the shortage of typing sera, whereas molecular high-throughput typing gained importance during the last years [13, 19–24]. We and others successfully applied MALDI-TOF MS for blood group screening of our donor population in order to identify rare donors who are negative for special HFA [25–27]. In the present study, our existing panel was

Table 1. Countries of origin of the probands

Country	Probands, <i>n</i>	Total, <i>n</i>
Syria	800	800
Iran	147	147
<i>Northern Africa</i>		41
Libya	1	
Morocco	19	
Tunisia	7	
Algeria	5	
Egypt	9	
<i>Arabian Peninsula</i>		123
Saudi Arabia	4	
Yemen	2	
Jordan	7	
Iraq	49	
United Arab Emirates	1	
Israel	6	
Lebanon	48	
Palestine	5	
Oman	1	
<i>German rare donor panel</i>		
Germany	2,821–20,529	

The total number of probands of the German rare donor panel was 20,529, but not each blood group was tested for each donor.

slightly modified and completed by PCR-SSP to type for blood group alleles in donors from Arabian countries and Iran.

Materials and Methods

Subjects

Between June 2016 and April 2018, a total of 1,111 blood samples were collected from volunteers originating from Syria, Iran, and further 14 countries in Northern Africa and the Arabian Peninsula (Table 1). In terms of a simplified description, this whole collective is named Arabian panel. Attribution to mother countries was performed due to self declaration. Out of these, 266 were Syrian volunteers that were recruited at social venues for refugees in Rhineland-Palatinate from June 2016 till October 2017. After informed consent had been obtained, they only gave 8 mL of EDTA anticoagulated blood for the study. The remaining 845 donors from different countries presented from July 2017 to April 2018 at regular blood collections of the German Red Cross Blood Service West in North Rhine-Westphalia, Rhineland-Palatinate, and Saarland. There was no preselection concerning ABO blood groups and Rhesus factors. All subjects had given informed consent, and the study on blood group frequencies in Arabian individuals was approved by the Ethics Committee of the Medical Chamber of Rhineland-Palatinate in Mainz, Germany.

A further 20,529 blood samples from regular repetitive blood donors of the German Red Cross Blood Service West were collected from June 2012 to June 2019. The aim of this continuing, subsequently called German rare donor panel is to identify donors with rare blood groups. The predominant majority of these donors

Table 2. Blood group SNPs included in the MALDI-TOF MS assays

Blood group system	ISBT No.	Blood group	Gene	Allele 1	Allele 2	NT 1	NT 2	Amino acid exchange	db SNP rs No.
<i>Assay 1 (German rare donor panel)</i>									
MNS	002	M/N	<i>GYPA</i>	<i>GYPA*01</i> (rf)	<i>GYPA*02</i>	71G, 72T	71A, 72G	Gly ²⁴ Glu	rs7687256 rs7658293
MNS	002	S/s	<i>GYPB</i>	<i>GYPB*03</i>	<i>GYPB*04</i> (rf)	143T	143C	Met ⁴⁸ Thr	rs7683365
Rhesus	004	C/C ^w	<i>RHCE</i>	<i>RHCE*C</i>	<i>RHCE*C^w</i>	122A	122G	Gln ⁴¹ Arg	rs138268848
Lutheran	005	Lu ^a /Lu ^b	<i>BCAM</i>	<i>LU*01</i>	<i>LU*02</i> (rf)	230A	230G	His ⁷⁷ Arg	rs28399653
Lutheran	005	Lu ^a /Lu ¹⁴	<i>BCAM</i>	<i>LU*02</i> (rf)	<i>LU02.14</i>	611T	611A	Met ²⁰⁴ Lys	rs28399656
Kell	006	Kp ^a /Kp ^b	<i>KEL</i>	<i>KEL*02.03</i>	<i>KEL*02</i> (rf)	841T	841C	Trp ²⁸¹ Arg	rs176059
Duffy	008	Fy ^a /Fy ^b	<i>ACKR1</i>	<i>FY*01</i>	<i>FY*02</i> (rf)	125G	125A	Gly ⁴² Asp	rs12075
Duffy	008	Fy ^b /Fy _{null}	<i>ACKR1</i>	<i>FY*02</i> (rf)	<i>FY*02N.01</i>	−67t	−67c (Promoter)	Protein absent on RBC	rs2814778
Kidd	009	Jk ^a /Jk ^b	<i>SLC14A1</i>	<i>JK*01</i>	<i>JK*02</i> (rf)	838G	838A	Asp ²⁸⁰ Asn	rs1058396
Cartwright	011	Yt ^a /Yt ^b	<i>ACHE</i>	<i>YT*01</i> (rf)	<i>YT*02</i>	1057C	1057A	His ³⁵³ Asn	rs1799805
Dombrock	014	Do ^a /Do ^b	<i>ART4</i>	<i>DO*01</i> (rf)	<i>DO*02</i>	793A	793G	Asn ²⁶⁵ Asp	rs11276
Colton	015	Co ^a /Co ^b	<i>AQP1</i>	<i>CO*01</i> (rf)	<i>CO*02.01</i>	134C	134T	Ala ⁴⁵ Val	rs28362692
Colton	015	Co ^a /Co _{null}	<i>AQP1</i>	<i>CO*01</i> (rf)	<i>CO*01N.06</i>	601G	601delG	Val ²⁰¹ stop	rs28362737
Vel	034	Vel/Vel _{null}	<i>SMIM1</i>	<i>VEL*01</i> (rf)	<i>VEL*-01</i>		64_80del	Gln ²² fs	rs566629828
<i>Assay 2 (Arabian panel)</i>									
MNS	002	S/s	<i>GYPB</i>	<i>GYPB*03</i>	<i>GYPB*04</i> (rf)	143T	143C	Met ⁴⁸ Thr	rs7683365
Rhesus	004	C/C ^w	<i>RHCE</i>	<i>RHCE*C</i>	<i>RHCE*C^w</i>	122A	122G	Gln ⁴¹ Arg	rs138268848
Rhesus	004	e/part. e or e+ ^w	<i>RHCE</i>	<i>RHCE*01</i> (rf)	<i>RHCE*697C>G</i>	697C	697G	Gln ²³³ Glu	rs142246017
Rhesus	004	e/part. e	<i>RHCE</i>	<i>RHCE*01</i> (rf)	<i>RHCE*733C>G</i>	733C	733G	Leu ²⁴⁵ Val	rs1053361
Lutheran	005	Lu ^a /Lu ^b	<i>BCAM</i>	<i>LU*01</i>	<i>LU*02</i> (rf)	230A	230G	His ⁷⁷ Arg	rs28399653
Lutheran	005	Lu ^a /Lu ¹⁴	<i>BCAM</i>	<i>LU*02</i> (rf)	<i>LU02.14</i>	611T	611A	Met ²⁰⁴ Lys	rs28399656
Kell	006	K/k	<i>KEL</i>	<i>KEL*01.01</i>	<i>KEL*02</i> (rf)	578T	578C	Met ¹⁹³ Thr	rs8176058
Kell	006	Kp ^a /Kp ^b	<i>KEL</i>	<i>KEL*02.03</i>	<i>KEL*02</i> (rf)	841T	841C	Trp ²⁸¹ Arg	rs176059
Kell	006	Js ^a /Js ^b	<i>KEL</i>	<i>KEL*02.06</i>	<i>KEL*02</i> (rf)	1790C	1790T	Pro ⁵⁹⁷ Leu	rs176038
Duffy	008	Fy ^a /Fy ^b	<i>ACKR1</i>	<i>FY*01</i>	<i>FY*02</i> (rf)	125G	125A	Gly ⁴² Asp	rs12075
Duffy	008	Fy ^b /Fy _{null}	<i>ACKR1</i>	<i>FY*02</i>	<i>FY*02N.01</i>	−67t	−67c	Protein absent on RBC	rs2814778
Kidd	009	Jk ^a /Jk ^b	<i>SLC14A1</i>	<i>JK*01</i>	<i>JK*02</i> (rf)	838G	838A	Asp ²⁸⁰ Asn	rs1058396
Dombrock	014	Do ^a /Do ^b	<i>ART4</i>	<i>DO*01</i> (rf)	<i>DO*02</i>	793A	793G	Asn ²⁶⁵ Asp	rs11276
Dombrock	014	Jo(a+)/ Jo(a−)	<i>ART4</i>	<i>DO*01</i> (rf) or <i>DO*02</i>	<i>DO*01.-05 or</i> <i>DO*02.-05</i>	350C	350T	Thr ¹¹⁷ Ile	rs28362798
Colton	015	Co ^a /Co ^b	<i>AQP1</i>	<i>CO*01</i> (rf)	<i>CO*02.01</i>	134C	134T	Ala ⁴⁵ Val	rs28362692
Indian	023	In ^a /In ^b	<i>CD44 (IN)</i>	<i>IN*01</i>	<i>IN*02</i> (rf)	137C	137G	Pro ⁴⁶ Arg	rs369473842

Each of both assays was performed in 1 well. For panel 2 (Arabian donors), *RHCE*667G>T*, *CO*601delG* (Co_{null}), *VEL*64-80del* (Vel_{null}), and *YT*01/YT*02* were determined by PCR-SSP. rf, reference allele; fs, frameshift.

is of Middle European origin, but very few individuals might have another ethnic background. However, blood donors do not regularly define their ethnicity in the donor questionnaire, which aggravates the identification of donors of non-Middle European origin. In the case that no unambiguous origin was available, this was deduced from the donor names. The rare blood group donors were preselected according to blood group O (rarely A, B, or AB), CCD, ee, ccD.EE, ccddee, and K negative.

DNA Isolation

DNA was isolated from EDTA anticoagulated blood either manually by the Gentra Puregene blood kit (Qiagen, Hilden, Germany) or with an automated magnetic bead technology (MSM1; Chemagen, Perkin Elmer, Baesweiler, Germany) according to the manufacturers' instructions. DNA concentration was adjusted to

15–25 ng/μL for the Arabian panel; for the German rare donor panel, it typically was in the range of 20–45 ng/μL without adjustment.

MALDI-TOF MS Genotyping

All samples were typed by in-house MALDI-TOF MS methods. Different multiplex assays were established to screen for regular blood donors within the German rare donor panel and for the study on blood group frequencies in Arabian donors (Table 2). The AgenaCx assay Design Suite V2.0 online tool (Agena Bioscience, Hamburg, Germany) was used as the basis to superplex the already existing German rare donor assay for specific needs for Arabian donors. In both cases, due to economic reasons, the precondition was to include as many SNPs in 1 well as possible, accepting the risk to lose single SNPs in favor of other new SNPs.

The basics of MALDI-TOF MS blood groups screening have been described before [23, 26]. All primers were synthesized and HPLC cleaned up by TibMolbiol (Berlin, Germany) for the Arabian panel and by Metabion (Munich, Germany) for the German rare donor panel. Primer sequences will be available upon request from the authors. Instructions how to prepare the primer mix, the master mix, and PCR cyclor protocol to generate the templates for the 2nd step had been provided as a Microsoft Excel (Microsoft, Redmond, WA, USA) spreadsheet by Agena Bioscience. After cleanup of the amplicon mix with shrimp alkaline phosphatase (included in the iPLEX® Pro kit), the mixture was used as template for the elongation of unextended primers.

The single base extension reaction using iPLEX® Pro reagents (Agena Bioscience) was performed according to the manufacturer's instructions. After cleaning the reaction with an anion exchange resin, the samples were spotted automatically onto Spectro chips in a MassARRAY Nano Dispenser RS1000 (Agena Bioscience).

Extended primer masses were determined in the MassARRAY Dx Analyzer 4 (Agena Bioscience). All data assigning relevant masses to the respective alleles could be read by the assay editor of the MassARRAY Typer 4.0 and 4.1 software, respectively. Translation of the determined genotyping data into predicted blood group information was performed by algorithms programmed to in-house Microsoft Excel spreadsheets, or in case of the German rare donor panel, csv files were directly embedded into the blood bank laboratory software system by the MassARRAY Typer 4.0 tool/Hemo ID™ Panel Report.

The German rare donor assay had been validated before by at least one alternative method like serology, commercial PCR-SSP reaction (BAG Healthcare, Lich, Germany, and Innotraining, Kronberg, Germany), or sequence-based typing, dependent on the availability of typing sera or commercial test kits. All blood group SNPs included within the MALDI-TOF MS assays are listed in Table 2. The study on blood groups in Arabian countries was validated using repeated testing of 70 donor samples with a known rare blood group typing based on either serology and/or PCR-SSP or Sanger sequencing. Sixteen biallelic polymorphisms were included. The known blood groups covered 29 of 32 alleles without any discrepancies. No pretyping results were available for *DO*01.-05* (encoding the antithetical glycoprotein of Jo^a) and the *IN*01* and *IN*02* alleles so that the presence of rare alleles during the screening should be confirmed by PCR-SSP or Sanger sequencing. One validation sample with a known *RHCE*c.733C>G* SNP additionally carried a heterozygous *ART4*c.350C>T* mutation that induces *DO*01.-05*. Three control samples with a known S-s-U- phenotype due to a deficiency in *GYPB* exons 2–5 were reported as “no call” results in MALDI TOF, which corresponds to the expected value.

The *SMIM1*c.64–80del* mutation, inducing the Vel_{null} phenotype, the *AQP1*c.601delG* mutation inducing the Co_{null} phenotype, and the *YT*01/YT*02* polymorphism that were included in assay 1 (German rare donors) were not compatible with the primer chemistry of assay 2 (Arabian donors), so that genotyping was performed separately by PCR-SSP. Additionally, *RHCE*c.667G>T* (*RHCE*01.07*, *RHCE*ceMO*) and the *GYPB* deletion that is associated with the S-s-U- phenotype were tested by in-house PCR-SSP. The S-s-U- PCR-SSP included 2 preparations specific for *GYPB* wild-type (wt) positions within introns 2 and 5, the latter being specific for *GYPB*270+5g*. In case of a homozygous *GYPB*270+5g>t* substitution, which induces a S-s-U^{weak} phenotype, the reaction would result in a homozygous amplicon loss, and the sample would be subjected to DNA sequencing. In short, 40–100 ng of DNA were added to a master mix including 0.5–1.0 U of Green Go Taq in Flexi buffer (Promega, Mannheim, Germany),

between 1.5 and 3.0 mM MgCl₂, 500 nM allele-specific sense and antisense primer each, 125 nM HGHI and HGHII internal control primer (all primers TibMolbiol), and 0.2 mM dNTP each (Roche, Mannheim, Germany). The Co_{null}-PCR reaction included CRP I and CRPII primers as internal controls. All primers are listed in Table 3.

Some rare results of the MALDI-TOF MS analysis like *RHCE* mutations, *KEL*02.06* or *DO*01.-05* were counter checked by Sanger sequencing. In the case of discrepancies between MALDI-TOF MS genotyping and serological phenotyping within the German rare donor panel, either commercial PCR-SSP or DNA sequencing were added for further determination.

Statistics

All data were calculated as percent samples with homo- or heterozygosity for the 2 alternative alleles referring to the number of samples with unambiguous results. Typing failures (no calls) were not included in the frequency calculation. Absolute allele frequencies within each group (German rare donors and Arabian donors, respectively) were calculated by direct allele counting according to Hardy-Weinberg proportions. In cases where only 1 allele was determined, homozygosity was postulated, and the allele was counted twice.

Differences in allele numbers (calculated per group) between the different Arabian panels and the German rare donor panel were calculated by $\chi^2 2 \times 2$ contingency table with Yates correction for frequencies of $n < 5$ and calculation in Microsoft Excel.

Results

Blood group genotyping by MALDI-TOF MS proved to be reliable, efficient, and time saving. Within the rare donor panel, 20,529 samples were included, but not each sample was tested for each allele. Some samples were repeatedly included as controls and thus were excluded for the final evaluation. Only 1 sample produced no call results in all but 1 of the tested blood groups. Related to the single SNP pairs, the rate of no calls ranged between 1 (0.01%) for *CO*01.01/CO*02.01* (Co^a/Co^b), *JK*01/JK*02* (Jk^a/Jk^b), *RHCE*C/RHCE*Cw* (C/C^w), *IN*01/IN*02* (In^a/In^b), and *KEL*01.01/KEL*02* (K/k) and 17 (1.5%) for *GYPB*03/GYPB*04* (Ss) (Table 4). The overall failure rate over all samples and all alleles was 0.4% for the Arabian panel. Except for *IN*01/IN*02* and the *CO*601delG* allele, 2 different genotypes could be observed for each polymorphism over all samples. As an example, the *DO*01/02* (*DO*A/DO*B*) cluster plot from 1 plate and the detail panes of 3 samples are shown in Figure 1.

Most apparent were differences in the *FY* frequencies. Twenty-two of 1,111 donors (2%) of the Arabian panel homozygously carried the *FY* promoter mutation *FY*-67t>c* (*FY*02.01N/02.01N*; Table 4). All these donors were also homozygous for *FY*02* (*FY*B*). Thus, *FY*-67t>c* was exclusively rated as *FY*02.01N*. Of the 180 samples with heterozygosity for the *FY*02.01N* allele, 98 (54%) were homozygous for the *FY*02* allele, too, whereas 82 (46%) carried *FY*01/02*. The overall allele frequen-

Table 3. Primer sequences for PCR-SSP reactions and sequencing

Gene	Primer name	Primer sequence	Usage
<i>RHCE</i>	CE_E5_F_Amp3	5'-ACTACCACATGAACCTGAG-3'	Amplification <i>RHCE</i> e5
	CE-E5_R_Amp4	5'-TATGTGTGCTAGTCCTGTTAGAC-3'	
	D_E5_F_Seq	5'-AGACCTTTGGAGCAGGAGTG-3'	Sequencing <i>RHCE</i> , e5, sense
<i>RHCE</i>	D_667T_F_Amp	5'-GTGGATGTTCTGGCCAAGTT-3'	PCR-SSP <i>RHCE</i> *667G, sense
	CE_667G_F_Amp	5'-GTGGATGTTCTGGCCAAGTG-3'	PCR-SSP <i>RHCE</i> *667G>T, sense
	CE_E5_R_Amp2	5'-TCTCACCTAGGTTTAGCCTCTGA-3'	PCR-SSP common, antisense
<i>RHCE</i>	CE_667G_F_Amp	5'-GTGGATGTTCTGGCCAAGTG-3'	PCR-SSP, sense
	CE_733G_R	5'-GTCACCACACTGACTGCTAC-3'	PCR-SSP, antisense
<i>ACKR1/FY</i>	FY_AF	5'-CTTTTGAAGTGCCTTTTCTTGG-3'	Amplification FY
	FY_AR	5'-CTTCCCCACCACTGTCCTAATC-3'	
	FY_S_F1	5'-GATGGAGGAGCAGTGAGAGTC-3'	Sequencing FY 5'UTR, sense
<i>ACHE/YT</i>	YTA_pos_se	5'-CATCAACGCGGGAGACTTCC-3'	PCR-SSP Yt ^a +, sense
	YTA_neg_se	5'-CATCAACGCGGGAGACTTCA-3'	PCR-SSP Yt ^a -, sense
	YT_com_anti-s	5'-GGGAGGACTTCTGGGACTTC-3'	PCR-SSP common, antisense
<i>ART4/DO</i>	ART4_E2_F	5'-CTGCAACCACATTCACCATC-3'	Amplification ART4 e2
	ART4_E2_R	5'-CCCAAAAACCCACTGAGAAA-3'	
	ART4_E2_F	5'-CTGCAACCACATTCACCATC-3'	Sequencing ART4 e2, sense
	ART4_E2_F2	5'-GTGGCAAAAAGCCCACTTAG-3'	Sequencing ART4 e2, sense
	ART4_E2_R	5'-CCCAAAAACCCACTGAGAAA-3'	Sequencing ART4 e2, antisense
<i>AQP1/CO</i>	CO-pos2_se	5'-GTCCTTTGGCTCCGCGG-3'	PCR-SSP CO*601G, sense
	CO-neg2_se	5'-GTCCTTTGGCTCCGCGT-3'	PCR-SSP CO*601delG, sense
	CO_2_anti-se	5'-TTCCTGTCCTCTGGCTGTCT-3'	PCR-SSP common, antisense
<i>SMIM1/Vel</i>	VEL-pos1_anti-se	5'-GCCTCTTCTGTGCTGGACA-3'	PCR-SSP Vel wt, antisense
	VEL-neg1_anti-se	5'-GCCTCTTCTGTGCTGGACT-3'	PCR-SSP Vel64-80del, antisense
	VEL_1_se	5'-CTCAGAGGGGGTCTTGACTG-3'	PCR-SSP common, sense
<i>GYPB/Ss</i>	GYPB_I5_G*	5'-TCGCCGACTGATAAAGGTGAG-3'	PCR-SSP wt i5, sense
	GYPB_I5_R2	5'-ATTTATGACAGTTCTGTTTCTCTTC-3'	PCR-SSP wt i5, antisense
	GYPB_E2_Def_F2	5'-CTCCGAAATCATTTTTGTGATGT-3'	PCR-SSP wt i2, sense
	GYPB_E2_Def_R	5'-CAGAGGCTAGAATTCCTCTGTAGTAA-3'	PCR-SSP wt i2, antisense

* Both, a missing *GYPB* exon 5 and a *GYPB**270+5g>t substitution, which induces a S-s-U^{weak} phenotype, will result in an amplicon loss. e, exon; i, intron.

cy in the Arabian panel was 0.101 for *FY**02.01N (all allele frequencies in Table 5). The highest frequency was found in Northern African donors with 0.375, and nearly 20% of them were homozygous for the mutation. This was in accordance with a higher *FY**B frequency in this group ($f = 0.720$) compared to the German rare donor panel ($f = 0.564$, $p < 0.01$). Within the German rare donor panel, only 0.8% ($n = 61$) were heterozygous for *FY**-67t>c ($f = 0.004$; $p < 0.001$ vs. the Arabian panel), with 23 (38%) of these being heterozygous for *FY**01 and *FY**02, while 38 (62%) carried an additional *FY**02 wt allele. No single German rare donor individual was found with the homozygous mutation. Deduced from the donor names, it could be assumed that 30 of these donors were not of Middle European origin with a special impact of the *FY**02/*FY**02N.01 genotype on African or Arabian individuals ($n = 17$). But even when these donors were in-

cluded, the differences in allele frequencies between the German rare donor panel and individuals from Syria or from Northern Africa were highly significant ($p < 0.001$ each, for the Syrian and the Northern African panel vs. the German rare donor panel).

A special focus was put on *RHCE* mutations within the Arabian panel. A total of 47 samples with *RHCE* exon 5 mutations other than *RHCE**c.676G(e) and *RHCE**c.676C(E) were found. Forty samples typed heterozygous for the *RHCE**c.733C>G mutation by MALDI-TOF MS, and 1 Syrian sample was homozygous. Applying a PCR-SSP approach with *RHCE**c.676G- and *c.733G-specific amplification primers (Table 3), it could be demonstrated that both SNPs were in 1 haplotype within each of these 41 samples, that is, the *RHCE**c.773G allele was a mutation of Rhesus e. The *RHCE**c.733G allele frequency was highest in Northern African probands with $f = 0.150$, followed

Table 4. Genotyping results of different blood group polymorphisms in individuals from Arabian countries and Iran and Germany

ISBT No.	Blood group polymorphism	Syria (n = 800), n (%)	Arabian Peninsula (n = 123), n (%)	Northern Africa (n = 41), n (%)	Iran (n = 147), n (%)	All Arabian/ Iranian (n = 1,111), n (%)	German rare donor panel	
							n (%)	total n
002	<i>GYPB*03/*03</i> (SS)	106 (13.5)	21 (17.2)	2 (4.9)	20 (13.8)	149 (13.6)	2,097 (10.3)	20,499
	<i>GYPB*03/*04</i> (Ss)	340 (43.3)	39 (32.0)	19 (46.3)	62 (42.8)	460 (42.1)	8,730 (42.7)	
	<i>GYPB*04/*04</i> (ss)	340 (43.2)	62 (50.8)	20 (48.8)	63 (43.4)	485 (44.3)	9,595 (47.0)	
	No call	14 (1.8)	1 (0.8)	0 (0.0)	2 (1.4)	17 (1.5)	77 (0.4)	
004	<i>RHCE*C/*C⁺</i>	795 (99.4)	122 (99.2)	40 (100)	144 (98.0)	1,101 (99.2)	7,322 (96.6)	7,579
	<i>RHCE*C/*C_w</i>	5 (0.6)	1 (0.8)	0 (0.0)	3 (2.0)	9 (0.8)	248 (3.3)	
	<i>RHCE*C_w/*C_w</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (0.1)	
	No call	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	1 (0.1)	3 (0.0)	
	<i>RHCE*c.667G/G</i> (wt)	797 (99.6)	122 (99.2)	41 (100)	147 (100)	1,107 (99.6)	n.t.	
	<i>RHCE*c.667G/T</i>	3 (0.4)	1 (0.8)	0 (0.0)	0 (0.0)	4 (0.4)	n.t.	
	<i>RHCE*c.667T/T</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	n.t.	
	No call	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	n.t.	
	<i>RHCE*c.697C/C</i> (wt)	794 (99.7)	122 (100)	40 (100)	145 (100)	1,101 (99.8)	n.t.	
	<i>RHCE*c.697C/G</i>	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.2)	n.t.	
	<i>RHCE*c.697G/G</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	n.t.	
	No call	4 (0.5)	1 (0.8)	1 (2.4)	2 (1.4)	8 (0.7)	n.t.	
	<i>RHCE*c.733C/C</i> (wt)	769 (97.0)	120 (98.4)	28 (70.0)	141 (97.9)	1,058 (96.3)	n.t.	
	<i>RHCE*c.733C/G</i>	23 (2.9)	2 (1.6)	12 (30.0)	3 (2.1)	40 (3.6)	n.t.	
	<i>RHCE*c.733G/G</i>	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	n.t.	
	No call	7 (0.9)	1 (0.8)	1 (2.4)	3 (2.0)	12 (1.1)	n.t.	
005	<i>LU*01/*01</i> (Lu ^a /Lu ^a)	1 (0.1)	1 (0.8)	0 (0.0)	0 (0.0)	2 (0.2)	26 (0.1)	20,499
	<i>LU*01/*02</i> (Lu ^a /Lu ^b)	19 (2.4)	4 (3.3)	2 (4.9)	8 (5.4)	33 (3.0)	1,311 (6.5)	
	<i>LU*02/*02</i> (Lu ^b /Lu ^b)	777 (97.5)	117 (95.9)	39 (95.1)	139 (94.6)	1,072 (96.8)	18,987 (93.4)	
	No call	3 (0.4)	1 (0.8)	0 (0.0)	0 (0.0)	4 (0.4)	175 (0.9)	
	<i>LU*02/*02</i> (Lu8/Lu8)	762 (95.4)	121 (99.2)	40 (100)	142 (96.6)	1,065 (96.1)	19,187 (96.5)	
	<i>LU*02/*02.14</i> (Lu8/Lu14)	37 (4.6)	1 (0.8)	0 (0.0)	5 (3.4)	43 (3.9)	680 (3.4)	
	<i>LU*02.14/*02.14</i> (Lu14/Lu14)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (0.1)	
006	No call	1 (0.1)	1 (0.8)	1 (2.4)	0 (0.0)	3 (0.3)	622 (3.0)	20,499
	<i>KEL*02/*02</i> (k/k)	747 (93.4)	114 (92.7)	38 (95.0)	137 (93.2)	1,036 (93.3)	n.t.	
	<i>KEL*02/*01.01</i> (k/K)	50 (6.3)	9 (7.3)	2 (5.0)	8 (5.4)	69 (6.2)	n.t.	
	<i>KEL*01.01.*01.01</i> (K/K)	3 (0.4)	0 (0.0)	0 (0.0)	2 (1.4)	5 (0.5)	n.t.	
	No call	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	1 (0.1)	n.t.	
	<i>KEL*02.03/*02.03</i> (Kp ^a /Kp ^a)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)	
	<i>KEL*02.03/*02</i> (Kp ^a /Kp ^b)	4 (0.5)	0 (0.0)	1 (2.5)	0 (0.0)	5 (0.5)	340 (1.7)	
	<i>KEL*02/*02</i> (Kp ^b /Kp ^b)	792 (99.5)	122 (100)	39 (97.5)	145 (100)	1,098 (99.5)	20,083 (98.3)	
	No call	4 (0.5)	1 (0.8)	1 (2.4)	2 (1.4)	8 (0.7)	70 (0.3)	
	<i>KEL*02.06/*02.06</i> (Js ^a /Js ^a)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
008	<i>KEL*02.06/*02</i> (Js ^a /Js ^b)	6 (0.8)	1 (0.8)	0 (0.0)	1 (0.7)	8 (0.7)	0 (0.0)	2,821
	<i>KEL*02/*02</i> (Js ^b /Js ^b)	792 (99.2)	122 (99.2)	40 (100)	145 (99.3)	1,099 (99.3)	2,790 (100)	
	No call	2 (0.3)	0 (0.0)	1 (2.4)	1 (0.7)	4 (0.4)	31 (1.1)	
	<i>FY*01/*01</i> (Fy ^a /Fy ^a)	139 (17.4)	27 (22.0)	6 (14.6)	39 (26.5)	211 (19.0)	3,951 (19.3)	20,510
	<i>FY*01/*02</i> (Fy ^a /Fy ^b)	384 (48.1)	48 (39.0)	11 (26.8)	70 (47.6)	513 (46.2)	9,896 (48.5)	
	<i>FY*02/*02</i> (Fy ^b /Fy ^b)	276 (34.5)	48 (39.0)	24 (58.5)	38 (25.9)	386 (34.8)	6,574 (32.2)	
	No call	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	89 (0.4)	
	<i>FY*02N.01/*02N.01</i> (Fy _{null})	10 (1.2)	4 (3.3)	8 (20.0)	0 (0.0)	22 (2.0)	0 (0.0)	
009	<i>FY*02N.01/*01</i> (Fy _{null} / Fy ^a)	75 (9.4)	3 (2.4)	3 (7.3)	1 (0.7)	82 (7.4)	23 (0.3)	7,579
	<i>FY*02N.01/*02</i> (Fy _{null} / Fy ^b)	75 (9.4)	8 (6.5)	11 (26.8)	4 (2.7)	98 (8.8)	38 (0.5)	
	<i>FY</i> wt	639 (80.0)	108 (87.8)	18 (45.0)	142 (96.6)	907 (81.8)	7,513 (99.2)	
	No call	1 (0.1)	0 (0.0)	1 (2.4)	0 (0.0)	2 (0.2)	5 (0.1)	
	<i>JK*01/*01</i> (Jk ^a /Jk ^a)	226 (28.3)	34 (27.6)	15 (37.5)	32 (21.8)	307 (27.7)	5,472 (26.8)	20,510
009	<i>JK*01/*02</i> (Jk ^a /Jk ^b)	393 (49.1)	67 (54.5)	21 (52.5)	90 (61.2)	571 (51.4)	10,225 (50.1)	
	<i>JK*02/*02</i> (Jk ^b /Jk ^b)	181 (22.6)	22 (17.9)	4 (10.0)	25 (17.0)	232 (20.9)	4,707 (23.1)	
	No call	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	1 (0.1)	106 (0.5)	

Table 4 (continued)

ISBT No.	Blood group polymorphism	Syria (n = 800), n (%)	Arabian Peninsula (n = 123), n (%)	Northern Africa (n = 41), n (%)	Iran (n = 147), n (%)	All Arabian/Iranian (n = 1,111), n (%)	German rare donor panel	
							n (%)	total n
011	<i>YT*01/*01 (Yt^a/Yt^a)</i>	607 (76.5)	105 (86.1)	41 (100)	127 (86.4)	878 (79.7)	18,431 (90.2)	20,499
	<i>YT*01/*02 (Yt^a/Yt^b)</i>	172 (21.7)	16 (13.1)	0 (0.0)	18 (12.2)	206 (18.8)	1,939 (9.5)	
	<i>YT*02/*02 (Yt^b/Yt^b)</i>	14 (1.8)	1 (0.8)	0 (0.0)	2 (1.4)	17 (1.5)	63 (0.3)	
	No call	7 (0.9)	1 (0.8)	0 (0.0)	0 (0.0)	8 (0.7)	66 (0.3)	
014	<i>DO*01/*01 (Do^a/Do^a)</i>	157 (19.7)	13 (10.7)	8 (20.0)	28 (19.2)	206 (18.6)	3,067 (15.0)	20,515
	<i>DO*01/*02 (Do^a/Do^b)</i>	384 (48.2)	75 (61.5)	22 (55.0)	76 (52.0)	557 (50.4)	9,522 (46.7)	
	<i>DO*02/*02 (Do^b/Do^b)</i>	255 (32.0)	34 (27.8)	10 (25.0)	42 (28.8)	342 (31.0)	7,820 (38.3)	
	No call	3 (0.4)	1 (0.8)	1 (2.4)	1 (0.7)	5 (0.5)	106 (0.5)	
	<i>DO*01/*01 (Jo^a+/Jo^a+) </i>	797 (99.7)	123 (100)	40 (100)	145 (100)	1,105 (99.8)	2,790 (100)	2,821
	<i>DO*01/*01.-05 (Jo^a+/Jo^a-) </i>	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.2)	1 (0.0)	
	<i>DO*01.-05/*01.-05 (Jo^a-/Jo^a-) </i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
015	No call	1 (0.1)	0 (0.0)	1 (2.4)	2 (1.4)	4 (0.4)	30 (1.1)	
	<i>CO*01/*01 (Co^a/Co^a)</i>	791 (98.9)	118 (95.9)	40 (100)	145 (98.6)	1,094 (98.6)	18,632 (91.6)	20,529
	<i>CO*01/02.01 (Co^a/Co^b)</i>	9 (1.1)	5 (4.1)	0 (0.0)	2 (1.4)	16 (1.4)	1,663 (8.2)	
	<i>CO*02.01/*02.01 (Co^b/Co^b)</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	46 (0.2)	
	No call	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	1 (0.1)	188 (0.9)	
	<i>CO*01/*01 (Co^a/Co^a)</i>	795 (100)	121 (100)	41 (100)	147 (100)	1,106 (100)	17,683 (100)	17,868
	<i>CO*01/*01N.06 (Co^a/Co_{null})</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	<i>CO*01N.06/*01N.06 (Co_{null}/Co_{null})</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	No call	5 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	5 (0.5)	185 (1.0)	
023	<i>IN*01/IN*01 (In^a/In^a)</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	n.t.	
	<i>IN*01/IN*02 (In^a/In^b)</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	n.t.	
	<i>IN*02/IN*02 (In^b/In^b)</i>	800 (100)	123 (100)	41 (100)	147 (100)	1,110 (100)	n.t.	
	No call	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.1)	n.t.	
034	<i>Vel*01/*01 (Vel/Vel)</i>	782 (97.9)	121 (98.4)	39 (95.1)	146 (99.3)	1,088 (98.0)	16,916 (96.2)	17,868
	<i>Vel*01/*-01 (Vel/Vel_{null})</i>	16 (2.0)	2 (1.6)	2 (4.9)	1 (0.7)	21 (1.9)	644 (3.7)	
	<i>Vel*-01/*-01 (Vel_{null}/Vel_{null})</i>	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	10 (0.1)	
	No call	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	298 (1.7)	

Results from PCR-SSP typing are italicized. Calculation of genotype percentages is based on the total number of samples with a definite result. n.t., not tested.

* Proband was not tested for C or c, but because the *RHCE**c.122A>G substitution in most cases is associated with C^w and not c^w, the polymorphism is reported as C/C^w. *FY**-67t>c was exclusively rated as *FY**02.01N.

by Syrians with 0.016, and lowest in individuals from the Arabian Peninsula with 0.008. Contrary, *RHCE**C^w was less frequent in the Arabian panel than in the German rare donor panel (Table 4; $p < 0.001$).

The *Vel**c.64–80del mutation (*VEL**-01) that is the main basis of the Vel_{null} phenotype was found with allele frequencies between 0.003 in donors from Iran and 0.024 in Northern African donors, which was in the same range as for the German donors ($f = 0.019$). All but 1 individual from Syria carried only 1 copy of the mutated allele, so that a Vel-positive phenotype was predicted.

The *LU**14 allele was present heterozygously in 37 out of 800 donors from Syria (4.6%) and 5 of 147 (3.4%) from Iran ($f = 0.023$ and 0.017, respectively), and thus the frequency was comparable with the German panel ($f =$

0.018). The *LU**B and *LU**A rates were comparable within the different groups.

Distribution of the *DO* alleles *DO**01 and *DO**02 showed higher differences between the Arabian panels than between these panels and the German rare donor panel (Table 4). Only 2 Syrian individuals (0.3%, allele frequency $f = 0.001$) heterozygously carried the *DO**01.-05 allele. In both samples, the mutation was confirmed by DNA sequencing. One sample was *DO**01/*01 and the other one was *DO**01/*02, but it is not known whether the *c.350C>T substitution in both samples was in 1 haplotype with *DO**01 or whether, alternatively, a rare *DO**02.-05 allele was present in the *DO**01/*02 donor.

Contrary to the German rare donor panel where no *KEL**02.06-positive sample was detected, 0.8% of the Syr-

Table 5. Allele frequencies within the different groups

ISBT No.	Allele	Syria (n = 800)	Arabian Peninsula (n = 123)	Northern Africa (n = 41)	Iran (n = 147)	All Arabian/ Iranian (n = 1,111)	German rare donor panel
002	<i>GYPB*03</i> (S)	0.351	0.332	0.280	0.352	0.346	0.316
	<i>GYPB*04</i> (s)	0.649	0.668	0.720	0.648	0.654	0.684
004	<i>RHCE*02</i> (C)	0.997	0.996	1.000	0.990	0.996	0.983
	<i>RHCE*02.08.01</i> (C ^w)	0.003	0.004	0.000	0.010	0.004	0.017
	<i>RHCE*c.667G</i> (wt)	0.998	0.996	1.000	1.000	0.998	n.t.
	<i>RHCE*c.667T</i>	0.002	0.004	0.000	0.000	0.002	n.t.
	<i>RHCE*c.697C</i> (wt)	0.999	1.000	1.000	1.000	0.999	n.t.
	<i>RHCE*c.697G</i>	0.001	0.000	0.000	0.000	0.001	n.t.
	<i>RHCE*c.733C</i> (wt)	0.984	0.992	0.850	0.990	0.981	n.t.
	<i>RHCE*c.733G</i>	0.016	0.008	0.150	0.010	0.019	n.t.
005	<i>LU*A</i> (<i>LU*01</i>)	0.013	0.025	0.024	0.027	0.017	0.034
	<i>LU*B</i> (<i>LU*02</i>)	0.987	0.975	0.976	0.973	0.983	0.966
	<i>LU*08</i>	0.977	0.996	1.000	0.983	0.981	0.982
	<i>LU*14</i> (<i>LU*02.14</i>)	0.023	0.004	0.000	0.017	0.019	0.018
006	<i>KEL*01.01</i> (K)	0.035	0.037	0.025	0.041	0.036	n.t.
	<i>KEL*02</i> (k)	0.965	0.963	0.975	0.959	0.964	n.t.
	<i>KP*B</i> (<i>KEL*02</i>)	0.997	1.000	0.987	1.000	0.998	0.992
	<i>KPA*</i> (<i>KEL*02.03</i>)	0.003	0.000	0.013	0.000	0.002	0.008
	<i>JS*A</i> (<i>KEL*02.06</i>)	0.004	0.004	0.000	0.003	0.004	n.t.
	<i>JS*B</i> (<i>KEL*02</i>)	0.996	0.996	1.000	0.997	0.996	n.t.
008	<i>FY*A</i> (<i>FY*01</i>)	0.414	0.415	0.280	0.503	0.421	0.436
	<i>FY*B</i> (<i>FY*02</i>)	0.586	0.585	0.720	0.497	0.579	0.564
	<i>Fy*–67t</i> (wt)	0.894	0.923	0.625	0.983	0.899	0.996
	<i>FY*02N.01</i> (<i>Fy*null</i>)	0.106	0.077	0.375	0.017	0.101	0.004
009	<i>JK*A</i> (<i>JK*01</i>)	0.528	0.549	0.637	0.524	0.534	0.519
	<i>JK*B</i> (<i>JK*02</i>)	0.472	0.451	0.363	0.476	0.466	0.481
011	<i>YT*A</i> (<i>YT*01</i>)	0.874	0.926	1.000	0.925	0.891	0.949
	<i>YT*B</i> (<i>YT*02</i>)	0.126	0.074	0.000	0.075	0.109	0.051
014	<i>DO*A</i> (<i>DO*01</i>)	0.438	0.414	0.475	0.452	0.438	0.384
	<i>DO*B</i> (<i>DO*02</i>)	0.562	0.586	0.525	0.548	0.562	0.616
	<i>DO*01</i> (Jo ^{a+})	0.999	1.000	1.000	1.000	0.999	1.000
	<i>DO*01–5</i> (Jo ^{a–})	0.001	0.000	0.001	0.000	0.001	0.000
015	<i>CO*A</i> (<i>CO*01</i>)	0.994	0.980	1.000	0.993	0.993	0.957
	<i>CO*B</i> (<i>CO*02.01</i>)	0.006	0.029	0.000	0.007	0.007	0.043
	<i>CO</i> wt	1.000	1.000	1.000	1.000	1.000	1.000
	<i>CO*01N.06</i> (Co _{null})	0.000	0.000	0.000	0.000	0.000	0.000
023	<i>IN*A</i> (<i>IN*01</i>)	0.000	0.000	0.000	0.000	0.000	n.t.
	<i>IN*B</i> (<i>IN*02</i>)	1.000	1.000	1.000	1.000	1.000	n.t.
034	<i>VEL*01</i> (Vel)	0.989	0.992	0.976	0.997	0.991	0.981
	<i>VEL*–01</i> (Vel _{null})	0.011	0.008	0.024	0.003	0.009	0.019

Number of donors tested in the German rare donor panel varies between 2,821 and 20,529 for the different blood groups. n.t., not tested.

ian donors and those from the Arabian Peninsula were heterozygous for *KEL*02.06/*02* (Js^a/Js^b; $p < 0.001$).

*YT*B* was more frequent in Syrian probands ($f = 0.126$) than in Northern Africans ($f = 0.000$) with 1.8% of the Syrians carrying *YT*B* homozygously. The frequency

within the German rare donor panel was in between ($f = 0.051$; $p < 0.001$ for Syrian vs. German rare donor panel).

The *GYPB*03* and **04* alleles (S and s) were distributed rather similar between the different groups with a slight overrepresentation of *GYPB*04* within Syrian do-

Fig. 1. Cluster plot and detail panes of MALDI-TOF MS analysis. The cluster plot of 1 plate for the *DO*01/*02* polymorphism is shown on the left side with the samples homozygous for the lower mass (5115.4 Da) in light blue, those with homozygosity for the higher mass (5131.4 Da) in orange, and the heterozygotes in green squares. Three of the “no call” results belong to negative control

samples. The detail panes of 3 samples (marked by red circles within the cluster plot) are shown on the right side. Peak heights and positions of the unextended primer (very low, because the primer was nearly completely consumed), the extended *DO*01*-specific primer (nucleotide A), and the extended *DO*02*-specific primer (nucleotide G) are marked.

nors ($p < 0.01$). One Northern African donor was typed as *GYPB*04/*04* (ss) by mass spectrometry, but the *GYPB* intron 5-specific PCR-SSP reaction with a wt *GYPB*270*+5g-specific primer was negative. DNA sequencing demonstrated a homozygous *GYPB*270*+5g>*t* mutation that induces the S-s-U^{weak} phenotype independent of the presence of the *GYPB*03* (S) or *GYPB*04* (s) allele. No donor with a complete lack of *GYPB* was identified by *GYPB* intron 2- and 5-specific PCR-SSP, but 17 samples produced no call results in the MALDI assay. In order to test for possible additional mutations within primer annealing sites that might cause an allele dropout, 16 of these samples were genotyped by DNA sequencing. Eleven samples typed as *GYPB*03/*04* and 5 as *GYPB*04/*04*. No additional mutation was found.

Two samples from the German rare donor panel that were determined as *GYPB*04/*04* (ss) by MALDI were

known to be positive for the presence of S (*GYPB*03*) from earlier serology typing. DNA sequencing in both samples identified the presence of both, *GYPB*03* (S) and *GYPB*04* (s) alleles and a heterozygous *GYPB*c.161G>A* substitution that is known to induce a Mit⁺ (MNS:24) phenotype. One of the 2 samples additionally exhibited a heterozygous *GYPB*249C>G* exchange that induces a p.Tyr⁸³stop.

All samples with a reported result were homozygous for the wt *IN*02/IN*02*. None of the 1,111 Arabian and 17,683 German rare donors samples carried the *CO*601delG* mutation that induces a Co_{null} phenotype (Table 4). Mass spectrometry demonstrated the presence of *CO*01* (*CO*A*) in each sample of the Arabian panel with allele frequencies between 0.993 and 1.000, whereas 0.2% of the German rare donors were homozygous for *CO*02.01* ($p < 0.001$ for the Arabian vs. the German rare donor collec-

tive). Between 0.0% (Northern Africa) and 4.1% (Arabian Peninsula) carried 1 *CO*02.01* (*CO*B*) copy, which is less than in the German rare donor panel (8.2%).

All other blood groups were equally distributed between the different groups.

Discussion

To the best of our knowledge, this is the first time that molecular typing for blood groups other than ABO and Rhesus CDE has been performed on a larger panel of blood donors from Arabian countries and Iran with a special emphasis on Syria. Identification of donors with rare blood groups is a challenge when the local donor population differs from the genetic background of patients. Since the increase in migration of people from Arabian countries, especially from Syria, to Europe in 2015, a higher number of patients suffering from hemoglobinopathies has been observed. Hemoglobinopathies have been addressed as a major migration-related aspect in blood supply [7]. Contrary to ABO and Rhesus CDE [9–12, 17, 28], frequency information on further blood groups in these countries is largely missing, except for a French-Afghan-Pakistan collaboration on 15 biallelic polymorphisms in 13 Afghan and Pakistani populations ($n = 599$) [13].

The results of the German rare donor panel with >20,000 samples served as controls but did not include each allele tested by the Arabian assay [25]. It enabled to spot rare alleles within the local donor population like *CO*01* negative ($n = 46$), *YT*01* negative ($n = 63$), *LU*02* negative ($n = 26$), *LU*08* negative ($n = 10$), *VEL*01* negative ($n = 10$), and *KEL*02.03* homozygous ($n = 1$).

The Arabian Peninsula was the initial site of the out-of-Africa migrations between 60,000 and 125,000 years ago. These indigenous Arabs are the most distant relatives of all other contemporary non-Africans and are direct descendants of the first Eurasian populations established by the out-of-Africa bottleneck migrations [29]. For this reason, we put a special focus on African blood group mutations that also might be present in modern Arabian populations at a higher frequency than in European populations.

We clearly demonstrated that especially the Fy silencing mutation *FY*-67t<c* is more frequent in Arabian countries than in our donor population. The frequency was highest within the small group of 41 individuals from Northern Africa (20% being homozygous for the mutation). Only the mother country but not the ethnicities of these individuals, who might be mostly of African black ancestry, is known. Because the Northern African group was the smallest, relevance of the observations is restricted. *FY*-67t<c* allele frequencies in our Arabian panels decreased from 0.375 (Northern Africa) to 0.017 (Iran) with growing distance from Central Africa. About 1.2% of Syr-

ian individuals were homozygous for *FY*02N.01*, which corresponds to the findings in Afghan and other western Asian populations [13, 28], but is lower than in Israeli Arabs [14]. Because each of these individuals carried at least 1 *FY*02* allele, the mutation in general was assigned to the *FY*02N.01* allele. However, it cannot completely be excluded that in single cases a *FY*01* allele was affected by the *FY*-67t>c* mutation (*FY*01N.01* allele), as it was demonstrated in rare cases in Papua New Guinea [18]. Interestingly, 61 of 7,579 German rare blood group donors also carried the mutation that was described as very rare in Central Europe [16, 18]. None of these donors was homozygous for the mutation, so that none would carry the Fy(a-b-) phenotype. Twenty-three of these were heterozygous for *FY*01* and *FY*02* with a predicted Fy(a+b-) phenotype, while 38 carried an additional *FY*02* wt allele that is predicted to result in the Fy(a-b+) phenotype. Our donor registry does not systematically record the ethnicity of donors. Half of the individuals with the *FY*-67t>c* mutation can be assigned to an African or Arabian ancestry due to their names, but 31 donors with typical German names remained. Of course, names are not an unequivocal proof for the ethnicity, but this might be a hint for the presence of the *FY* silencing mutation in donors with Middle European ancestry as it had been calculated even before the correct mutation had been decoded [28]. On the other hand, these findings clearly demonstrate that a small minority of individuals represented in the German rare donor panel is not of Middle European ancestry, as also demonstrated for a Swiss cohort [23].

Our MALDI-TOF MS panel did not include typing of Rhesus C, c, E, and e because these blood groups can be easily determined by serology. *RHCE* mutations like *RHCE*c.667G>T* (p.Val²²³Phe), **c.697C>G* (p.Gln²³³Glu), and **c.733C>G* (p.Leu²⁴⁵Val) are rather frequent in African individuals and were therefore included in molecular screening [30–32]. Especially the *RHCE*c.733C>G* substitution was detected in 41 individuals of the Arabian panel ($f = 0.019$), and, in each case, a *RHCE*c.676G* (*RHCE*e*)-specific PCR-SSP approach demonstrated the presence of *RHCE*c.676G* and **c.733G* in 1 haplotype. The substitution that can be associated with different further *RHCE* mutations is reported in partial c, partial e phenotypes that can be of clinical significance [18]. In contrast, the *RHCE*c.122A>G* substitution (*C^w*) was less frequent in the Arabian panel.

Between 5.0% (Northern Africans) and 7.3% (Arabian Peninsula) of donors from the Arabian Panel were positive for the *KEL*01.01* allele that codes for k (cellano; $f = 0.025$ – 0.041), which is in contrast to previous findings of up to 25% in Arabs [18] but corresponds with allele frequencies of about 0.034 in Western Asia [28]. Antibodies to Js^b are very rare but can cause hemolytic transfusion reactions [33]. Eight individuals with heterozygosity for

the *KEL*02.06* allele ($f = 0.004$; encoding Js^a) were identified within the Arabian panel, which is in the range of a study on Israeli Arabs [14]. The allele is very rare within Caucasians (<0.01%) but rather frequent within Blacks [18]. In contrast to a Swiss study, no carrier of the *KEL*02.06* allele was detected within the German rare donor panel [24].

Intrapanel frequency variation in *YT*01* (Yt^a) and *YT*02* (Yt^b) was higher within the Arabian panel than the German rare donor panel with *YT*02* frequencies between 0.000 in Northern Africans up to 0.126 in Syrians and 0.051 in Germans. The frequency in Syrians is within the same range as for Israelis [18]. Homozygosity for *YT*02* might enable immunization to Yt^a, but the antibodies are supposed to be of minor clinical effects [18].

Samples with homozygosity for the *VEL*-01* allele with a predicted Vel-negative phenotype showed a comparable frequency in both the Arabian and the German rare donor panel with about 0.055%, which seems to be within the same range as data reported from different regions in Switzerland and Southwestern Germany [34, 35]. We did not test for further reported *SMIM1* mutations like rs1175550 and rs143702418, which both induce mutations within the noncoding region of intron 2 and independently modulate Vel antigen expression [36].

*IN*01* and *CO*01N.06* were not detected in any sample, not even within the Iranian collective, although these mutations seem to have originated in Southern Asia or Iran [18, 37]. This again can be attributed to the rather low number of samples from Iran. Because no *IN*01* samples were available for validation of the assay, it could only be deduced from the peak heights and the areas under the peaks that the assay per se did work, but we cannot exclude that we might have missed an *IN*01*-positive sample. The *CO*01N.06* allele was not at all detected. The mutation was described before in German and Spanish Roma with a Co(a-b-) phenotype so that samples were available for validation [38]. Because Roma migrated from north/northwestern India to Europe about 1,500 years ago via the Near or Middle East and the Balkans, presence of the mutation at least within individuals from Iran was considered [37], but the absolute number of individuals was too low taking into account an allele frequency of 0.0036 in Spanish Roma [39]. We aimed to analyze at least 1,000 samples of Arabian donors in order to identify alleles with a frequency of 0.05 with sufficient probability. The complete panel of 1,111 donors achieves this requirement, but discrimination between smaller subcollectives cannot warrant identification of rare alleles.

Because in rare cases the U-negative phenotype has been the cause of hemolytic transfusion reactions in African individuals, and *GYPB* mutations can either induce the S-s-U- or the S-s-U+^{var} phenotype [18, 40, 41], we included both, the MALDI-TOF MS screening of the *GYPB*03/*04*

(Ss) polymorphism and PCR-SSP determination of the wt *GYPB*270+5g* in intron 5 in our screening of the Arabian panel. One Northern African donor was typed as *GYPB*04/*04* (ss) by mass spectrometry, but the *GYPB* intron 5 (counting including pseudo exon 3)-specific PCR-SSP reaction with a *GYPB*270+5g*-specific primer was negative. The suspected *GYPB*270+5g>t* mutation that predicts the S-s-U+^{weak} phenotype was confirmed by DNA sequencing. This fact clearly demonstrates that both, single base extension reactions and PCR-SSP only are designed to detect specific SNPs. Polymorphisms other than those covered by the specific assay may remain undetected, and thus phenotypes can only be predicted but not unambiguously determined. The *GYPB*270+5g>t* allele frequency could not be determined because we only tested for the wt allele and thus only detected the homozygous mutation. Two further samples from the German rare donor panel attracted attention because the samples were known as S positive (*GYPB*03*) from serological testing but typed as *GYPB*04/*04* by MALDI-TOF MS. DNA sequencing in both samples identified the presence of both, *GYPB*03* (S) and *GYPB*04* (s) alleles. An additional heterozygous *GYPB*c.161G>A* substitution seems to have induced an allele dropout and a false-negative *GYPB*04* result. One of the 2 samples additionally carried a heterozygous *GYPB*c.249C>G* (Tyr⁸³stop) mutation, but it is not known which of the 2 alleles was affected by the truncation; 17 samples from the Arabian panel failed in MALDI-TOF MS but were positive in the *GYPB*270+5g* PCR-SSP reaction, and demonstrated regular *GYPB*03* and **04* alleles in DNA sequencing, thus excluding amplification failures due to mutations within the MALDI primer annealing sites.

Allele dropouts caused by mutations within the primer annealing sequences, as shown for the *GYPB*c.161G>A* substitution and further unrecognized mutations in general, might be considered disadvantages of molecular typing that are designed to detect SNPs. For the majority of the Arabian panel samples, no phenotype data except for ABO, Rhesus, and Kell exist, and thus false-negative results caused by allele dropouts may be undetected. However, extended blood group typing by serology is not applicable for high sample numbers, especially when rare typing sera are not available. During the last decade, different molecular strategies for extended blood group typing have been developed. These include multiplex PCR-SSP and TaqMan reactions, PCR oligonucleotide extension assays with fluorescent terminator nucleotides, and pooled capillary electrophoresis with fluorescent dyes [19, 42–44]. MALDI-TOF MS has been applied to type thousands of blood donors [23, 24, 34]. Besides the high accuracy and interlaboratory concordance, this methodology has the advantage of a high flexibility for the user that enables adjusted combinations of blood group SNPs within 1 multiplex assay [23,

24] that correspond to the requirements of the blood service. We introduced MALDI-TOF MS genotyping in our blood service in 2012 and, over the years, adapted the assay design to include as many clinically relevant blood group SNPs as possible within 1 well in order to lower the costs per allele. Up to 36 SNPs and more can be included provided that the assay designer can assign amplicon primers and single base extension primers that do not impede each other and enable mass discriminations of at least 16 Da within a detection range of 4,300–9,000 Da. Failure rates within the Arabian panel with 0.1–1.5% were very low and comparable to published data [34].

Next-generation sequencing has been introduced to perform high-throughput multiplex blood group typing with a coverage of either whole genes or at least complete exon sequencing [45–48]. Despite the rather time-consuming procedure, the rapidly decreasing costs make next-generation sequencing attractive for blood group genotyping because it minimizes detection failures due to allele dropout and does not only identify known blood group SNPs of interest, but can also detect novel alleles that might be of clinical relevance [49]. However, the phenotype of new or rare alleles can only be predicted and requires confirmation by means of serology.

Altogether, we presented clinically relevant blood group frequencies in individuals from Arabian countries and Iran in comparison to the local German donor population. The study may help to provide transfusion centers with information regarding the transfusion support of patients from these regions.

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Statement of Ethics

All subjects provided informed consent, and the study on blood group frequencies in Arabian individuals was approved by the Ethics Committee of the Medical Chamber of Rhineland-Palatinate in Mainz, Germany.

Disclosure Statement

The authors have no conflict of interest to disclose.

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Author Contributions

B.K.F. initiated and coordinated the study, evaluated data and wrote the manuscript. V.S. analyzed samples, acquired and evaluated data, and carefully revised the manuscript. B.J. initiated and coordinated the German rare donor study, evaluated data and carefully revised the manuscript. A.O. and T.Z. initiated and supervised the study, discussed the topic and revised the manuscript. O.O. coordinated and supervised provision of Arabian donor samples. A.J. and M.S. organized the provision of samples from Syrian donors, managed the laboratory work flow and data acquisition, and performed the experiments.

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