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Genotyping of 28 blood group alleles in blood donors from Mali: Prediction of rare phenotypes

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ABSTRACT

We determined the frequencies of clinically relevant blood group alleles in 300 blood donors from Mali. Multiplex test based on xMAP technology was used to investigate six blood group systems (RH, KEL, MNS, FY, JK, DO, HPA) and complementary analysis were conducted for MNS and RH systems. Polymorphisms that affect the specificity of molecular tests leading to discrepant genotype results are discussed. Antigen expressions were predicted showing that 50% of donors expressed at least one traditional low prevalence antigen, and 11.6% lacked the ability to express at least one high prevalence antigen compatible with Dob-, HPA1a-, S-s-U-, Jsb-, RH:-31 and/or RH:-34 phenotypes.

1. Introduction

The International Society of Blood Transfusion (ISBT) has designated more than 300 inherited blood group antigens on the surface of human red cells. Red blood groups are polymorphic and the distribution of antigens varies among different populations and ethnic groups. Ethnic variations in red blood cell (RBC) antigens can be a source of alloimmunization, especially in migrant populations. A 3.9% alloimmunization rate was reported in the general American population [1] but a higher rate was observed in sickle cell disease (SCD) patients treated in the USA or France (around 40%) [2,3]. This was suggested to result from various types of blood group polymorphisms. The first involves differences between donors and recipients of the common but highly immunogenic antigens C, E, Fy^a, Jk^b and S of the Rh, Duffy, Kidd and MNS systems, respectively; these antigens are more prevalent in Caucasians than in Blacks [4]. The second involves the absence of high-prevalence antigens, which may or may not be associated with the expression of low-prevalence antigens [5]. Thirdly, partial antigens (mainly for D, C, c, e and U^{var}) can be associated with a risk of alloimmunization against the missing immunogenic epitopes of the protein, when the carrier is exposed to the complete protein [2]. Knowledge of blood group antigen distribution in continental Africa and countries with African migrants would help to improve transfusion safety. Blood group antigen frequencies quoted for Africans are often based on data from Afro-Caribbean populations in the United States who have Caucasian admixture [6] that do not reflect the regional differences found throughout the continent of Africa.

Molecular basis of almost all blood group antigens has been determined to provide the alternative to predict blood group phenotype from genomic DNA with a high degree of accuracy. Typing for RBC polymorphisms at the DNA level is important in transfusion medicine [7–9]. Genotyping circumvents the limitations of hemagglutination in patients and through multiplexing enables extensive typing of donors. Many molecular techniques for blood group genotyping that have been developed are dedicated to patients or donors [10–18].

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A few genotyping investigations were conducted in continental sub Saharan Africa [19–21] but large-scale genotyping data are lacking for African populations. In order to determine the frequencies of clinically relevant blood group alleles in Mali, we investigated 300 blood donors from Bamako, located in south-west of Mali. The survey was conducted by using a multiplex genotyping kit allowing the detection of 28 alleles belonging to RH, Kell, MNS, Duffy, Kidd, Dombrock and HPA systems together with some *RHCE* polymorphisms relevant in Africa.

We attempted to achieve two main objectives: First, we aimed to evaluate the utility of multiplex tools combining the detection of antigens considered clinically relevant together with *RHCE* polymorphisms for African population in countries hosting migrant populations. Second, we aimed to raise awareness on alloimmunization against rare antigens among medical staff in Mali.

2. Materials and methods

2.1. Blood samples

Ethylenediaminetetraacetate (EDTA) blood samples were collected from 300 blood donors at the Centre National de Transfusion Sanguine, Bamako. Participants provided written informed consent. Study and consent protocols were approved by the *Comité d'éthique institutionnel de l'Institut National de Recherche en Santé Publique* in Mali.

2.2. Genomic DNA extraction and blood group genotyping

Genomic DNA was isolated from 200 µl of whole blood using the QIAmp Blood DNA Mini-kit (Qiagen, Courtaboeuf, France) according to manufacturer instructions.

Genotyping of six blood group systems and HPA platelet was performed by the reverse sequence specific oligonucleotide (PCR-RSSO) method using the Lifecodes RBC kit (Gen-Probe, Belgium) according to the manufacturer's instructions. This multiplex assay method based on Luminex xMAP technology was designed for detection of the following alleles: *RHCE*C/*c, RHCE*E/*e, KEL*01/*02/*03/*04/*06/*07/*21, GYPA*01/*02, GYPB*03/*04/*03N.01/*03N.03, FY*01/*02/*02M.01/*02N.01, JK*1/*2/*2N.06, DO*01/*02* and *HPA*1a/*1b*. Eight SNPs in *RHCE* gene are also investigated, that is, 106G > A, 122A > G, 667G > T, 697C > G, 712C > G, 733C > G, 1006C > T, 1025C > T. Briefly, the technique consisted of asymmetric PCR-amplification of 50–150 ng of DNA in a final volume of 25 µl containing 10 µl of master mix Lifecodes RBC and 0.25 µl of Taq DNA polymerase (Gen-Probe). Amplification was performed on a Biometra thermocycler (Labgene) and includes an initial denaturation step of 4 minutes at 94 °C followed by 45 cycles (30 s at 94 °C, 45 s at 51 °C, 2 minutes 30 s at 65 °C) and a terminal elongation of 5 minutes at 65 °C. PCR amplicons were hybridized to complementary allele-specific oligonucleotide probes immobilized on fluorescent-coded microsphere beads. At the same time, the biotinylated PCR products were labeled with phycoerythrinconjugated streptavidin and acquisition was performed immediately on a Luminex 100. Genotype determination and data analysis were performed automatically using the Gen-Probe RBC MatchIT!-RBC software (Lifecodes, Belgium) according to the manufacturer's instructions.

2.3. Complementary investigation

GYP(B-A) gene rearrangement encoding Dantu antigen was detected by PCR (2300 bp) using a forward (5'-cctcctttcttctcattatttttttacatg) primer specific of *GYPB* and reverse primers (5'-ataaaccctcttagagctgttcagat) specific of *GYPA*. Amplification of *RHCE* exon 6 (forw: 5'-cctgtaatcccaatattttggaaa; rev: 5' bacacccctgtagcctggt; product size 831 bp) was used at internal control. *GYP* and *RHCE* primers were used at 100 nM and 200 nM respectively. Amplification was performed on 100 ng of genomic DNA in the presence of PCR buffer, 2 mM $MgCl_2$, 40 ng/µl BSA, 0.2 mM of each dNTP, 0.05 unit of Taq DNA-polymerase (Life Technologies, Saint-Aubin, France). PCR included a 5-minute denaturation step at 94 °C followed by amplification cycles consisting of 30 s at 94 °C, 30 s at 62 °C, and 1 min at 72 °C. PCR products were analyzed on 1% agarose gel and visualized using Sight DNA stain (Euromedex, Strasbourg, France).

PCR-amplification of *GYPB* exons 4–5 was performed using a primer-pair that generates a 2194 pb product (forw: 5'-gaaattactaatggtaagactgacac; rev: 5'ctgtttctcttctgagtttaactg). PCR performed with 100 ng of genomic DNA in the presence of PCR buffer, 2 mM $MgSO_4$, 0.2 mM of each dNTP, 0.05 unit of Platinium Taq DNA-polymerase (Life Technologies, Saint-Aubin, France), 200 nM of each primer. PCR included a 2 min denaturation step at 94 °C followed by amplification cycles consisting of 30 s at 94 °C, 30 s at 57 °C, and 1 min at 68 °C. PCR product was purified and cloned in pGEMT vector (Promega, Charbonnieres, France) according to manufacturer's instructions. Clones were sequenced using the Sanger technique (GATC Biotech Konstanz, Germany).

Complementary investigation in RH system consisted of sequencing of *RHD* and *RHCE* [21], detection of *RHCE*Ce-D(4)-Ce* allele [22] and specific amplification of *(C)ce^s type 1* [23].

2.4. Comparisons and statistical analysis

Allele frequencies were compared between blood donors in Mali and literature data using Fisher's exact test (http://aoki2.si.gunma-u.ac.jp/exact/exact.html). Differences with p value ≤ 0.05 were considered as statistically significant.

3. Results

3.1. KEL, Duffy, Kidd, MNS, Dombrock and HPA-1 genotypes and alleles frequencies

A cohort of 300 blood samples from Malian donors was investigated for blood group alleles. The genotypes and alleles frequencies are reported in Table 1 and Table 2 respectively. Only two samples were genotyped as heterozygous for *KEL*01/*02* (0.7%); the others were *KEL*02/*02*. *KEL*06* allele was detected in a total of 44 samples (two of them being homozygous).

In the Duffy system, 99% of the samples were homozygous for the *FY*02N.01* allele encoding a Fy(a-b-) phenotype.