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# Towards a Regional Registry of Extended Typed Blood Donors: Molecular Typing for Blood Group, Platelet and Granulocyte Antigens

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**Keywords**
Molecular blood typing · Genotyping · Polymerase chain reaction · High- and low-prevalence antigens · Granulocytes · Platelets · Blood groups

**Summary**
**Background:** The provision of compatible blood products to patients is the most essential task of transfusion medicine. Besides ABO and Rh, a number of additional blood group antigens often have to be considered for the blood supply of immunized or chronically transfused patients. It also applies for platelet antigens (HPA) and neutrophil antigens (HNA) for patients receiving platelet or granulocyte concentrates. Here, we describe the molecular screening for a number of blood group, HPA, and HNA alleles. Based on the screening results we are building up a regional blood donor registry to provide extended matched blood products on demand. **Methods:** We developed and validated TaqMan™ PCR and PCR-SSP methods for genetic markers defining 37 clinically relevant blood group antigens (beyond ABO and Rh), 10 HPA, and 11 HNA. Furthermore, we describe a feasible method for fast molecular screening of the HNA-2$^{null}$ phenotype. All data were statistically evaluated regarding genotype distribution. Allele frequencies were compared to ExAC data from non-Finnish Europeans. **Results:** Up to now more than 2,000 non-selected regular blood donors in south-west Germany have been screened for blood group, HPA, and HNA alleles. The screening results were confirmed by serology and PCR-SSP methods for selected numbers of samples. The allele frequencies were similar to non-finnish Europeans in the ExAC database except for the alleles encoding the S, HPA-3b and HNA-4b antigens, with significantly lower prevalence in our cohort, as well as the LU14 and the HNA-3b antigens, with a higher frequency compared to the ExAC data. We identified 71 donors with rare blood groups such as Lu(a+b–), Kp(a+b–), Fy(a–b–) and Vel–, and 169 donors with less prevalent HPA or HNA types. **Conclusion:** Molecular screening for blood group alleles by using TaqMan™ PCR is an effective and reliable high-throughput method for establishing a rare donor registry.



## Introduction

The provision of optimally matched blood products with regard to antigen compatibility is an ongoing challenge in transfusion medicine. Patients who repeatedly need red blood cell concentrates (RBCs) due to e.g. hemoglobinopathy or other diseases and also patients who have developed red cell antibodies often have to be transfused with RBCs compatible not only for ABO and Rh but also for several additional blood group antigens [1]. Blood recipients who are negative for high-prevalence antigens such as Vel are at risk for immunization and subsequent hemolytic transfusion reactions when transfused with antigen-positive blood. On the other hand, blood donors positive for low-prevalence antigens can cause

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antibody formation in antigen-negative patients [2]. Antibodies to low-prevalence antigens are of risk in pregnancy as they can cause a hemolytic disease of the fetus and the newborn (HDFN) and are often not detected in prenatal antibody screening. It also applies for the immunization to human platelet alloantigens (HPA) causing fetal neonatal alloimmune thrombocytopenia (FNAIT) or neutrophil antigens (HNA) causing neonatal immune neutropenia (NIN) [3, 4]. Furthermore, transfusion-associated acute lung injury (TRALI) can be caused by antibodies to HNA antigens.

With the advent of high-throughput screening in molecular blood typing, there is increasing information regarding the prevalence of rare blood groups and other antigens. Several methods have been developed and used for screening larger numbers of blood donors for common and rare antigens [5–13]. These studies add further information regarding the frequency of blood group alleles and regional differences in the availability of donors with rare blood groups. These approaches have led to the establishment of regional or national rare donor registries [14–17]. As national legal and logistical considerations can cause problems and critical delay for import and export of rare blood, it might be reasonable to establish such registries on a local level as well. Our institute is located in the Rhein-Neckar metropolitan region in south-west Germany, an area which is home to a population with different ethnic background [18, 19]. This in turn may lead to a higher incidence of diseases such as thalassemia, which require frequent blood transfusions [20]. In addition, those ethnic differences might well translate into a higher incidence of rare blood groups as well [21].

To improve our ability to provide rare blood RBCs on a regional level, it is important to continue the screening activities and to test significant part of our donor population for alleles coding for rare blood groups. We also focused on the genotypes for the most relevant HPAs and HNAs in order to improve the availability of donors for antigen-selected platelet and granulocyte concentrates and for diagnostic purposes.

## Material and Methods

*DNA Samples of Blood Donors*

This study was performed in a urban blood donor cohort from the southwestern part of Germany. Donors gave written consent to provide additional 4.5 ml EDTA blood samples, and the use of the blood samples for research purposes was approved by the ethics committee II of Heidelberg University, Medical Faculty Mannheim. DNA was isolated in our laboratory from pseudonymized EDTA-anticoagulated blood samples using a commercial system for 96 well plates (Invisorb Blood Mini HTS 96 Kit; Stratec Biomedical AG, Berlin, Germany).

*Genotyping*

We developed TaqMan™ PCR methods and PCR with sequence-specific primers (PCR-SSP) for 21 blood group-defining SNPs, 5 SNPs defining HPAs, 3 SNPs defining the HNA-3a/b, HNA-4a/b and HNA-5a/b and the *CD177* haplotype causing the HNA-2$^{null}$ phenotype (table 1). The *FCGR3B*01*, **02,* and **03* alleles encoding the HNA-1a/b/c/d antigens were screened by PCR-SSP using a previously published protocol [22] and slightly modified primers (table 1). For each genetic marker, we established PCR-SSP methods according to a standard protocol [23] and with primers listed in table 1. The *A4GALT* SNP rs2143918 has a 100% correlation with the $P_1/P_2$ phenotype in all populations, except for rare individuals of African descent [24]. This SNP was addressed for P genotyping.

For screening by TaqMan™ PCR we used commercial assays (Life Technologies, Darmstadt, Germany), except for the alleles encoding the M/N, VEL, and HNA-2 antigens. All TaqMan™ PCR analyses were performed by endpoint fluorescence detection on a ABI 7000 real-time PCR cycler using the standard program for genotyping (Applied Biosystems, Darmstadt, Germany). The method for VEL screening was described before [12]. For the M/N antigens we developed primers specific for *GYPA* and probes specific for the corresponding *GYPA*01* and *GYPA*02* alleles (table 1).

Homozygosity for a nonsense mutation c.787A>T in *CD177* was identified as the most frequent cause of the HNA-2$^{null}$ phenotype [25]. But genotyping is hampered by the *CD177P1* pseudogene with identical DNA sequence in the corresponding region. The 787A>T mutation can also occur in the *CD177P1* gene but does not affect the HNA-2 phenotype. In order to differentiate between the *CD177*c.787A>T* and the *CD177P1*787A>T* genotype, a long-range PCR for the specific amplification of the *CD177* gene followed by PCR-SSP was described [26]. However, this method is not appropriate for screening of samples in larger scales. We developed a TaqMan™ PCR method with primers and probes listed in table 1 by which individuals homozygous for the 787A>T mutation in both genes *CD177* and *CD177P1* can be identified.

*Validation*

The results from TaqMan™ PCR screening were verified by PCR-SSP for significant numbers of samples representing the different genotypes and for all samples with rare genotypes. Donors homozygous for the *CD177*c.787A>T* mutation were re-analyzed using the long-range and PCR-SSP method described recently [26] with slightly modified primers for the PCR-SSP (table 1). As far as available genotyping data were confirmed by serology for some of the antigens such as MNS, P, K/k, Au$^{a/b}$, Vel, HPA-1, and HPA-5. In all validation samples the serologically determined phenotype matched the genotype. In addition, 8 donors homozygous for the *CD177*c.787A>T* mutation identified by the TaqMan™-PCR method were phenotyped for the HNA-2 antigen (flow cytometry using the monoclonal anti-CD177 MEM-166) and the HNA-2$^{null}$ phenotype was confirmed in all samples.

*Statistics*

The typing data for each genetic marker was reviewed with regard to deviation from the Hardy-Weinberg equilibrium. The minor allele frequencies (MAF) in our donor cohort were compared to the allele frequencies of non-Finnish Europeans in the ExAC database [27]. Significance of differences was calculated by using appropriate tests in the SPSS software package (SPSS Vers. 12.0; IBM, Armonk, NY, USA).

## Results

Blood donors of our institute have been enrolled in the genotyping study since June 2016 irrespective of age, gender or ABO and Rh blood group (table 2). Using TaqMan™ PCR, the donors were screened for alleles defining 37 blood group antigens, 10 HPAs, and 11 HNAs including the HNA-2$^{null}$ phenotype. In total, 2,084 donors were typed for all antigens (table 3). Some antigens have already been typed in higher numbers of donors.

The most frequent cause of the HNA-2$^{null}$ phenotype is the *CD177* haplotype carrying the nonsense mutation *c.787A>T* (linked to additional 4 SNPs) in exon 7 [25]. However, the *CD177P1* pseudogene with almost identical DNA sequence in the exon 4 to 9 region can also harbor this haplotype making *CD177*-specific genotyping difficult. Pre-amplification of the *CD177* gene

**Table 1.** Primers for PCR-SSP and TaqMan™-PCR assays

| Antigen system | dbSNP No. | Primer sequences for PCR-SSP (5'-3') | Specificity | TaqMan™ PCR assay* |
|---|---|---|---|---|
| MNS (MN) | rs7687256,<br>rs7658293 | rev: GGCAAGAATTCCTCCATAGTAG<br>for: CATATCAGCATTAAGTACCACTGGT<br>for: CATATCAGCATTAAGTACCACTGAG | GYPA<br>c.71G, c.72T<br>c.71A, c.72G | (self-designed assay[1]) |
| MNS (Ss) | rs7683365 | for: caaaatatgattaagaaaaggaaacccg<br>rev: cgatggacaagttgtccca<br>rev: cgatggacaagttgtcccg | GYPB<br>c.143T<br>c.143C | C__34183121_10 |
| P1PK (P1/P2) | rs2143918 | rev: CACAAAGAACCTGGCTTCTCG<br>for: CACATCTTTCCTGGGAAGGAATT<br>for: CACATCTTTCCTGGGAAGGAATG | A4GALT<br>+2857T<br>+2857G | C__16072990_20 |
| LU (Lu$^{a/b}$) | rs28399653 | for: CCAGGGAGACCCATAACAAG<br>rev: TCTCAGCCGAGGCTAGGT<br>rev: TCTCAGCCGAGGCTAGGC | BCAM<br>c.230A<br>c.230G | C__25764173_10 |
| LU (LU8/14) | rs28399656 | rev: GAGGTCAAAGGCCAGCACAG<br>for: CTCTCCCAGAGGGCTACAT<br>for: GATCTCTCCCAGAGGGCTACAA | BCAM<br>c.611T<br>c.611A | C__32363989_10 |
| LU (Au$^{a/b}$) | rs1135062 | rev: ggttaagctatggtggtcattgc<br>for: ccatgtcttccacttcggca<br>for: ccatgtcttccacttcggcg | BCAM<br>c.1615A<br>c.1615G | C___1846428_10 |
| KEL (K/k) | rs8176058 | for: GGGAGATGGAGATGGAAATGG<br>rev: GACTCATCAGAAGTCTCAGCG<br>rev: GACTCATCAGAAGTCTCAGCA | KEL<br>c.578C<br>c.578T | C_____1719_20 |
| KEL (Kp$^{a/b}$) | rs8176059 | rev: AGGGCACTAGGAGGAAGAAG<br>for: CTTGTCAATCTCCATCACTTCAC<br>for: CTTGTCAATCTCCATCACTTCAT | KEL<br>c.841C<br>c.841T | C__25596888_20 |
| KEL (Js$^{a/b}$) | rs8176038 | rev: ggcccttgacacttgcatac<br>for: tgcctgggggctgcct<br>for: tgcctgggggctgccc | KEL<br>c.1790T<br>c.1790C | C__25596899_20 |
| KEL (11/17) | rs61729034 | rev: CCTTAGAGGAGGGACACAAAG<br>for: GGCAAGCTCTTCCAGATGGT<br>for: GGCAAGCTCTTCCAGATGGC | KEL<br>c.905T<br>c.905C | C__27862879_10 |
| FY (Fy$^{a/b}$) | rs12075 | rev: GGCACCACAATGCTGAAGAG<br>for: CTTCCCAGATGGAGACTATGG<br>for: CTTCCCAGATGGAGACTATGA | DARC<br>c.125G<br>c.125A | C___2493442_10 |
| FY (Fy$^{0}$) | rs2814778 | rev: CAAAGGGAGGGACACAAGAG<br>for: CCTCATTAGTCCTTGGCTCTTAT<br>for: CCTCATTAGTCCTTGGCTCTTAC | DARC<br>-67T<br>-67C | C__15769614_10 |
| JK (Jk$^{a/b}$) | rs1058396 | rev: AGACAGCAAGTGGGCTCAAG<br>for: TCTTTCAGCCCCATTTGAGG<br>for: GTCTTTCAGCCCCATTTGAGA | SLC14A1<br>c.838G<br>c.838A | C___1727582_10 |
| DI (Di$^{a/b}$) | rs2285644 | rev: TCCTGCCTGCCCTAGTTCTG<br>for: GGGTGGTGAAGTCCACGCT<br>for: GGTGGTGAAGTCCACGCC | SLC4A1<br>c.2561T<br>c.2561C | C__26654865_10 |
| DI (Wr$^{a/b}$) | rs75731670 | rev: TGGGAGAATGCCAGGGAAAG<br>for: CACTGGGCTTGCGTTCCA<br>for: CACTGGGCTTGCGTTCCG | SLC4A1<br>c.1972A<br>c.1972G | C___2259533_10 |
| YT (Yt$^{a/b}$) | rs1799805 | rev: GGGAGGACTTCTGGGACTTC<br>for: CATCAACGCGGGAGACTTCC<br>for: CATCAACGCGGGAGACTTCA | ACHE<br>c.1057C<br>c.1057A | C___8786419_20 |
| SC (SC1/2) | rs56025238 | rev: CCCTTATATTCCGGCATCAGATC<br>for: CTCTCTCCCTCTGGCCCG<br>for: CTCTCTCCCTCTGGCCCA | ERMAP<br>c.169G<br>c.169A | C__32292873_10 |

*Table 1 continued on next page*

**Table 1.** Continued

| Antigen system | dbSNP No. | Primer sequences for PCR-SSP (5'-3') | Specificity | TaqMan™ PCR assay* |
|---|---|---|---|---|
| DO (Do$^{a/b}$) | rs11276 | for: CTCCACATCCCTCCTGAAAG<br>rev: GTTGACCTCAACTGCAACCAGTT<br>rev: GTTGACCTCAACTGCAACCAGTC | ART4<br>c.793A<br>c.793G | C___2687344_20 |
| CO (Co$^{a/b}$) | rs28362692 | rev: GCACGGAAGATGCTGATCTGG<br>for: GGGGAACAACCAGACGGC<br>for: GGGGAACAACCAGACGGT | AQP1<br>c.134C<br>c.134T | C__27536484_20 |
| LAN (+/–) | rs149202834 | rev: AGGTGTACCTGGCTCCTTTC<br>for: TTTAGCCTGTGGGTGCTGC<br>for: TTTAGCCTGTGGGTGCTGT | ABCB6<br>c.574C<br>c.574T | C_168788417_10 |
| VEL (+/–) | rs566629828 | rev: CCAAAGGCTGCGGTTTGCTG<br>for: GCAGCAGGGACGGAGTCA<br>for: GCAGCAGGGACGGAGTCC | SMIM1<br>c.64-80ins<br>c.64-80del | see [7] |
| HPA-1a/b | rs5918 | rev: gtgcaatcctctggggact<br>for: gacttacaggccctgcctct<br>for: acttacaggccctgcctcc | ITGB3<br>ITGB3*176T<br>ITGB3*176C | C____818008_30 |
| HPA-2a/b | rs6065 | rev: gccagcgacgaaaatagagg<br>for: cccccagggctcctgac<br>for: cccccagggctcctgat | GP1BA<br>GP1BA*482C<br>GP1BA*482T | C__11442703_10 |
| HPA-3a/b | rs5911 | rev: accagagagcctgctcactac<br>for: ggactgggggctgcccat<br>for: ggactgggggctgcccag | ITGA2B<br>ITGA2B*2621T<br>ITGA2B*2621G | C___3017440_10 |
| HPA-5a/b | rs1801106 | rev: ggcagtacactatacattcaactc<br>for: aaggaagagtctacctgtttactatcaaag<br>for: aaggaagagtctacctgtttactatcaaaa | ITGA2<br>ITGA2*1600G<br>ITGA2*1600A | C__27862812_10 |
| HPA-15a/b | rs10455097 | for: cagtattatgaccttatgatgacctattc<br>rev: gttacttcaaattcttggtaaatcctgg<br>rev: gttacttcaaattcttggtaaatcctgt | CD109<br>CD109*2108C<br>CD109*2108A | C___3226894_10 |
| HNA-1a | rs448740<br>rs368410676 | for: cacagtggtttcacaatgagaa<br>rev: atggacttctagctgcacc | FCGR3B*01 | none |
| HNA-1b/d | rs527909462<br>rs5030738 | for: gcctcaatggtacagcgtgctt<br>rev: ctgtcgttgactgtggcag | FCGR3B*02 | none |
| HNA-1b/c | rs527909462<br>rs5030738 | for: gcctcaatggtacagcgtgctt<br>rev: ctgtcgttgactgtggcaT | FCGR3B*03 | none |
| HNA-2 (+/–) | rs1164364335 | for: catggagaaggtgacctttgag<br>rev: CAACAGTGCTGCAGCCTTT<br>rev: CAACAGCGCTGCAGCTTTA | CD177<br>CD177*787A<br>CD177*787T | (self-designed assay[2]) |
| HNA-3a/b | rs2288904 | rev: ctgcatggagcagaggatgg<br>for: GAGTGGCTGAGGTGCTTCG<br>for: GGAGTGGCTGAGGTGCTTCA | SLC44A2<br>SLC44A2*461G<br>SLC44A2*461A | C__11789692_10 |
| HNA-4a/b | rs1143679 | rev: aaggaggtctgacggtgaag<br>for: ctcatgcgagcccatccg<br>for: ctcatgcgagcccatcca | ITGAM<br>ITGAM*230G<br>ITGAM*230A | C___2847895_1_ |
| HNA-5a/b | rs2230433 | rev: tccaccttgcggaaggagagtc<br>for: atcatcccccacagatccag<br>for: catcccccacagatccac | ITGAL<br>ITGAL*2372G<br>ITGAL*2372C | C__25754090_10 |

*TaqMan™-PCR assays were commercially available (Life Technologies) under the given assay number; [1]self-designed TaqMan™-PCR assay for MN genotyping with forward primer CTCAGTCACCTCGTTCTTAATC, reverse primer GGCAAGAATTCCTCCATAGTAG, FAM-probe CACTGGTGTGGCAA (M), VIC-probe CACTGAGGTGGCAA (N); [2]self-designed TaqMan™-PCR assay for HNA-2 genotyping with forward primer CCCTCAGGACTCACATCAAC, reverse primer CTGAGTGGATGGTGGTCTTC, FAM-probe CAGCCTTTTGTCCC (CD177 wild type), VIC-probe CAGCTTTAGGTCGC (CD177 null variant).

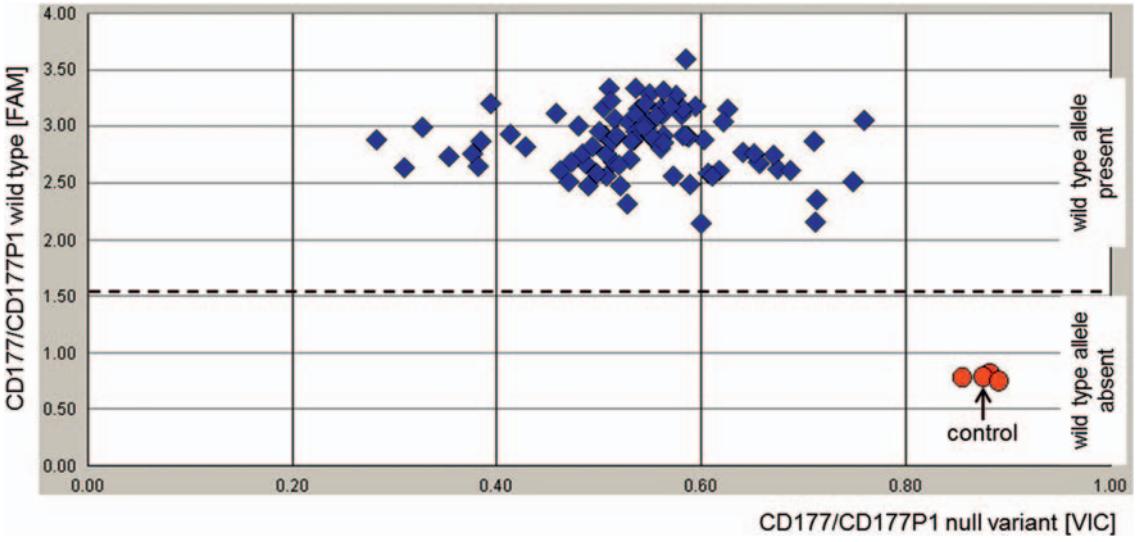


**Fig. 1.** Representative result of TaqMan™ PCR-based typing of 96 samples for the *c.787A>T* mutation in *CD177* and *CD177P1*. Primers were designed to amplify a 88 bp fragment from both genes *CD177* and *CD177P1*. The FAM-labeled probe detects the wild-type allele and the VIC-labeled probe is specific for the null haplotype including the *c.787A>T* mutation. FAM signals below the threshold (dashed line) indicate absence of the wild type allele in both genes and, therefore, indicate the HNA-2$^{null}$ phenotype. Control: donor sample with confirmed homozygosity for the *c.787A>T* mutation by *CD177* genomic sequencing and confirmed HNA-2$^{null}$ phenotype by flow cytometry. In addition to the control three donors revealed absence of the wild type allele, i.e. homozygosity for the *c.787A>T* mutation. As expected, the HNA-2$^{null}$ phenotype was confirmed for these samples.

**Table 2.** Demographic characteristics of the screened donor population

| | % of all donors |
|---|---|
| Age, years | |
| <20 | 6.7 |
| 20–29 | 37.8 |
| 30–39 | 15.9 |
| 40–49 | 17.2 |
| 50–59 | 14.5 |
| >60 | 7.9 |
| Sex | |
| Male | 60.4 |
| Female | 39.6 |
| Blood groups | |
| O pos | 31.0 |
| O neg | 11.4 |
| A pos | 30.8 |
| A neg | 8.9 |
| B pos | 9.1 |
| B neg | 2.7 |
| AB pos | 4.8 |
| AB neg | 1.3 |

using long-range PCR followed by PCR-SSP typing of the *c.787A>T* mutation was described as a genotyping method [26]. Due to the long-range PCR this method is, however, time-consuming and cost-intensive. With our fast (70 min) and easy TaqMan™ PCR method we were able to screen 3,399 donors within a short period of time and identified 84 donors (2.47%) homozygous for the null haplotype in both genes *CD177* and *CD177P1* (fig. 1). Because this screening method does not distinguish between *CD177* and *CD177P1*, individuals homozygous for the *c.787A>T* mutation in the *CD177* gene, but with one or two wild-type alleles in the *CD177P1* gene, are not identified as HNA-2$^{null}$. For all 84 donors we could confirm the *CD177*c.787T* homozygosity using the *CD177*-specific long-range PCR and PCR-SSP. For 8 of the donors we obtained fresh blood samples and confirmed the HNA-2$^{null}$ phenotype by flow cytometry (data not shown). Thus, we describe here a suitable method for fast molecular screening of the HNA-2$^{null}$ phenotype.

The prevalence of the different genotypes was within the expected range, with a few exceptions. For each measured distribution, basic probability testing was performed using a chi-square test comparing the observed distribution of genotypes to the expected distribution based on observed allele frequencies and the Hardy-Weinberg equilibrium. All but two results (Au$^{a/b}$ and Fy$^{null}$) were in line with the expected genotype frequencies. For the Au$^{a/b}$-encoding SNP we identified a trend ($p = 0.054$) for more heterozygotes (933 vs. 869) and less Au$^{b}$ homozygotes (151 vs. 183) as expected. Re-typing of 144 selected samples using PCR-SSP confirmed the result from TaqMan™ PCR. In addition, phenotyping of 11 samples for the Au$^{a/b}$ antigens by means of serology also confirmed the genotyping results. The overrepresentation of Au$^{a/b}$ donors in our cohort could be a characteristic of our donor population or is just a coincidental finding. The promoter mutation *FY*-67T>C* was screened for identification of the Fy$^{null}$ phenotype. Based on the allele frequency of 0.0139 for the mutation, we could expect one homozygote in 5,164 donors. We found 6 homozygotes in 2,084 typed donors leading to a significant deviation ($p < 0.0001$) from the Hardy-Weinberg equilibrium. Most likely, the Fy$^{null}$ donors are of African ancestry because the mutation is very common in this population.

The MAF of blood group antigens, HPAs, and HNAs in our study cohort were compared to the data of non-Finnish Europeans in the ExAC exome sequencing study [27]. The S antigen encoding *GYPB*c.143T* allele, the HPA-3b antigen encoding *ITGA2B*c.2621G* allele, and the HNA-4b antigen encoding *ITGAM*c.230A* allele was significantly less prevalent in our cohort (table 4). The *LU*c.611T*

**Table 3.** Results from genotype screening of blood donors

| Antigen system | dbSNP No. | Allele | Genotype | Phenotype[1] | Number of donors | % of donors |
|---|---|---|---|---|---|---|
| *Blood groups* | | | | | | |
| MNS | rs7687256, | *GYPA*01* | *c.71G, c.72T* | M+N– | 589 | 28.26 |
| (002) | rs7658293 | *GYPA*02* | *c.71G/A, c.72T/G* | M+N+ | 1,078 | 51.58 |
| | | | *c.71A, c.72G* | M–N+ | 420 | 20.15 |
| MNS | rs7683365 | *GYPB*03* | *c.143T* | S+s– | 212 | 9.33 |
| (002) | | *GYPB*04* | *c.143T/C* | S+s+ | 995 | 43.77 |
| | | | *c.143C* | S–s+ | 1,066 | 46.90 |
| P1PK | rs2143918[2] | *A4GALT*P1.01* | *+2857T* | $P_1$ | 555 | 26.58 |
| (003) | | *A4GALT*P2.01* | *+2857T/G* | $P_1$ | 1,017 | 48.56 |
| | | | *+2857G* | $P_2$ | 518 | 24.86 |
| LU | rs28399653 | *LU*01* | *c.230A* | Lu(a+b–) | 4 | 0.19 |
| (005) | | *LU*02* | *c.230A/G* | Lu(a+b+) | 153 | 7.34 |
| | | | *c.230G* | Lu(a–b+) | 1,927 | 92.47 |
| LU | rs28399656 | *LU*02* | *c.611T* | LU:8,–14 | 1,987 | 95.35 |
| (005) | | *LU*02.14* | *c.611T/A* | LU:8,14 | 96 | 4.61 |
| | | | *c.611A* | LU:–8,14 | 1 | 0.05 |
| LU | rs1135062 | *LU*02* | *c.1615A* | Au(a+b–) | 1,000 | 47.98 |
| (005) | | *LU*02.19* | *c.1615A/G* | Au(a+b+) | 933 | 44.77 |
| | | | *c.1615G* | Au(a–b+) | 151 | 7.25 |
| KEL | rs8176058 | *KEL*01* | *c.578C* | K+k– | 7 | 0.25 |
| (006) | | *KEL*02* | *c.578C/T* | K+k+ | 222 | 8.08 |
| | | | *c.578T* | K–k+ | 2,518 | 91.67 |
| KEL | rs8176059 | *KEL*02* | *c.841C* | Kp(a–b+c–) | 2,047 | 98.22 |
| (006) | | *KEL*02.03* | *c.841C/T* | Kp(a+b+c–) | 36 | 1.73 |
| | | | *c.841T* | Kp(a+b–c–) | 1 | 0.05 |
| KEL | rs8176038 | *KEL*02* | *c.1790T* | Js(a–b+) | 2,083 | 99.95 |
| (006) | | *KEL*02.06* | *c.1790T/C* | Js(a+b+) | 1 | 0.05 |
| | | | *c.1790C* | Js(a+b–) | 0 | 0.00 |
| KEL | rs61729034 | *KEL*02* | *c.905T* | KEL:11,–17 | 2,078 | 99.71 |
| (006) | | *KEL*02.17* | *c.905T/C* | KEL:11,17 | 6 | 0.29 |
| | | | *c.905C* | KEL:–11,17 | 0 | 0.00 |
| FY | rs12075 | *FY*01* | *c.125G* | Fy(a+b–) | 402 | 19.29 |
| (008) | | *FY*02* | *c.125G/A* | Fy(a+b+) | 998 | 47.89 |
| | | | *c.125A* | Fy(a–b+) | 684 | 32.82 |
| FY | rs2814778 | *FY*01N.01 or* | *-67T* | | 2,032 | 97.50 |
| (008) | | *FY02N.01* | *-67T/C* | | 46 | 2.21 |
| | | | *-67C* | Fy(a–b–) | 6 | 0.29 |
| JK | rs1058396 | *JK*01* | *c.838G* | Jk(a+b–) | 564 | 27.06 |
| (009) | | *JK*02* | *c.838G/A* | Jk(a+b+) | 999 | 47.94 |
| | | | *c.838A* | Jk(a–b+) | 521 | 25.00 |
| DI | rs2285644 | *DI*01* | *c.2561T* | Di(a+b–) | 0 | 0.00 |
| (010) | | *DI*02* | *c.2561T/C* | Di(a+b+) | 3 | 0.11 |
| | | | *c.2561C* | Di(a–b+) | 2,746 | 99.89 |
| DI | rs75731670 | *DI*02* | *c.1972A* | Wr(a+b–) | 0 | 0.00 |
| (010) | | *DI*02.03* | *c.1972A/G* | Wr(a+b+) | 3 | 0.14 |
| | | | *c.1972G* | Wr(a–b+) | 2,081 | 99.86 |
| YT | rs1799805 | *YT*01* | *c.1057C* | Yt(a+b–) | 2,427 | 88.64 |
| (011) | | *YT*02* | *c.1057C/A* | Yt(a+b+) | 295 | 10.77 |
| | | | *c.1057A* | Yt(a–b+) | 16 | 0.58 |
| SC | rs56025238 | *SC*01* | *c.169G* | SC:1,–2 | 2,727 | 99.27 |
| (013) | | *SC*02* | *c.169G/A* | SC:1,2 | 20 | 0.73 |
| | | | *c.169A* | SC:–1,2 | 0 | 0.00 |
| DO | rs11276 | *DO*01* | *c.793A* | Do(a+b–) | 319 | 15.31 |
| (014) | | *DO*02* | *c.793A/G* | Do(a+b+) | 1,029 | 49.38 |
| | | | *c.793G* | Do(a–b+) | 736 | 35.32 |

*Table 3 continued on next page*

**Table 3.** Continued

| Antigen system | dbSNP No. | Allele | Genotype | Phenotype[1] | Number of donors | % of donors |
|---|---|---|---|---|---|---|
| CO (015) | rs28362692 | *CO*01* | *c.134C* | Co(a+b–) | 1,931 | 92.66 |
| | | *CO*02* | *c.134C/T* | Co(a+b+) | 151 | 7.25 |
| | | | *c.134T* | Co(a–b+) | 2 | 0.10 |
| LAN (033) | rs149202834 | *ABCB6*01* | *c.574C* | Lan+ | 2,064 | 99.04 |
| | | *ABCB6*01N.13* | *c.574C/T* | Lan+ | 20 | 0.96 |
| | | | *c.574T* | Lan– | 0 | 0.00 |
| VEL (034) | rs566629828 | *VEL*01* | *c.64-80ins* | Vel+ | 2,653 | 96.54 |
| | | *VEL*-01* | *c.64-80ins/del* | Vel+ | 94 | 3.42 |
| | | | *c.64-80del* | Vel– | 1 | 0.04 |
| *Platelet antigens (HPA)* | | | | | | |
| HPA-1 | rs5918 | *ITGB3*176T* | *c.176T* | HPA–1(a+b–) | 1,764 | 72.03 |
| | | *ITGB3*176C* | *c.176T/C* | HPA–1(a+b+) | 636 | 25.97 |
| | | | *c.176C* | HPA–1(a–b+) | 49 | 2.00 |
| HPA-2 | rs6065 | *GP1BA*482C* | *c.482C* | HPA–2(a+b–) | 2,040 | 82.93 |
| | | *GP1BA*482T* | *c.482C/T* | HPA–2(a+b+) | 395 | 16.06 |
| | | | *c.482T* | HPA–2(a–b+) | 25 | 1.02 |
| HPA-3 | rs5911 | *ITGA2B*2621T* | *c.2621T* | HPA–3(a+b–) | 909 | 38.47 |
| | | *ITGA2B*2621G* | *c.2621T/G* | HPA–3(a+b+) | 1,116 | 47.23 |
| | | | *c.2621G* | HPA–3(a–b+) | 338 | 14.30 |
| HPA-5 | rs1801106 | *ITGA2*1600G* | *c.1600G* | HPA–5(a+b–) | 1,925 | 81.33 |
| | | *ITGA2*1600A* | *c.1600G/A* | HPA–5(a+b+) | 422 | 17.83 |
| | | | *c.1600A* | HPA–5(a–b+) | 20 | 0.84 |
| HPA-15 | rs10455097 | *CD109*2108C* | *c.2108C* | HPA–15(a+b–) | 598 | 24.28 |
| | | *CD109*2108A* | *c.2108C/A* | HPA–15(a+b+) | 1,227 | 49.82 |
| | | | *c.2108A* | HPA–15(a–b+) | 638 | 25.90 |
| *Neutrophil antigens (HNA)* | | | | | | |
| HNA-1 | rs448740 | *FCGR3B*01* | *c.227A* | HNA–1(a+b–c–d–) | 349 | 12.71 |
| | rs368410676 | *FCGR3B*02* | *c.227A/c.147T,266C* | HNA–1(a+b+c–d+) | 1,174 | 42.75 |
| | rs527909462 | *FCGR3B*03* | *c.147T,266C* | HNA–1(a–b+c–d+) | 1,100 | 40.06 |
| | rs5030738 | | *c.227A/c.147T,266A* | HNA–1(a+b+c+d–) | 41 | 1.49 |
| | | | *c.227A/c.147T,266C/A* | HNA–1(a+b+c+d+) | 64 | 2.33 |
| | | | *c.147T,266C/A* | HNA–1(a–b+c+d+) | 18 | 0.66 |
| HNA-2 | rs1164364335 | *CD177/CD177P1* | *c.787A, c.787A/T* | HNA–2(+/–) | 3,315 | 97.53 |
| | | | *c.787T* | HNA–2(–) | 84 | 2.47 |
| HNA-3 | rs2288904 | *SLC44A2*461G* | *c.461G* | HNA–3(a+b–) | 1,329 | 58.42 |
| | | *SLC44A2*461A* | *c.461G/A* | HNA–3(a+b+) | 819 | 36.00 |
| | | | *c.461A* | HNA–3(a–b+) | 126 | 5.58 |
| HNA-4 | rs1143679 | *ITGAM*230G* | *c.230G* | HNA–4(a+b–) | 1,797 | 79.06 |
| | | *ITGAM*230A* | *c.230G/A* | HNA–4(a+b+) | 454 | 19.97 |
| | | | *c.230A* | HNA–4(a–b+) | 22 | 0.97 |
| HNA-5 | rs2230433 | *ITGAL*2372G* | *c.2372G* | HNA–5(a+b–) | 1,151 | 50.66 |
| | | *ITGAL*2372C* | *c.2372G/C* | HNA–5(a+b+) | 934 | 41.11 |
| | | | *c.2372C* | HNA–5(a–b+) | 187 | 8.23 |

Phenotype deduced from the genotype; [2]rs2143918 has a 100 % correlation with the P1/P2 phenotype in all populations except for rare individuals of African descent [21]. It was therefore used to determine the P1/P2 genotype even though it is not the causative mutation.

allele encoding the LU14 antigen and the *SLC44A2*c.461A* allele encoding the HNA-3b antigen showed a higher frequency compared to the ExAC data.

By screening of more than 2,000 donors we found a number of rare blood types, such as Lu(a+b–), Kp(a+b–), $Fy^{null}$, Vel–, HPA-1(a–b+), and HNA-$2^{null}$ (table 5).

## Discussion

Molecular screening of the local blood donor population is an approach to enable a compatible blood supply on demand. We describe a TaqMan™ PCR-based typing of the corresponding genetic markers (mostly SNPs). This method is feasible, as it is associated

**Table 4.** Minor allele frequencies in our donor population compared to the non-Finnish European population in the ExAC database

| Antigen | dbSNP No. | Minor allele | MAF in our cohort | MAF in ExAC | Significance* |
|---|---|---|---|---|---|
| *Blood groups* | | | | | |
| S | rs7683365 | *GYPB*c.143T* | 0.3121 | 0.3802 | **<0.001** |
| Lu(a) | rs28399653 | *LU*c.230A* | 0.0390 | 0.0382 | 0.935 |
| LU14 | rs28399656 | *LU*c.611T* | 0.0234 | 0.0146 | **0.048** |
| Au(b) | rs1135062 | *LU*c.1615G* | 0.2962 | 0.2993 | 0.809 |
| K | rs8176058 | *KEL*c.578T* | 0.0430 | 0.0412 | 0.753 |
| Kp(a) | rs8176059 | *KEL*c.841T* | 0.0091 | 0.0105 | 0.634 |
| Js(a) | rs8176038 | *KEL*c.1790C* | 0.0002 | 0.0005 | 0.548 |
| KEL17 | rs61729034 | *KEL*c.905C* | 0.0014 | 0.0018 | 0.726 |
| Fy(a) | rs12075 | *FY*c.125G* | 0.4325 | 0.4244 | 0.632 |
| Jk(b) | rs1058396 | *SLC14A1*c.838A* | 0.4897 | 0.4912 | 0.950 |
| Di(a) | rs2285644 | *SLC4A1*c.2561T* | 0.0005 | 0.0004 | 0.823 |
| Wr(a) | rs75731670 | *SLC4A1*c.1972A* | 0.0007 | 0.0002 | 0.114 |
| Yt(b) | rs1799805 | *ACHE*c.1057A* | 0.0597 | 0.0502 | 0.211 |
| Sc2 | rs56025238 | *ERMAP*c.169A* | 0.0036 | 0.0050 | 0.628 |
| Do(a) | rs11276 | *ART4*c.793A* | 0.4000 | 0.3849 | 0.348 |
| Co(b) | rs28362692 | *AQP1*c.134T* | 0.0372 | 0.0398 | 0.623 |
| LAN- | rs149202834 | *ABCB6*c.574T* | 0.0048 | 0.0030 | 0.454 |
| *Platelet antigens (HPA)* | | | | | |
| HPA-1b | rs5918 | *ITGB3*c.176C* | 0.1499 | 0.1553 | 0.629 |
| HPA-2b | rs6065 | *GP1BA*c.482T* | 0.0904 | 0.0865 | 0.697 |
| HPA-3b | rs5911 | *ITGA2B*c.2621G* | 0.3792 | 0.4443 | **<0.001** |
| HPA-5b | rs1801106 | *ITGA2*c.1600A* | 0.0976 | 0.0918 | 0.553 |
| HPA-15a | rs10455097 | *CD109*c.2108C* | 0.4919 | 0.4975 | 0,752 |
| *Neutrophil antigens (HNA)* | | | | | |
| HNA-3b | rs2288904 | *SLC44A2*c.461A* | 0.2358 | 0.2094 | **0.048** |
| HNA-4b | rs1143679 | *ITGAM*c.230A* | 0.1095 | 0.1769 | **<0.001** |
| HNA-5b | rs2230433 | *ITGAL*c.2372C* | 0.2879 | 0.2856 | 0.889 |

*Bold numbers indicate significant differences ($p < 0.05$) of MAF between both populations.

**Table 5.** Rare blood types identified in the screened donor cohort

| Phenotype* | Number of donors |
|---|---|
| *Negative for HP blood groups* | |
| Lu(a+b–) | 4 |
| LU:–8,14 | 1 |
| K+k– | 7 |
| Kp(a+b–) | 1 |
| Fy(a–b–) | 6 |
| Yt(a–b+) | 16 |
| Co(a–b+) | 2 |
| Vel– | 1 |
| *Positive for LP blood groups* | |
| Js(a+) | 1 |
| KEL:17 | 6 |
| Di(a+) | 3 |
| Wr(a+) | 3 |
| SC:2 | 20 |
| *HPA / HNA* | |
| HPA–1(a–b+) | 49 |
| HPA–2(a–b+) | 25 |
| HPA–5(a–b+) | 20 |
| HNA–$2^{null}$ | 84 |
| HNA–4(a–b+) | 22 |

*Negative for high-prevalence (HP) antigens; positive for low-prevalence (LP) antigens.

with limited workload and can be easily adapted with regard to higher throughput of samples and additional alleles.

Up to now we obtained 72,337 genotypes from 33 genetic markers in more than 2,000 donors, and it is an ongoing screening program including all new donors at our institute. Based on the genotyping data we were able to deduce the phenotypes for 37 blood group antigens, 10 HPAs, and 11 HNAs including the HNA-$2^{null}$ phenotype. The complete antigen profile could be achieved for 2,084 donors. Some of the antigens are already typed in higher numbers of donors such as Vel (2,748 donors), $Yt^{a/b}$ (2,738), HPA-1 (2,449), HNA-1 (2,746), HNA-2 (3,399) and others (table 3).

In this study we also describe a feasible method for fast and easy molecular screening to identify HNA-$2^{null}$ individuals. The method is based on TaqMan™ PCR with endpoint fluorescence detection and can be easily adapted to medium or high throughput. Using the 96-well format in standard PCR cyclers more than 1,000 donors can be typed per day. CAVE: only individuals homozygous for the null mutation *c.787A>T* in both genes *CD177* and *CD177P1* are typed as HNA-$2^{null}$. If the mutation is homozygous in *CD177* but heterozygous or absent in *CD177P1*, these individuals are also HNA-$2^{null}$, but are not recognized by the TaqMan™ PCR method. Other null variants of CD177 are also not detected.

Most of the allele frequencies observed in our cohort were similar to the frequencies reported for non-Finnish Europeans in the ExAC database [27]. Only 5 of the 25 alleles with clear information in the database revealed a significant difference. This probably results from the demographic characteristics of the urban area in which our institute is located. As of 2016, the population of Mannheim includes 44.7% immigrants, of which at least 70% belong to countries with a predominantly Caucasian population [18]. About 12% have Asian ancestry and 5% are African. However, we do not know the portion of immigrants in our donor population. At least for the $Fy^{null}$ phenotype that is most prevalent in Africans and which we found in 6 of our donors (0.3%), we can assume a significant number of donors with African ancestry. Another example could be the *SLC44A2*c.461A* allele encoding the HNA-3b antigen. According to ExAC data it is more prevalent in East Asians (0.3091) than in non-Finnish Europeans (0.2094), and the MAF was 0.2358 in our cohort. Thus, the differences in allele frequencies in our donor population compared to the general European population could be attributed to a certain number of donors with African or Asian ancestry.

Immigration to Central Europe in general may increase the number of patients with sickle cell anemia or other hemoglobinopathies. The transfusion therapy of such patients is challenging because of a frequent blood demand and because of antigens with different prevalence in other populations. The concept of extended matching in transfusion therapy of such patients is appropriate to prevent alloimmunization events [28–30]. Especially, patients who were already immunized by past transfusion could benefit from extensive antigen matching beyond C/c, E/e, and K [31, 32]. As recently reported in a large cohort of transfused patients, the most immunogenic antigens were in order K, E, $C^W$, e, $Jk^a$, c, and $Fy^a$ [33]. Among the 2,084 donors typed for these antigens, we could identify 22 donors negative for K, E, $Jk^a$, c, and $Fy^a$ as well as 22 donors negative for D, K, E, $Jk^a$, and $Fy^a$. Three donors were negative for a number of clinically relevant antigens: A, B, C, D, E, K, $Jk^a$, $Fy^a$, M, and S.

Based on our experiences we attempt to establish a regional registry of extended typed blood donors to provide fresh RBC units with special blood types on demand. Similar concepts for the extended matched blood supply are established in different countries worldwide [34–37].

## Disclosure Statement

The authors declare no conflict of interest.

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