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Low-Frequency Blood Group Antigens in Switzerland

Christoph Gassner[a] Frauke Degenhardt[b] Stefan Meyer[a] Caren Vollmert[c] Nadine Trost[a] Kathrin Neuenschwander[a] Yvonne Merki[a] Claudia Portmann[a] Sonja Sigurdardottir[a] Antigoni Zorbas[d] Charlotte Engström[d] Jochen Gottschalk[d] Soraya Amar el Dusouqui[e] Sophie Waldvogel-Abramovski[e] Emmanuel Rigal[e] Jean-Daniel Tissot[f] Caroline Tinguely[g] Simon M. Mauvais[h] Amira Sarraj[h] Daniel Bessero[i] Michele Stalder[i] Laura Infanti[j] Andreas Buser[j] Jörg Sigle[k] Tina Weingand[l] Damiano Castelli[m] Monica C. Braisch[n] Jutta Thierbach[n] Sonja Heer[o] Thomas Schulzki[o] Michael Krawczak[p] Andre Franke[b] Beat M. Frey[d]

[a] Blood Transfusion Service Zürich, Swiss Red Cross (SRC), Department of Molecular Diagnostics & Research (MOC), Schlieren, Switzerland;
[b] Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany;
[c] Agena Bioscience GmbH, Hamburg, Germany;
[d] Blood Transfusion Service Zürich, SRC, Schlieren, Switzerland;
[e] Blood Transfusion Service Genève, SRC, Geneva, Switzerland;
[f] Blood Transfusion Service Vaud, SRC (recently merged with Interregional Blood Transfusion, SRC, Ltd., Bern), Lausanne, Switzerland;
[g] Interregional Blood Transfusion, SRC, Ltd., Bern, Switzerland;
[h] Blood Transfusion Service Neuchâtel-Jura, SRC, Neuchâtel, Switzerland;
[i] Blood Transfusion Service Valais, SRC (recently merged with Interregional Blood Transfusion, SRC, Ltd., Bern), Sion, Switzerland;
[j] Blood Transfusion Service beider Basel, SRC, Basel, Switzerland;
[k] Blood Transfusion Service Aargau-Solothurn, SRC, Aarau, Switzerland;
[l] Blood Transfusion Service Zentralschweiz, SRC, Luzern, Switzerland;
[m] Blood Transfusion Service Svizzera Italiana, SRC, Lugano, Switzerland;
[n] Blood Transfusion Service Ostschweiz, SRC, St. Gallen, Switzerland;
[o] Blood Transfusion Service Graubünden, SRC, Chur, Switzerland;
[p] Institute for Medical Informatics and Statistics, Christian-Albrechts-University Kiel, Kiel, Germany

Keywords

Blood groups · Low-frequency antigen · High-frequency antigen · Rare donor panel/program · Rare/molecular blood group · Blood group allele · Population genetics · Switzerland

Summary

Background: High-frequency blood group antigens (HFA) are present in >90% of the human population, according to some reports even in >99% of individuals. Therefore, patients lacking HFA may become challenging for transfusion support because compatible blood is hardly found, and if the patient carries alloantibodies, the cross-match will be positive with virtual every red cell unit tested. **Methods:** In this study, we applied high-throughput blood group SNP genotyping on >37,000 Swiss blood donors, intending to identify homozygous carriers of low-frequency blood group antigens (LFA). **Results:** 326 such individuals were identified and made available to transfusion specialists for future support of patients in need of rare blood products. **Conclusion:** Thorough comparison of minor allele frequencies using population genetics revealed heterogeneity of allele distributions among Swiss blood donors which may be explained by the topographical and cultural peculiarities of Switzerland. Moreover, geographically localized donor subpopulations are described which contain above-average numbers of individuals carrying rare blood group genotypes.

Christoph Gassner, Frauke Degenhardt, and Stefan Meyer contributed equally to this work.

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Fax +49 761 4 52 07 14
Information@Karger.com
www.karger.com

Accessible online at:
www.karger.com/tmh

Dr. Christoph Gassner
Blood Transfusion Service, SRC, Zurich
Molecular Diagnostics & Research (MOC)
Rütistrasse 19, 8952 Schlieren, Switzerland
c.gassner@zhbsd.ch

Introduction

One of the challenges in transfusion medicine is to provide compatible blood for patients negative for a high-frequency blood group antigen (HFA) and who have an alloantibody against the antigen [1]. Such HFA negativity may either be caused by a complete lack of the protein serving as carrier of a certain blood group system with concomitant negativity for all its associated antigens, or by the lack of only one specific antigen, because of the presence of an identical variant blood group protein inherited on both parental haplotypes. Low-frequency blood group antigens (LFA) are the less frequent antithetic variants of HFAs. LFAs do not create a major transfusion problem from the aspect of finding compatible donors. However, a potentially dangerous antibody to an LFA could remain undetected if a full cross-match analysis was not performed [2].

The Kell blood group system is an exemplary model for the above-mentioned model. The system is highly polymorphic and expresses 36 antigens, all encoded on a type II glycoprotein of 732 amino acids encoded by the *KEL* gene and its allelic variants [3, 4]. Some of the Kell antigens have been classified into antithetical pairs, each represented by one HFA and its correspondent LFA, e.g. K and k or Kp^a and Kp^b, others being independently expressed or having unknown antithetical partners. KEL2 or k+, formerly also named 'Cellano', is a HFA and its antithetical variant is the LFA: KEL1 or K+. As a result, K+ k– homozygous individuals are encountered only rarely, e.g. at an exemplary frequency of 1 per 1,371 Swiss [5]. While, K+ k– individuals still express other Kell antigens, e.g. Kp^b or KEL11, none of the Kell antigens are expressed on cells of the Kell-null phenotype, K_0, which arise from homozygous or compound heterozygous *KEL*-inactivating mutations [6–8]. K_0 individuals are exceedingly rare and may only be found at frequency fewer than 1 individual per 1 million Austrians [6].

Some blood types are extremely scarce worldwide, and requests for transfusion are particularly difficult to fulfill. On the other side of the frequency spectrum, e.g. where prevalence of certain antigens is shifting towards 'public antigen frequency', a generally accepted numerical definition for 'rarity' is lacking. For instance, D– blood is common in Caucasians (approximately 15% of the population), but it is rare in Asia (less than 1%) [2]. In some nations, a blood type with a prevalence of 1 in 100 is considered rare, whereas in other countries, the same status requires antigen prevalence of less than 1 in 5,000. Moreover, in some programs, rare donors are exclusively determined by being negative for single HFAs, whereas in other programs, donors negative for a combination of several public (common) red cell antigens are also recognized as being rare. As a consequence, the definition of a 'rare donor' is widely different, as vividly demonstrated by the existent variety of national rare donor programs [9].

Traditionally, red cell antigens have been identified by serology. Recent advances in molecular biology made it possible to genotype most of the blood group antigens employing high-throughput technology platforms [10]. Also, today, blood group antigens without suitable anti-sera – such as Scianna and Dombrock – are screened for using high-throughput genotyping methods [11, 12].

The presented project was conducted for the main purpose to ensure the supply of rare blood units to the Swiss population. MALDI-TOF MS was adapted and used to genotype 37,253 Swiss blood donors by a customized 'RARE module' covering 26 blood group single nucleotide polymorphisms (SNPs) including 22 antithetical HFA/LFA pairs (table 1) [13].

Material and Methods

Samples

Between 2012 and 2014, samples from 37,253 blood donors were collected at different sites throughout Switzerland. Donor samples were provided by 11 regional blood transfusion services (BTSs) headed in Geneva (n = 1,348), Lausanne (n = 1,526), Neuchâtel (n = 1,029), Sion (n = 760), Basel (n = 1,222), Aarau (n = 855), Luzern (n = 2,770), Zurich (n = 24,058), Lugano (n = 768), St. Gallen (n = 1,476), and Chur (n = 1,441). In 2012, blood donations were collected by 13 independent BTSs, most of them covering more than their administrative-regional area, also known as 'cantons'. As a consequence, respective inhabitant numbers and deduced 'coverage' of every BTS given need to be considered as approximate (table 2). The ethical approval of the study was waived by the ethical committee of the Canton of Zurich, and all donors explicitly permitted genetic laboratory investigations by written consent.

Blood Group Polymorphisms, SNPs, Analyzed by MALDI-TOF MS 'RARE Module'

For automated DNA extraction, magnetic bead technology was used (Chemagen; Perkin Elmer, Baesweiler, Germany). Assay design for all SNPs (table 1), quality control of the primer mixes, and MALDI-TOF MS-based genotyping was done as described previously [5].

Prior to implementation of the 'RARE module' into routine use, molecular typing performance was validated by assessing a panel of 95 natural and artificial, reference DNAs representing all blood group specificities of the module. Additionally, every individual typing batch was controlled for specificity, using the identical reference DNA panel. Reference DNAs representing the blood group phenotypes, Lu(a+b–), KK, Kp(a+b–) and Yt(a–b+), and heterozygous phenotypes representative of Lu(08+14+), Au(a+b+), Js(a+b+), KEL(11+ 7+) and Do(a+b+) were provided by BTS Zurich [5, 14–16]. Sample material of individuals with phenotypes Di(a+b+), Wr(a+b+), Co(a–b+), and Kn(a–b+) was given by Susanne Kilga-Nogler (Zentralinstitut für Bluttransfusion und Immunologische Abteilung, Innsbruck, Austria). Indian, In(a+b–), reference DNAs were provided by Joyce Pool (International Blood Group Reference Laboratory, NHS Blood and Transplant, Bristol, UK). Sample material of Vel– individuals was provided by Christof Jungbauer (Austrian Red Cross, Blood Service for Vienna, Lower Austria and Burgenland, Vienna, Austria).

Artificially synthesized control DNAs were used in cases where natural blood group DNA was not available and were generated by standard PCRs using one mutated and one regular amplification primer each, in order to cover the respective polymorphic SNPs. Artificial DNA fragments were generated for the following LFAs and their antithetical HFA partners: SC2 (*SC*02*), LW^b (*LW*07*), Hy– (*DO*02.-04*), Jo(a–) (*DO*01.-05*), McC^b (*KN*01.06*), Vil+ (*KN*01.07*), and all Cromer antigens [17–21]. Before use, artificial DNA fragments were titrated to equimolar copy concentrations as found in genomic DNA extracts from donor samples before validation.

Provision of Diagnostic Anti-Sera

Rare diagnostic anti-sera for serotyping by standard techniques, such as anti-Di^b, anti-Co^a, anti-LW^a, and anti-Js^a, were made available by exchange programs or were provided by sources as described previously [5, 22].

Data Sources, Statistical Methods, and Allele Frequency Calculation

Population data of Switzerland was taken from the report published annually by the Schweizerische Eidgenossenschaft, 'Bundesamt für Statistik' (table 2) [23].

Table 1. Specificities included in the matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS) based 'RARE module'

Blood group system	ISBT #	Blood group	Gene (HGNC)	Chromo-some	Allele name 1	Allele name 2	CDS[§] position	CDS on mRNA accession number	nt 1	nt 2	Amino acid exchange	dbSNP rs number	Anti-gens	HFA/ LFA	Allele ct.	SNP ct.	Plex W1	Plex W2
ABO	001	ABO A vs O1	*ABO*	9q34.2	*ABO*A(wt)*	*ABO*O.01*	261	NM_020469.2	G	del G	fsThr88Pro	rs8176719	2	–	2	1	–	W2
ABO	001	ABO A vs O2	*ABO*	9q34.2	*ABO*A(wt)*	*ABO*O.02*	802	NM_020469.2	G	A	Gly268Arg	rs41302905	–	–	1	1	–	W2
ABO	001	ABO A vs B	*ABO*	9q34.2	*ABO*A(wt)*	*ABO*B*	803	NM_020469.2	G	C	Gly268Ala	rs8176747	1	–	1	1	W1	–
Lutheran	005	Lu^a / Lu^b	*BCAM*	19q13.32	*LU*01*	*LU*02 (wt)*	230	NM_005581.4	A	G	His77Arg	rs28399653	2	1	2	1	W1	–
Lutheran	005	Lu8 / Lu14	*BCAM*	19q13.32	*LU*02.14*	*LU*02 (wt)*	611	NM_005581.4	A	T	Lys204Met	rs28399656	2	1	1	1	W1	–
Lutheran	005	Au^a / Au^b	*BCAM*	19q13.32	*LU*02.19*	*LU*02 (wt)*	1615	NM_005581.4	G	A	Ala539Thr	rs1135062	2	–	1	1	–	W2
Kell	006	K / k	*KEL*	7q34	*KEL*01*	*KEL*02 (wt)*	578	NM_000420.2	T	C	Met193Thr	rs8176058	2	1	2	1	W1	–
Kell	006	Kp^a / Kp^b	*KEL*	7q34	*KEL*02.03*	*KEL*02 (wt)*	841	NM_000420.2	T	C	Trp281Arg	rs8176059	2	1	1	1	–	W2
Kell	006	K11 / K17	*KEL*	7q34	*KEL*02.17*	*KEL*02 (wt)*	905	NM_000420.2	C	T	Ala302Val	rs61729034	2	1	1	1	W1	–
Kell	006	Js^a / Js^b	*KEL*	7q34	*KEL*02.06*	*KEL*02 (wt)*	1790	NM_000420.2	C	T	Pro597Leu	rs8176038	2	1	1	1	W1	–
Diego	010	Di^a / Di^b	*SLC4A1*	17q21.31	*DI*01*	*DI*02 (wt)*	2561	NM_000342.3	T	C	Leu854Pro	rs2285644	2	1	2	1	W1	–
Wright	010	Wr^a / Wr^b	*SLC4A1*	17q21.31	*DI*02.03*	*DI*02 (wt)*	1972	NM_000342.3	A	G	Glu658Lys	rs75731670	2	1	1	1	–	W2
Cartwright	011	Yt^a / Yt^b	*ACHE*	7q22.1	*YT*01 (wt)*	*YT*02*	1057	NM_001302621.1	C	A	His353Asn	rs1799805	2	1	2	1	W1	–
Scianna	013	SC1, SC2	*ERMAP*	1p34.2	*SC*01 (wt)*	*SC*02*	169	NM_001017922.1	G	A	Gly57Arg	rs56025238	2	1	2	1	–	W2
Dombrock	014	Do^a / Do^b	*ART4*	12p12.3	*DO*01*	*DO*02 (wt)*	793	NM_021071.2	A	G	Asn265Asp	rs11276	2	–	2	1	–	W2
Dombrock	014	Hy+ / Hy-	*ART4*	12p12.3	*DO*02.–04*	*DO*02 (wt)*	323	NM_021071.2	T	G	Val108Gly	rs28362797	2	1	1	1	W1	–
Dombrock	014	Jo(a+) / Jo(a–)	*ART4*	12p12.3	*DO*01.–05*	*DO*02 (wt)*	350	NM_021071.2	T	C	Ile117Thr	rs28362798	2	1	1	1	–	W2
Colton	015	Co^a / Co^b	*AQP1*	7p14.3	*CO*01.01 (wt)*	*CO*02*	134	NM_198098.2	C	T	Ala45Val	rs28362692	2	1	2	1	W1	–
Landst.-Wien.	016	LW^a / LW^b	*ICAM-4*	19p13.2	*LW*05 (wt)*	*LW*07*	299	NM_001544.4	A	G	Gln100Arg	rs77493670	2	1	2	1	W1	–
Cromer	021	Cr^a / Cr^{a-}	*CD55*	1q32.2	*CROM*–01*	*CROM*01 (wt)*	679	NM_000574.3	C	G	Pro227Ala	rs60822373	2	1	2	1	W1	–
Cromer	021	Tc^a / Tc^b	*CD55*	1q32.2	*CROM*01.03*	*CROM*01 (wt)*	155	NM_000574.3	T	G	Leu52Arg	rs28371588	2	1	1	1	W1	–
Cromer	021	Tc^a / Tc^c	*CD55*	1q32.2	*CROM*01.04*	*CROM*01 (wt)*	155	NM_000574.3	C	G	Pro52Arg	rs28371588	1	1	1	–	W1	–
Knops	022	Kn^a / Kn^b	*CR1*	1q32.2	*KN*01 (wt)*	*KN*02*	4681	NM_000573.3	G	A	Val1561Met	rs41274768	2	1	2	1	W1	–
Knops	022	McC^a / McC^b	*CR1*	1q32.2	*KN*01.06*	*KN*01 (wt)*	4768	NM_000573.3	G	A	Glu1590Lys	rs17047660	2	1	1	1	W1	–
Knops	022	Vil+ / Vil–	*CR1*	1q32.2	*KN*01.07*	*KN*01 (wt)*	4801	NM_000573.3	G	A	Gly1601Arg	rs17047661	2	1	1	1	–	W2
Indian	023	In^a / In^b	*CD44*	11p13	*IN*01*	*IN*02 (wt)*	137	NM_001001391.1	C	G	Pro46Arg	rs369473842	2	1	2	1	W1	–
Vel	034	Vel+ / Vel-	*SMIM1*	1p36.32	*VEL*–01*	*VEL*01*	c.64-80 del	NM_001163724.2	del 17 bp	-	Ser22Glnfs*	rs566629828	2	1	2	1	–	W2
Gender	n.a.	female/male	*GYG2/ paralog*	Xp22.33/ Yp11.2	*GYG2*Xfemale*	*GYGpar*Ymale*	i2+3291	NG_021257.1 (female)[+] AC002992.1 (male)[+]	C	A	–	no rs	–	–	2	1	W1	–
Gender	n.a.	female/male	*AMEL/ paralog*	Xp22.2/ Yp11.2	*AMEL*Xfemale*	*AMEL*Ymale*	i3-7/ i4-140	NG_012040.1 (female)[+] NG_008011.1 (male)[+]	T	C	–	no rs	–	–	2	1	–	W2
SUM excluding SNPs for gender determination													50	22	40	26		

[§]CDS =coding sequence, + genomic sequences.

Table 2. Geographic organisation of the 13 Blood Transfusion Services (BTSs) of Switzerland in 2015. Demographic data on Swiss cantons [23]; their BTSs and headquarter locations; origin and number of blood donor samples, sampling coverage of Swiss population

Canton Name (local language)	Canton abbreviation	BTS headquarter	Number of Inhabitants	Coverage per BTS	Donor tested	% of Swiss	% of coverage
Genève	GE	Geneva	484,736	484,736	1,348	5.82	6.96
Vaud	VD	Lausanne	773,407	773,407	1,5260	9.29	11.10
Neuenburg/Neuchâtel	NE	Neuchâtel	178,107	250,889	1,0290	3.01	3.60
Jura	JU		72,782				
Wallis/Valais	VS	Sion	335,696	335,696	760	4.03	4.82
Basel-Stadt	BS	Basel	191,817	475,048	1,222	5.70	6.82
Basel-Landschaft	BL		283,231				
Aargau	AG	Aarau	653,675	920,093	855	11.05	13.21
Solothurn	SO		266,418				
Luzern	LU	Luzern	398,762	600,392	2,770	7.21	8.62
Obwalden	OW		37,076				
Nidwalden	NW		42,420				
Zug	ZG		122,134				
Zürich	ZH	Zurich	1,466,424	1,967,782	24,058	23.63	28.25
Schwyz	SZ		154,093				
Schaffhausen	SH		79,836				
Thurgau	TG		267,429				
Ticino	TI	Lugano	351,946	351,946	768	4.23	5.05
St. Gallen	SG	St. Gallen	499,065	569,582	1,476	6.84	8.18
Appenzell Ausserrhoden	AR		54,543				
Appenzell Innerrhoden	AI		15,974				
Graubünden	GR	Chur	196,610	236,638	1,441	2.84	3.40
Glarus	GL		40,028				
Swiss areas covered			6,966,209	6,966,209	37,253	83.66	100.00
Freiburg/Fribourg	FR		307,461	307,461	0	3.69	
Bern	BE		1,017,483	1,053,456	0	12.22	
Uri	UR		35,973		0	0.43	
Swiss areas uncovered			1,360,917	1,360,917	0	16.34%	
Total Switzerland			8,327,126	8,327,126	37,253	100.00%	

Absolute allele frequencies were calculated by direct allele counting (table 3) according to Hardy-Weinberg proportions for all samples originating from the greater area covered by BTS Zurich and are given as 'minor allele frequencies' (MAF) [24]. Allele frequencies of individual BTSs were calculated by direct allele counting as described above and, in order to give an averaged 'Swiss allele frequency', statistically corrected (weighted) according to the number of inhabitants of the areas covered by the respective BTSs (tables 2, 4). Additionally, for each allele, the mean, standard deviation (SD) and coefficient of correlation (CV) across all cantons is given (supplementary table 1; available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=490714*). The coefficient of correlation is calculated by division of the SD of the mean and is a measure of dispersion that is independent of the scale. We also checked for correlation between sample size and MAF using the rank correlation coefficient by Spearman [25].

To visualize overall similarities in relative blood group frequencies among BTSs, we performed a principal coordinate analysis (PCoA) on the relative blood frequencies using the R-package ape 5.0. Cantons with comparable blood group frequency will cluster closely together in this analysis, whereas those with unequal blood group frequency will spread apart. In population genetics, a measure of population structure is the fixation index (here F_{ST} [26]) that is usually calculated on SNPs or microsatellite data. F_{ST} can take a value between 0 and 1. In simplified terms, the smaller the F_{ST} the more similar the genetic background and vice versa. Here we use F_{ST} to elucidate population substructures based on blood group antigens. The PCoA was performed on the minor blood group alleles having a frequency of >0.1% across all cantons, using an Eucledian distance measure, and on F_{ST} using it as distance measure. Since we used the F_{ST} as distance measure for the second PCoA, we calculated the variance explained by the first two PCoA components across only the positive eigenvalues. To visualize pairwise (comparison of each canton to the rest) F_{ST} values, we plotted a heatmap using the gplots package 3.0.1 of R. In short, a heatmap plots the differences of the input values using different color intensities. Dendrograms are constructed using hierarchical clustering to depict overall similarities of features using the complete linkage algorithm [27]. We also performed pairwise Fisher's exact tests for each blood group on the contingency tables listing the absolute frequencies of the respective homozygous and heterozygous antigen counts for the analyzed cantons. We adjusted for the sample size of the different panels as described (supplementary table 2; available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=490714*) [28]. Moreover, we adjusted for multiple testing using the correction proposed by Benjamini and Hochberg [29].

Results

MALDI-TOF MS-Based 'RARE Module'

The 'RARE module' consisted of two multiplex reactions, comprising a total of 26 biallelic or triallelic SNP assays for the simultaneous analysis of 13 blood group genes and 40 of their alleles, rep-

Table 3. Genotyping results for ISBT 005 (Lutheran) to ISBT 013 (Scianna) and for ISBT 014 (Dombrock) to ISBT 034 (Vel)[a]

	Blood group system																								
	Luthe-ran	Luthe-ran	Luthe-ran	Kell	Kell	Kell	Kell	Diego	Wright	Cart-wright	Sci-anna	Dom-brock	Dom-brock	Dom-brock	Col-ton	Landst.-Wiener	Cro-mer	Cro-mer	Cro-mer	Knops	Knops	Knops	In-dian	Vel
ISBT number	005	005	005	006	006	006	006	010	010	011	013	014	014	014	015	016	021	021	021	022	022	022	023	034
Frequent antigen	Lu[b]	Lu8	Au[b]	k	Kp[b]	K11	Js[b]	Di[b]	Wr[b]	Yt[a]	SC:1	Do[b]	Hy+	Jo(a+)	Co[a]	LW[a]	Cr[a]	Tc[a]		Kn[a]	McC[a]	Vil-	In[b]	Vel+
Rare antigen	Lu[a]	Lu14	Au[a]	K	Kp[a]	K17	Js[a]	Di[a]	Wr[a]	Yt[b]	SC:2	Do[a]	Hy-	Jo(a-)	Co[b]	LW[b]	Cr[a-]	Tc[b]	Tc[c]	Kn[b]	McC[b]	Vil+	In[a]	Vel-
Geneva																								
AA	1,237	1,304	623	1,212	1,313	1,313	1,313	1,318	1,294	1,163	1,319	507	1,323	1,318	1,239	1,318	1,302	1,318		1,268	1,311	1,296	1,307	1,297
Aa	78	16	575	106	16	6	6	3	3	151	12	623	2	3	80	3	1	1	2	53	8	29	0	33
aa	1	0	132	1	1	0	0	0	0	7	0	201	0	0	1	0	0	0	0	0	2	6	0	1
invalid	32	28	18	29	18	29	29	27	51	27	17	17	23	27	28	27	45	27		27	27	17	41	17
Lausanne																								
AA	1,397	1,451	721	1,392	1,478	1,487	1,486	1,493	1,502	1,324	1,495	533	1,510	1,478	1,390	1,491	1,407	1,489		1,421	1,471	1,487	1,490	1,459
Aa	83	40	649	101	32	6	6	0	0	162	15	735	1	2	100	2	2	0	3	70	8	21	2	52
aa	1	0	138	0	1	0	0	0	0	5	0	242	0	0	3	0	0	0	0	1	0	3	0	0
invalid	45	35	18	33	15	33	34	33	24	35	16	16	15	46	33	33	117	34		34	47	15	34	15
Neuchâtel																								
AA	957	1,002	502	957	1,002	1,026	1,026	1,028	999	909	1,020	361	1,028	1,027	956	1,024	1,027	1,026		972	1,024	1,018	1,023	1,002
Aa	69	25	416	69	27	2	2	0	0	116	8	499	1	0	68	4	0	0	1	55	4	10	1	27
aa	0	1	105	2	0	0	0	0	0	1	0	162	0	0	4	0	0	0	0	1	0	1	0	0
invalid	3	1	6	1	0	1	1	1	30	3	1	7	0	2	1	1	2	2		1	1	0	5	0
Sion																								
AA	685	732	376	692	742	745	744	746	755	659	748	275	755	742	699	741	716	744		723	742	752	746	755
Aa	53	13	313	50	13	1	2	0	0	85	7	375	0	0	46	5	0	0	1	23	0	3	0	0
aa	1	1	66	2	0	0	0	0	0	2	0	104	0	0	1	0	0	0	0	0	0	0	0	0
invalid	21	14	5	16	5	14	14	14	5	14	5	6	5	18	14	14	44	15		14	18	5	14	5
Basel																								
AA	982	1,057	516	997	1,033	1,064	1,060	1,080	1,027	955	482	379	1,047	1,064	1,004	1,073	1,007	1,073		1,024	1,063	1,047	1,079	475
Aa	82	22	426	69	18	3	1	0	0	122	1	522	0	0	75	7	0	0	4	56	2	9	0	9
aa	0	0	100	3	0	0	0	0	0	3	0	154	0	0	1	0	0	0	0	0	0	0	0	0
invalid	158	143	180	153	171	155	161	142	195	142	739	167	175	158	142	142	215	145		142	157	166	143	738
Aarau																								
AA	790	841	411	781	833	844	844	849	854	758	854	288	855	850	776	843	850	849		792	849	854	846	817
Aa	60	9	368	68	22	6	6	1	1	91	1	417	0	0	69	7	0	0	1	57	1	1	0	38
aa	0	0	76	1	0	0	0	0	0	2	0	150	0	0	2	0	0	0	0	1	0	0	0	0
invalid	5	5	0	5	0	5	5	5	0	4	0	0	0	5	8	5	5	5		5	5	0	9	0

Table 3. *continued on next page*

Table 3. Continued

	Blood group system																						
	Luthe-ran	Luthe-ran	Luthe-ran	Kell	Kell	Kell	Kell	Diego	Wright	Cart-wright	Sci-anna	Dom-brock	Dom-brock	Dom-brock	Col-ton	Landst.-Wiener	Cro-mer	Cro-mer	Cro-mer	Knops	Knops	Knops	In-dian	Vel
Luzern																								
AA	2,514	2,661	1,333	2,495	2,664	2,697	2,712	2,716	2,710	2,446	898	975	2,724	2,719	2,494	2,701	2,715	2,717		2,535	2,719	2,728	2,708	876
Aa	197	58	1,147	222	72	24	8	1	2	261	4	1,306	4	1	219	19	0	0	0	184	1	8	1	26
aa	6	0	252	4	1	0	0	0	0	12	0	456	0	0	3	0	0	0	0	1	0	0	0	0
invalid	53	51	38	49	33	49	50	53	58	51	1,868	33	42	50	54	50	55	53		50	50	34	61	1,868
Zurich																								
AA	22,013	23,287	11,766	21,953	23,237	23,628	23,591	23,698	23,120	21,075	14,681	8,451	23,717	23,713	21,971	23,536	23,630	23,677		22,459	23,684	23,680	23,702	14,753
Aa	1,678	440	9,773	1,693	527	66	74	36	9	2,567	98	11,427	7	13	1,705	201	14	4	44	1,259	36	115	3	26
aa	29	5	2,207	50	4	0	0	1	0	92	0	3,911	0	0	31	1	0	0	0	18	2	6	0	3
invalid	338	326	312	362	290	364	393	323	929	324	9279	269	334	332	351	320	414	333		322	336	257	353	9,276
Lugano																								
AA	718	745	322	698	749	758	754	753	728	674	380	269	762	760	691	757	753	756		726	759	759	758	382
Aa	43	12	335	56	12	0	1	0	1	80	3	382	0	0	61	4	0	0	0	35	1	4	0	0
aa	0	0	105	4	0	0	0	0	0	6	0	112	0	0	2	0	0	0	0	0	0	0	0	1
invalid	7	11	6	10	7	10	13	15	39	8	385	5	6	8	14	7	15	12		7	8	5	10	385
St. Gallen																								
AA	1,361	1,435	745	1,349	1,444	1,460	1,453	1,460	1,435	1,297	1,458	491	1,447	1,462	1,347	1,450	1,461	1,460		1,395	1,462	1,458	1,454	1,404
Aa	99	27	596	113	22	1	8	1	0	158	7	724	0	0	113	12	1	0	1	65	0	7	0	60
aa	2	0	124	0	0	0	0	0	0	6	0	249	0	0	2	0	0	0	0	1	0	0	0	0
invalid	14	14	11	14	10	15	15	15	41	15	11	12	29	14	14	14	14	15		15	14	11	22	12
Chur																								
AA	1,343	1,398	636	1,301	1,396	1,411	1,419	1,422	1,421	1,249	393	516	1,426	1,422	1,318	1,415	1,420	1,417		1,360	1,421	1,421	1,421	395
Aa	76	24	628	119	33	12	3	0	0	166	2	665	3	0	100	7	1	2	1	60	0	9	0	0
aa	2	0	161	1	0	0	0	0	0	7	0	247	0	0	2	0	0	0	0	2	0	0	0	0
invalid	20	19	16	20	12	18	19	19	20	19	1,046	13	12	19	21	19	20	21		19	20	11	20	1,046
Total																								
AA	33,997	35,913	17,951	33,827	35,891	36,433	36,402	36,563	35,845	32,509	23,728	13,045	36,594	36,555	33,885	36,349	36,288	36,526		34,675	36,505	36,500	36,534	23,615
Aa	2,518	686	15,226	2,666	794	127	117	42	16	3,959	158	17,675	18	19	2,636	271	19	7	58	1,917	61	216	7	271
aa	42	7	3466	68	7	0	0	1	0	143	0	5988	0	0	52	1	0	0	0	25	4	16	0	5
invalid	696	647	610	692	561	693	734	647	1,392	642	13,367	545	641	679	680	632	946	662		636	683	521	712	13,362
% Invalid	1.87	1.74	1.64%	1.86	1.51	1.86	1.97	1.74%	3.74	1.72	n.a.	1.46	1.72	1.82	1.83	1.70	2.54	1.78		1.71	1.83	1.4%	1.9%	n.a.
Overall	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253	37,253		37,253	37,253	37,253	37,253	37,253

[a]Top to bottom: Numbers of frequent homo- (AA), hetero- (Aa), and rare homozygotes(aa) and invalid typings for each BTS, identified by their respective head quarter locations, ordered according to geographical latitude from West to East. Left to right: antigens tested, ordered according to the ISBT number of the respective blood group system. Donors with rare blood group phenotypes are underlayed in gray (excluding blood group system Knops) and are summing up to a total of 326 (line 'rare homo' of total numbers). Only 23,886 and 23,848 blood donors, were analyzed for Scianna rs56025238 and Vel rs566629828, respectively. Donor samples not tested for these antigens are indicated as n.t.

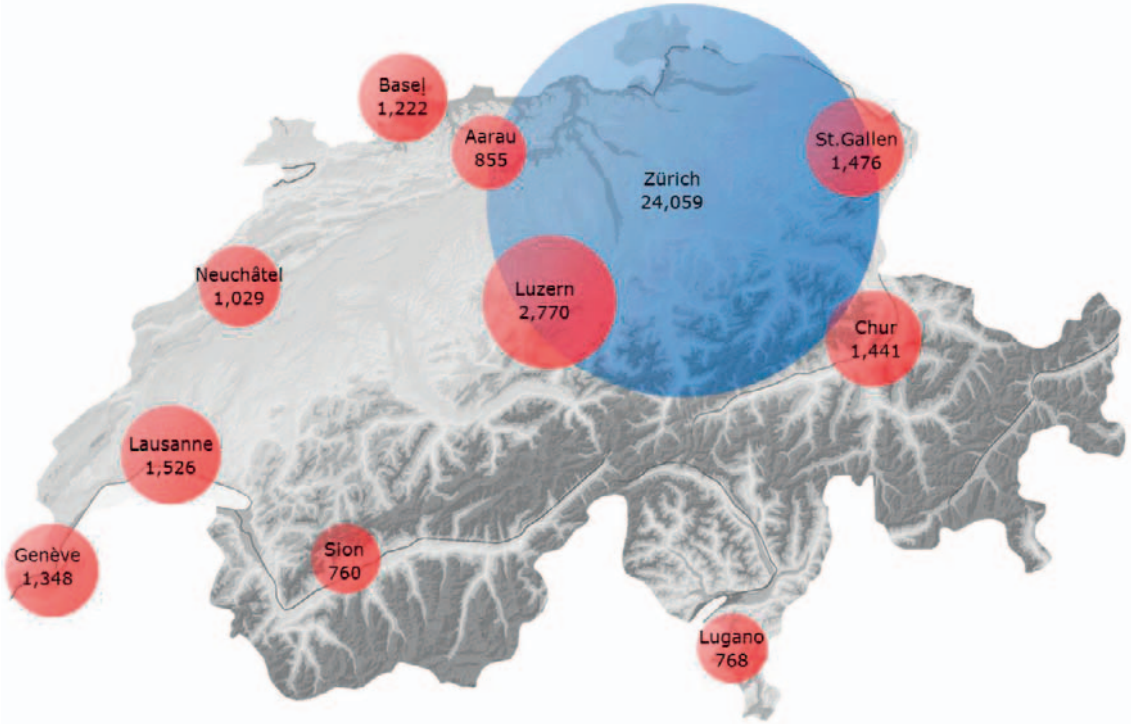

Fig. 1. Origin of blood donor samples and BTSs, identified by their headquarter locations (local language), participating in this project. The respective head quarter locations are given with their approximate geographical location and in their local languages. The area of each circle is correspondent to the number of samples investigated for each BTS (also see table 2). Approximately two-thirds of the analyzed blood donors (n = 24,058) were from the greater area of Zurich (blue circle), whereas the other third (n = 13,195) were from another 10 different blood transfusion services distributed throughout Switzerland (red circles), summing up to a total of 37,253 individual blood donor samples investigated in the course of this project. Topographically, Swiss Alps are shown in dark grey. Lugano and Sion are located south and Chur within the Swiss Alps.

resenting a total of 50 blood group antigens, most of them described as HFAs or LFAs (table 1). Genotyping for Scianna (SC1/SC2, rs56025238) and Vel (Vel+/Vel–, rs566629828) only became available in a later revised version of the RARE module and after the description of the genetic background of Vel negativity [30]. Therefore, only 23,886 and 23,848 blood donors were analyzed for Scianna rs56025238 and Vel rs566629828, respectively. All other SNPs were analyzed on 37,253 DNA samples. Approximately two-thirds of the analyzed blood donors (n = 24,058) were from the greater area of Zurich, whereas the other third (n = 13,195) was provided by the other 10 BTSs (fig. 1, table 2). Approximately 84% of the Swiss population, e.g. 6.966 of the total 8.327 million inhabitants are covered by all 11 BTSs. Therefore, roughly 1 of every 200, or 37,253 of 8.327 million Swiss individuals were assessed by our approach.

Both multiplexes included additional assays, one each for gender determination, and three additional assays for the specific detection of *ABO* SNPs, located at coding nucleotide positions 261, 802 and 803. The respective assays served as quality control measure, e.g. to link DNA samples to their available donor phenotype data, thereby allowing for the exclusion of serial sample mistake. Comparison of ABO geno- and serotyping will be published elsewhere (manuscript in preparation). Calling failures, caused by samples with either a negative result for all SNPs or for only a single SNP assay failure, were excluded from the finally analyzed data set and ranged between 1.40% and 3.73% per assay (average 1.87%, median 1.78%; table 3, bottom line).

Rare Blood Donors Negative for HFAs and Homozygous Positivity for LFAs

The identified individuals with rare blood group antigen constellations had genotypes, known to encode for the blood group phenotypes Lu(a+b–) (n = 42), Lu(8–14+) (n = 7), K+k– (n = 68), Kp(a+b–) (n = 7), Di(a+b–) (n = 1), Yt(a–b+) (n = 143), Co(a–b+) (n = 52), LW(a–b+) (n = 1), Vel– (n = 5), summing up to a total of n = 326 (table 3). Knops blood group antigens are defined by clinically insignificant antibodies, but are notoriously difficult to identify [2]. Therefore, individuals with predicted phenotypes Kn(a–b+) (n = 25), McC(a–b+) (n = 4), heterozygous for Vil+/– (n = 216) ,and homozygous for Vil+ (n = 16) are listed separately from the above. All donors beside those showing rare Knops phenotypes were reported to the Swiss Rare Donor File [31]. Successive samples of rare genotype carriers were used for serological confirmation of genotype. So far, the reinvestigated individuals with the following phenotypes were: Lu(a+b–) (n = 15 of 42), K+k– (n = 26 of 68), Kp(a+b–) (n = 2 of 7), Di(a+b–) (n = 1 of 1), Yt(a–b+) (n = 52 of 143), Co(a-b+) (n = 18 of 52).

Blood Group Allele Frequencies

Genotyping allowed for the identification of frequent and rare homozygous and heterozygous genotypes, the later in some cases not detectable by serologic testing. For instance, using standard serological methods, Vel positivity is undistinguishable in between *VEL*01/ VEL*–01* heterozygotes and *VEL*01/ VEL*01* homozygotes. On a molecular and statistical basis however, heterozygotes

Table 4. Allele frequency data given for the minor allele of each SNP as minor allele frequency (MAF) for the greater area of Zurich or Switzerland[a]

	Blood group system											
	Lutheran	Lutheran	Lutheran	Kell	Kell	Kell	Kell	Diego	Wright	Cartwright	Scianna	Dombrock
Rare allele	*LU*01*	*LU*02.14*	*LU*02.19*	*KEL*01*	*KEL*02.03*	*KEL*02.17*	*KEL*02.06*	*DI*01*	*DI*02.03*	*YT*02*	*SC*02*	*DO*01*
c.nt position	230	611	1615	578	841	905	1790	2561	1972	1057	169	793
Rare nt.	A	A	G	T	T	C	C	T	A	A	A	A
rs number	rs28399653	rs28399656	rs1135062	rs8176058	rs8176059	rs61729034	rs8176038	rs2285644	rs7531670	rs1799805	rs56025238	rs11276
MAF Zurich only	0.03659	0.00948	0.29872	0.03783	0.01125	0.00139	0.00156	0.00080	0.00019	0.05795	0.00332	0.40458
MAF Swiss covered (weighted)	0.03451	0.00930	0.30523	0.03853	0.01064	0.00196	0.00184	0.00043	0.00028	0.05796	0.00298	0.40412
Delta % ZH vs. Swiss	6	2	−3	−2	6	−29	−15	88	−30	0	11	0
	Blood group system											
	Dombrock	Dombrock	Colton	Landst. Wiener	Cromer	Cromer	Cromer	Knops	Knops	Knops	Indian	Vel
Rare allele	*DO*02.–04*	*DO*01.–05*	*CO*02*	*LW*07*	*CROM*–01*	*CROM*01.03*	*CROM*01.04*	*KN*02*	*KN*01.06*	*KN*01.07*	*IN*01*	*VEL*–01*
c.nt position	323	350	134	299	679	155	155	4681	4768	4801	137	c.64-80del
Rare nt.	T	T	T	G	C	T	C	A	G	G	G	del 17 bp
rs number	rs28362798	n.a.	rs28362692	rs77493670	rs60822373	rs28371588	rs28371588	rs41274768	rs17047660	rs17047661	rs369473842	rs566629828
MAF Zurich only	0.00023	0.00027	0.03727	0.00428	0.00030	0.00008	0.00093	0.02728	0.00084	0.00267	0.00006	0.00108
MAF Swiss covered (weighted)	0.00025	0.00025	0.03790	0.00321	0.00023	0.00007	0.00072	0.02662	0.00112	0.00405	0.00013	0.01022
Delta % ZH vs. Swiss	−6	11%	-2%	33%	29%	28%	29%	2%	-25%	-34%	-50%	-89%

[a]Left to right: antigens investigated, ordered according to the ISBT number of the respective blood group system.

will be recognized and are much more frequent as compared to rare homozygotes. Therefore, genotyping data provided exact blood group allele frequency estimates. The MAF of all blood group SNPs from all BTSs are shown in supplementary table 3 (available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=490714*). MAF data, separately calculated for the donor panel from the greater area of Zurich, and an average Swiss MAF, averaging data of all BTSs according to the number of inhabitants covered by the respective BTSs, are given in table 4.

The most common alleles are encoding the two public antigens of Do^a and Do^b (mean MAF of 0.40 (0.39, 0.42)) and Au^a and Au^b (MAF of 0.31 (0.29, 0.36)), followed by Yt^b (MAF 0.059 (0.052, 0.063)) with borderline frequency and by K with a clear LFA value (MAF of 0.039 (0.0338, 0.043)),. Alleles with very low frequency are encoding In^a (MAF 0.0001, (0.0007)) and Cr(a–) (MAF 0.0002 (0.0007)). In general, differences in allele frequencies can be observed for all analyzed blood group SNPs. These differences are more pronounced for some blood groups than for others.

Looking at the very rare alleles (MAF < 0.1%), a comparably high variability of (CV > 0.8) across cantons can be observed which may be linked to the fact, that for these even a small changes of MAF has a higher impact on overall frequency. All of the following predicted antigens, Jo(a–), McC^b, Wr^a, Hy–, Vel–, Tc^c, Di^a, are observed in some but not all cantons (table 3). For Vel+/Vel– we saw a correlation of $R^2 < 0.7$ between sample size and MAF, which explains the observed frequency differences across the cantons by differences in sample sizes. To some extent, this is also true for KEL11/17 with a Spearman correlation coefficient of 0.6. The highest variability across the more frequent rare blood group antigens (MAF > 1%) is seen for Kp^a and Kn^b followed by Lu^a.

Analysis of Inter-Cantonal Blood Group Variability

The PCoA analyses, both on the relative frequencies of the blood group antigens (fig. 2) and the $F_{ST,}$ were performed to visualize frequency differences in the different cantons to facilitate their investigation (supplementary tables 4 and 5; available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=490714*). The F_{ST} is used to determine population differences because of genetic differentiation and is usually applied to SNP data. Figure 2 shows the four outlying cantons TI (headed in Lugano), GE (Geneva), VS (Sion), and AG (Aarau) (for abbreviations of canton names, their areas covered and location of headquarters see table 2). It was performed on 13 blood group antigens with mean MAF of 0.1% across the cohorts, e.g. Lu^a, Lu14 and Au^b, of the Lutheran system, K, Kp^a, Js^a and Kel17 of the Kell system, Kn^b, McC^b and Vil+ of the Knops system as well as on Yt^b, Co^b and Do^a (Do^a and Do^b antigens have 'public' allele frequency). Figure 3 shows the values of the F-Statistics in a heatmap for the blood groups with rare antigen frequencies of >0.1%. Overall F_{ST} were very low, ranging from 0.79×10^{-5} for the comparison between BL and BS (Basel) and AG (Aarau) to 80.8×10^{-5} for the comparison between VS (Sion) and TI (Lugano), showing that genetic population structures, as was to be expected, are very similar between the cantons. ZH (Zurich), NE (Neuchâtel), and VD (Lausanne) clustered together with NE at the

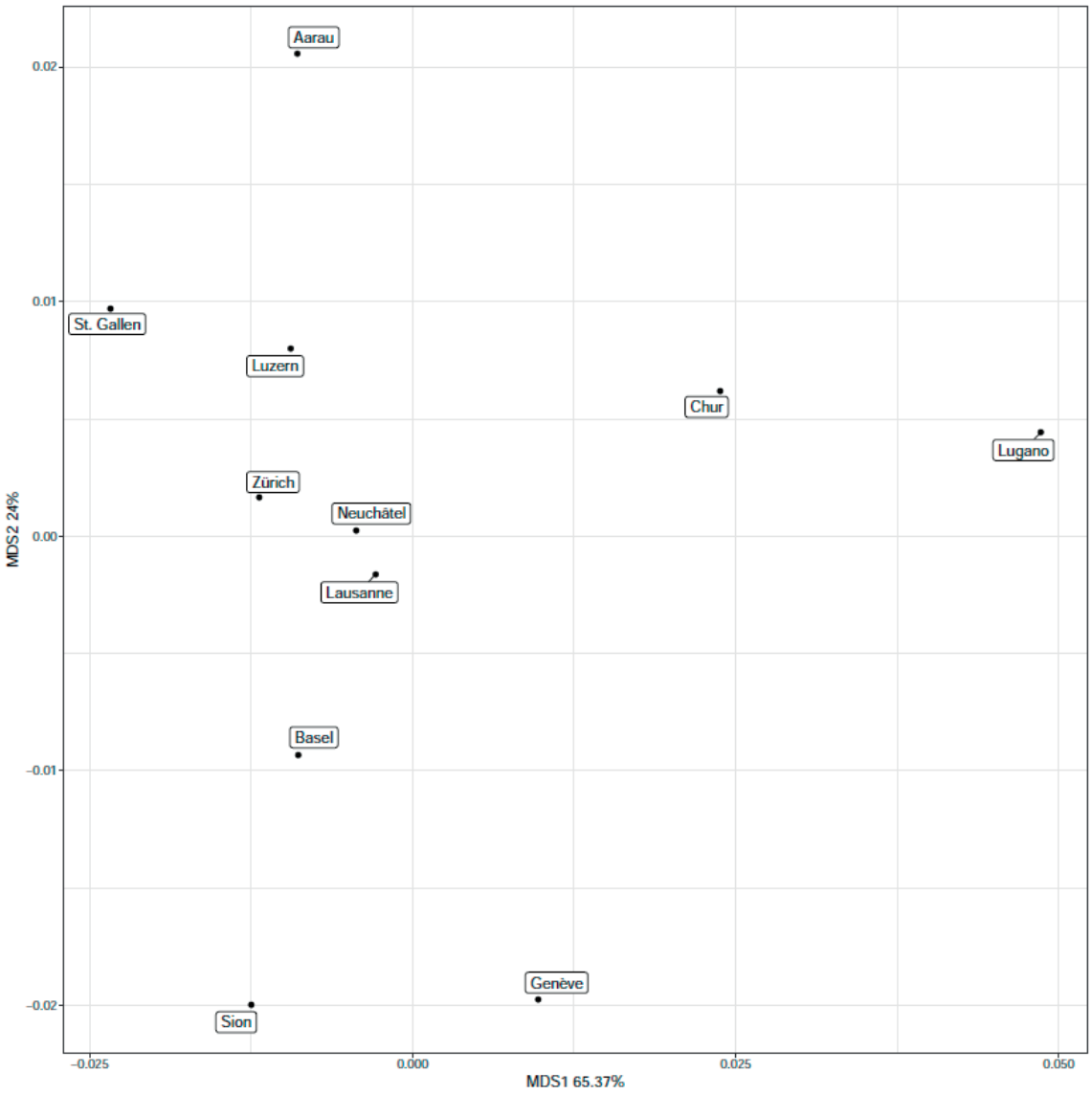

Fig. 2. PcoA analysis of the MAF using only blood group antigens with a minimal allele frequency of 0.1% or higher, across all head quarter locations of the participating BTS. Genetic blood group profiles of samples collected from BTSs headquartered in Sion, Lugano, Aarau, Geneva, and Chur cluster far away from the other Swiss regions investigated. Except for Aarau, this overlaps well with local languages spoken and geographical profiles of the other headquarters representative of the respective cantons (table 2). All but Sion, Geneva and Lugano are located in a region with a German language profile (beside some areas of Graubünden, with its capital Chur, and its inhabitants, still cultivating Raetho-Romanic language). Additionally, Lugano, Chur and Sion are clearly separated from the other areas investigated by the Swiss Alps , reaching maximal altitudes of up to 4,634 m above sea level in Switzerland (fig. 1).

center, with mean F_{ST} of 4.8×10^{-5} and 10.7×10^{-5}. On the outposts are Lugano with a mean F_{ST} of 58.42×10^{-5}, Sion, Aarau, Chur and Geneva.

The differences between the cantons based on the antigen frequencies (here always the rarer of the alleles is analyzed) were mainly driven by Au^b (Geneva and Lugano), Kn^b, Vil+ (Geneva), and McC^b (fig. 3). After sample size correction using pairwise Fisher Exact Test, Au^b remains significantly associated with differences between the cantons (see supplementary table 6; available at *http://content.karger.com/ProdukteDB/produkte.asp?doi=490714*). For the Zurich samples, we had access to the ZIP codes of all donors. With this, we were able to classify them into probands living in extremely elevated (>1,500 m) and extremely low (<300 m) regions of the greater cantonal area of Zurich. For these samples, we tested for differences in antigen frequencies according to the altitude above sea level of the region of living with no significant results (data not shown). Of note, out of the total 24,058 samples analyzed, only 40 belonged to the 'extreme height' set.

Discussion

This country-wide search for rare blood group antigens was conducted to increase the pool of the Swiss rare blood donors and thus to improve the supply of rare blood units. In total 326 Swiss blood donors with rare or extremely rare blood group antigen constellations were identified. Thanks to this pool of rare blood donors, it will be possible to provide rare donor blood on demand, without the need for frozen stocks of respective erythrocytes. Once, the expected recognition of molecular specificities had been validated, the 'mission' could be accomplished without the need of commercially available typing sera, e.g. diagnostic antibodies, some of them directed against Au^b, Di^a, Co^b, Do^a, Js^a and SC2 notoriously being unobtainable [11, 12].

Molecular blood group typing also allowed for correct identification of heterozygotes for all antigens investigated, thereby delivering exact frequency data. Frequencies of certain antigens differed pronouncedly among different regions of Switzerland. Based on

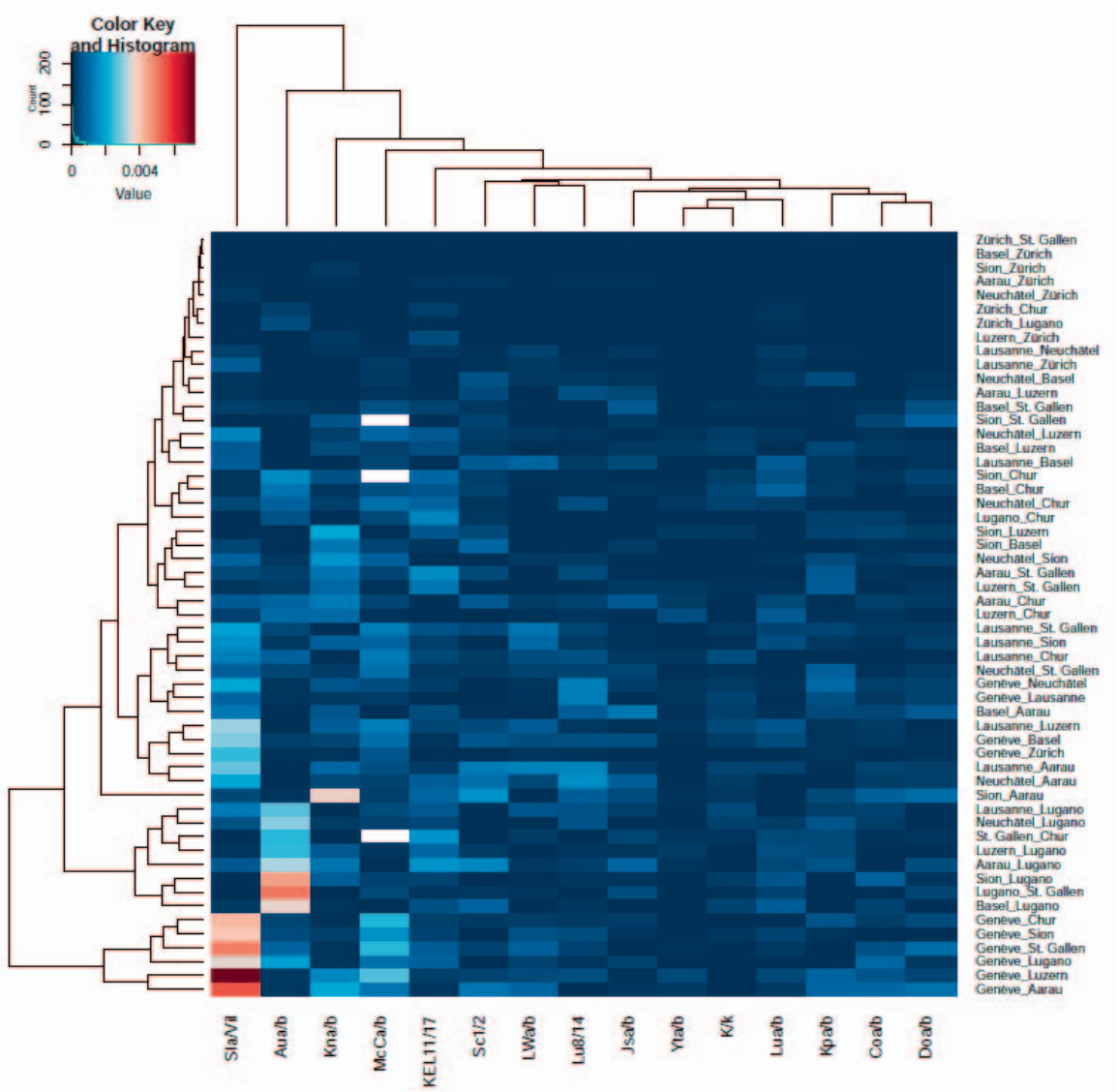

Fig. 3. Heatmap of the pairwise F-Statistics F_{ST} across all cantons. Larger values indicate a higher degree of genetic variation within the comparison. The row dendrogram shows two main clusters with comparisons for Geneva and Lugano and a third broader cluster comprised of comparisons made to Geneva, Aarau, Chur, and Sion. The main differences are driven by $Au^{a/b}$ antigens, member of the Lutheran, and the Vil+/–, $Kn^{a/b}$ and $McC^{a/b}$ antigens of the Knops blood group system.

these results, targeted searches for certain rare phenotypes focused on regions with expected higher occurrence of the respective rare antigens are possible in the future. For instance, individuals negative for Vel were to be expected once among 2,025 inhabitants covered by BTS of Aarau, in comparison to one Vel– among 146,689 inhabitants, expected by Hardy-Weinberg proportions, of the Italian part of Switzerland and represented by the BTS headed in Lugano [24]. Of course, it is expected, that the same local antigen frequencies will be observed in the local patients. Thereby, such frequencies have important clinical implications, on one hand with respect to the expected occurrence of the respective antigens among patients and on the other hand, and as an indirect result, with respect to the prevalence of respective antibodies directed against them.

Previous genetic studies on the European population have revealed a substructure within Switzerland [32, 33]. Using a genome-wide set of common markers, the existing 'language clusters' (fig. 4) were re-identified within Switzerland. These studies showed (again) that genetic distance varies with geographic distance and that language is an important barrier for reproduction. Geographically, TI (Lugano), and VS (Sion) are located south, whereas GR (Chur) lies within the massive mountain wall of the Swiss Alps (fig. 1). This 'wall' reaches maximal altitudes of up to 4634 m (peak Dufourspitze) above sea level. First inspections with respect to *VEL*-01* allele prevalence, seemed to show that it is much rarer south than in the north of the Alps. However, the greater cantonal area of ZH (Zurich) north of the Alps proved this hypothesis statistically wrong, or alternatively, and purely speculative, suggested this region having predominantly been populated from the southern part of the Alps. Additionally, Switzerland also has a very distinct language profile with four spoken languages, French, German, Italian and Rhaeto-Romanic, also known as 'Romansch' (fig. 4) [34]. Of the analyzed cantons, GE, VD, NE, and some parts of JU and VS belong to the French, TI and some parts of GR to the Italian, and some parts mainly located in GR and partially in TI to the Rhaeto-Romanic speaking regions, whereas all other cantons are in regions

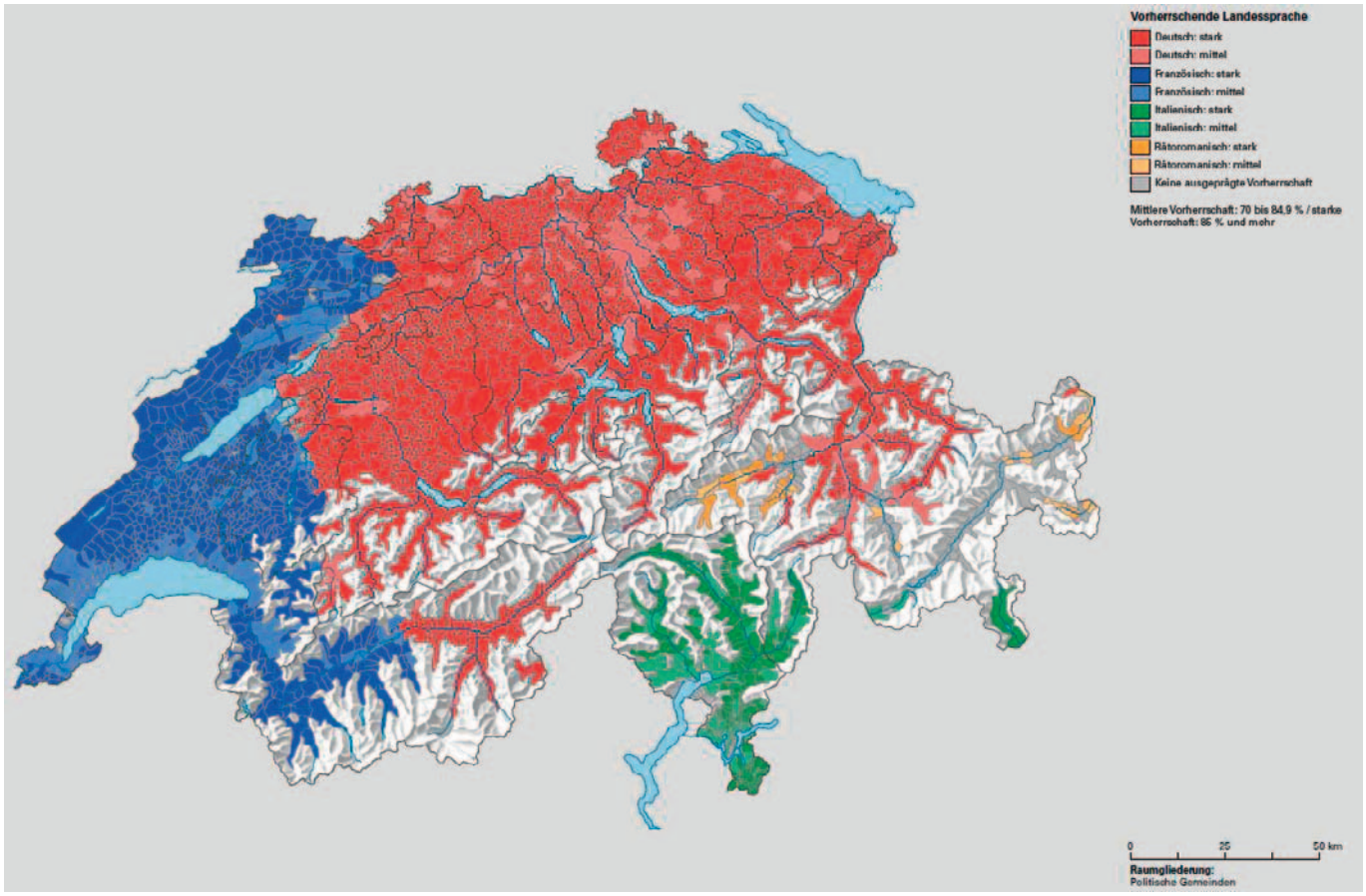

Fig. 4. Languages of Switzerland [34].

where the generally spoken language is German. This language profile may also be reflected in the ethnic and genetic background of the local blood donors investigated. The cantons BS and BL with their capitol of Basel, for instance, are both located close to France but have a German language profile and cluster between the cantons with German and French language profiles. GR with its capitol Chur and TI with its capitol Lugano both share Italian and Rhaeto-Romanic influences and cluster closest together.

However, frequency data need to be interpreted with caution. The rarer certain alleles are observed and the smaller respective donor panels are, the less reliable the frequency estimates might be calculated. For instance, the only Vel– allele carrier identified in the Italian-speaking part of Switzerland also typed phenotypically Vel– and was homozygous for *VEL*-01/ VEL*-01*. Thereby this individual represented a highly significant statistical outlier, without any further *VEL*01/VEL*-01* heterozygotes among 383 other individuals investigated and donating blood in the area of Lugano. There is a chance that this individual is a retired Swiss citizen and 'refugee' from the cold winter climate of Aarau. Similarly, the only Di(a+b–) individual identified within all 37,235 donors investigated, turned out to be an immigrant from Peru.

Heterozygous SNP carriers may also represent an important resource for further scientific analysis of blood group antigen genetics. In heterozygotes, each mutation affecting the allelic expression would become directly visible on the phenotypic level. Previously, the underlying principle had been used to identify K_0 alleles among apparently *KEL*01/KEL*02* heterozygotes, but with a discrepant K+k– phenotype [6]. Accordingly, among the total of 2,518 apparently *LU*A/LU*B* heterozygotes identified in the course of this study, 500 were reinvestigated by serology. All showed a congruent Lu(a+b+) phenotype, suggesting a low frequency of ill expressed Lutheran alleles within Switzerland (data not shown, manuscript in preparation). In addition, the data set allowed for new observations with respect to allelic Lutheran haplotypes, For example, among the samples investigated, six *LU*A* homozygous samples were identified which also were proven to be homozygous for *LU*19*. Sequence analysis proved the existence of this new *LU* allele, now being recognized by the ISBT as *LU*01.19* [4, 16].

The present study is an example for the magnitude of information delivered by applying high-throughput blood group genotyping. Gathered data provided new scientific insights into blood group genetics, completed allele frequency data for practical use, and delivered newly identified blood donors with rare and very rare antigen constellations, now available for the provision of rare donor blood.

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Author Contributions

N.T., K.N., Y.M., C.P., and S.S., performed experiments.

C.G., S.M., C.V., F.D., M.K., C.E, A.K. and J.G., performed experiments and analyzed data.

S. AelD., S.WA., C.T., JD.T., SM.M., A.S., JD.B., M.S., L.I., A. B., J.S., B.W., D.C., MC.B, J.T., S.H. and T.S., contributed essential material and collected data.

C.G., S.M., C.V., BM.F, F.D., M.K., A.F. discussed the results and commented on the manuscript.

C.G., S.M., C.V. and BM.F. designed the study.

C.G. and S.M. supervised the study.

C.G., F.D. and BM.F. wrote the manuscript.

C.G. and F.D made the tables and figures.

All authors revised and edited the manuscript.

Disclosure Statement

Christoph Gassner is an employee of the Blood Transfusion Service Zurich, SRC, and acts as a consultant for inno-train GmbH, Kronberg i.T., Germany. Caren Vollmert is employed at Agena Bioscience GmbH, Hamburg, Germany. All other authors do not disclose any competing interests.

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