Screening for the SMIM1*64_80 del Allele in Blood Donors in a Population from Southern Brazil

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SUMMARY

Background and Objectives Serological screening for the Vel– phenotype is complex given the large individual variation in the levels of expression of the Vel antigen, and the polyclonal anti-human sera of immunised persons, when available, show heterogeneous reactivity levels. Studies of the SMIM1 gene have enabled the development of several molecular methodologies that will be crucially important for the screening of different populations, including Brazilians. To evaluate the deletion of 17 bp in the SMIM1 gene in a population from the south of Brazil, 448 unrelated blood donors from 7 regions comprising the haemotherapy network in the state of Santa Catarina were evaluated between August 2011 and March 2014.

Materials and Methods DNA samples from these donors were analysed employing a 5' nuclease real-time polymerase chain reaction (PCR) assay targeting the 17 bp deletion in the SMIM1 gene.

Results Among the 448 samples analysed, 10 (2.23%) harboured the 17 bp deletion of the gene SMIM1, and all were heterozygote for the SMIM1*64_80 del allele.

Conclusion The allelic frequency found differed from those observed in other Caucasian populations. This difference can be explained by the ethnic make-up of each Caucasian population. The data obtained are important to characterise the correct phenotype of the donor as the serological assay results are not reliable due to variations in the expression intensity of the Vel antigen in heterozygote donors for the SMIM1*64_80 del allele. Moreover, the tool used in this study is of great value for identifying a donor Vel– phenotype and supplying a possible need for transfusion.

Key words: blood groups, donors, genotyping, transfusion.

The Vel antigen was described by Sussman & Miller (1952) and is restricted to the erythroid lineage. It is a high-frequency antigen expressed in the red blood cells of most people, but it may be missing in rare cases (Sussman & Miller, 1952). The overall frequency of the Vel– phenotype in the Caucasian population is approximately 0.025%, and it has been described in other populations such as Black, Asian and Canadian Indian populations (Reid et al., 2012; Daniels, 2013).

The Vel– phenotype is one of the most difficult blood types to supply in many countries. This is partly due to the low frequency of the Vel– blood type but also to the lack of systematic screening for Vel in blood donors (Ballif et al., 2013). Few donor screenings have been carried out as the serological screening for the Vel– phenotype is complex due to the large individual variation in Vel expression levels and polyclonal anti-human sera of immunised (anti-Vel) persons available that show heterogeneous reactivity levels. While the Vel– phenotype is rare, many examples of anti-Vel developed after transfusion or pregnancy have been reported (Sussman & Miller, 1952; Van Gammern et al., 2008; Linz et al., 2010; Reid et al., 2012). Anti-Vel is well known for its aggressive haemolytic activity. Due to the clinical significance of anti-Vel and the limitations of serological tests for the Vel antigen, researches have been made to identify the genetic basis of the Vel blood type, and the results have been successful (Ballif et al., 2013; Storry et al., 2013; Haer-Wigman et al., 2015).

Recent investigations on the molecular mechanism responsible for the Vel– phenotype, that is, the deletion of 17 nucleotides in exon 3 of SMIM1 gene, which codes for the Vel blood group system, resulted in the transfer of the Vel antigen from collection 212 to a new blood group system: number 34 (Ballif et al., 2013; Cvejic et al., 2013; Storry et al., 2013; Storry, 2014; Haer-Wigman et al., 2015). Such knowledge allowed several molecular methodologies to be developed, which will be
important to screen various populations, such as Brazilians, that have large racial heterogeneity. Knowing the molecular bases of the genes responsible for coding antigens, besides being important to develop molecular biology methods, identify mutations, understand polymorphisms and find new alleles and new systems, also works as a tool to search for rare donors (Reid, 2003; Reid & Denomme, 2011).

Thus, this study aimed to genotype the deletion of 17 bp in the SMIM1 gene in samples of voluntary blood donors in the state of Santa Catarina, southern Brazil, and identify possible donors with the Vel– phenotype often misidentified by serological methods due to the weak expression of the antigen in the erythrocytes.

MATERIALS AND METHODS

A total of 448 blood donors were selected from the Center of Hematology and Hemotherapy of Santa Catarina (HEMOSC). All participants provided written informed consent before sample collection. This study was approved by the Human Research Ethics Committee of the Federal University of Santa Catarina and HEMOSC (no. 2270/2011). Next, aliquots of ethylenediaminetetraacetic acid (EDTA)-anticoagulated peripheral venous blood were collected from healthy blood donors between August 2011 and March 2014.

Genomic DNA Extraction

A total of 5 mL of peripheral blood were collected in EDTA tubes and centrifuged (2500 × g for 10 min). Buffy coat was transferred to a clean tube, and DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen, Chattlesworth, CA, USA) following the manufacturer’s protocol. DNA concentration and quality were evaluated in a NanoVue™ Plus spectrophotometer (GE Healthcare Life, Uppsala, Suécia).

Specific Real-time PCR Analysis for Vel– Negative Genotype

PCR primers were designed spanning 127 base pairs from exon 3 of the SMIM1 gene, using the software Prime 3. An internal probe to this amplicon was synthesised, and its 3’ end was designed to hybridise specifically to the nucleotide sequence existing only in individuals carrying the 17-bp deletion (GenBank NM_001163724).

The primers and probe were synthesised and packaged together as a PrimeTime qPCR assay (IDT, Skokie, Il, USA). The real-time PCR was performed in accordance to a protocol previously described by Dezan et al. (2016) as follows: 10 μL of TaqMan Universal Master Mix (Applied Biosystems), 1 μL of primers/probe solution (final concentration = 500 nM of primers and 250 nM of probe, labelled with the FAM fluorophore) in addition to 9 μL of DNA (40 ng μL⁻¹) extracted from the buffy coat, yielding a final reaction volume of 20 μL (Dinardo et al., 2014). PCR analysis was performed using the StepOne Real-time PCR System (Applied Biosystems) programmed for an incubation of 50 °C for 2 min and 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min (Fig. 1).

PCR-RFLP Vel Genotyping

Samples found positive for the 17-bp deletion were further submitted to a conventional PCR and restriction fragment length polymorphism (RFLP) analysis, aiming to verify the zygosity of the reactive blood donors. A fragment encompassing exons 3 and 4 of the SMIM1 gene was amplified from genomic DNA by PCR based on primers previously described (Ballif et al., 2013). PCR amplification was carried out in a Mastercycler personal thermal cycler (Eppendorf, Hamburg, Germany) in 50-μL final volume reactions containing 200 ng of genomic DNA, 50 pmol of each primer, 2 nmol of each dNTP and 1-0 U of Taq DNA Polimerase (Invitrogen Life Technologies®, Grand Island, NY, USA). The amplification programme consisted of an initial step at 95 °C for 5 min, 35 cycles at 95 °C for 1 min, 62 °C for 1 min and 72 °C for 1 min and a final extension step at 72 °C for 7 min. RFLP analysis was carried out with 2 μL from the PCR reaction amplicon in a 10-μL reaction containing 3 U of Sty I restriction endonuclease (New England Biolabs Beverly, MA, USA) in 1 NE Buffer 3 at 37 °C for 12 h, and revealed by 3-0% agarose gel electrophoresis in 1 TBE buffer.

The PCR fragment products observed in agarose gel electrophoresis were 1200, 800 and 400 bp corresponding, respectively, to heterozygote of the SMIM1*64_80 del allele (Wild type/SMIM1*64_80 del genotype), 1200 bp for homozygote of the SMIM1*64_80 del allele (SMIM1*64_80 del/ SMIM1*64_80 del genotype) and 800 and 400 bp for homozygote of the Wild type allele (Wild type/Wild type genotype). PCR fragments products indicating heterozygous and homozygous SMIM1*64_80 del allele were determined using reference samples.

Serology Analysis

Expression levels of the Vel antigen in samples that were positive (heterozygous) for 17-bp deletion were evaluated by serology. All donors with positive real-time PCR results were tested using 50 μL of 0.8% red blood cell suspension added to the LISS/Coombs (Grifols S.A.) test gel card, to which 25 μL of anti-Vel human serum were then added. Positive and negative controls were performed in parallel. The macroscopic agglutination analysis was performed following previously established protocols (Dinardo et al., 2014).

rs1175550 SNP genotyping

Genotyping to identify rs1175550 SNP in SMIM1 that was reported to be associated with expression levels of the Vel antigen was performed in all samples classified as heterozygous for c.64_80del. PCR-SSP was performed using the following primers: rs1175550-R1 5’-CTCAGGCAAGCCCTGACC-3’ (antisense), rs1175550-F1g 5’-TCAGGGGCTGCAGCCT

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AGG -3′ (sense) and rs1175550-F1a 5′-TCAGGGCTGCAGCCTAGA-3′ (sense), designed and previously described (Wieckhusen et al., 2015). PCR amplification was carried out in a Mastercycler personal thermal cycler (Eppendorf, Hamburg, Germany) in 50-μL final volume reactions containing 50–150 ng mL⁻¹ of genomic DNA, 10 pmol of forward and reverse primers, 2 nmol of each dNTP and 1·0 U of Taq DNA Polimerase (Platinum Invitrogen Life Technologies®, Grand Island, NY, USA). PCR conditions were as follows: 10 min at 95 °C, 40 cycles of 30 s at 95 °C, 30 s at 65 °C and 60 s at 72 °C, followed by 3 min at 72 °C. Amplification product (160 bp) was identified on 2% agarose gels containing GelRed DNA stain (Biotium, Hayward, CA, USA). Results were documented using a gel documentation (ImageQuant LAS 500, Piscataway, NJ, USA).

### RESULTS

**Real-time PCR Analysis**

Among the 448 blood donor DNA samples analysed, 10 (2.23%) were positive for the 17-bp deletion (64_80 del) of the SMIM1 gene in at least one allele (10/448 = 2.23%). All positive samples were repeated in triplicate to confirm the results, and the observed average Ct was 32.97.

A total of 10 (2.23%) positive samples were characterised as heterozygotes for the SMIM1*64_80 del allele using RFLP-PCR as described above.

Serological analysis of the Vel antigen for the 10 positive samples characterised as heterozygotes by RFLP-PCR showed reactivity for the Vel antigen ranging from one to three crosses (Table 1).

### DISCUSSION

The SMIM1 gene codes for the protein SMIM1, which is responsible for expressing the Vel antigen of the new Vel Blood Group System, has been recently described and has been the target of studies by several researchers. Knowing the genetic bases responsible for expression of the Vel antigen allowed...
molecular assays to be applied for the first time to determine the frequency of the SMIM1*64_80 del allele – which corresponds to the Vel− phenotype in homozygosity or for Vel+ when in heterozygosity. In this study, a cohort of 448 blood donors of seven regions that represent the state of Santa Catarina were tested for the SMIM1*64_80 del allele. The frequency obtained for the SMIM1*64_80 del allele was 1-12%, of the Wild type/SMIM1*64_80 del genotype was 2.23%, and no sample was found homozygous for the deletion. According to the literature, the frequency of the Vel− phenotype or homozygosity for SMIM1*64_80 del in Caucasians is 0-025% (Reid et al., 2012).

It is estimated that, to find this phenotype, 5000 blood donor samples would have to be analysed (Reid et al., 2012; Daniels, 2013). Dezan et al. (2016) showed it is possible to find a donor with Vel− phenotype in a study with 4680 blood donors through a strategy developed to identify the SMIM1*64_80 del allele in blood donors from viral NAT screening plasma pools.

As for the frequency of the SMIM1*64_80 del allele in Brazil, this study found a frequency of 1-12%, i.e. higher than the frequency of 0-22% found among blood donors in the state of São Paulo, southeast Brazil. This difference could be explained by the strong influence of immigration in that state, which continuously receives Afro-descendant immigrants. Studies carried out on African Blacks showed a frequency of 0-56% of the allele (Haer-Wigman et al., 2015).

Caucasian populations from the Netherlands who were also studied presented a frequency of 1-46%, which is similar to the one found in the present study (Haer-Wigman et al., 2015). These frequencies can be explained by the similarity of the ethnic make-up of the Caucasian populations studied. The Brazilian population is one of the most heterogeneous in the world, being composed of ethnicities from three continents over a period of five centuries: the European colonisers, mainly from Portugal and Italy; African slaves; and local Amerindian populations (Carvalho-Silva et al., 2001; Parra et al., 2003; Guelsin et al., 2010). The present study evaluated samples from the State of Santa Catarina in the southern region of Brazil, which represents an interracial mixture of European, African and Amerindian genes. According to Manta et al. (2013), the population of the state of Santa Catarina is predominantly of European origin (79.7%), with a contribution from Africans (11-4%) and Amerindians (8-9%) (Manta et al., 2013).

The frequency of the SMIM1*64_80 del allele in the population studied was higher than those observed in other populations who reported frequencies of 0-09 in African Americans, 0-56 in African Blacks and 0-60 in Chinese populations (Storry et al., 2013, Haer-Wigman et al., 2015). In this study, the role played by rs1175550 on the expression of Vel antigen among nine Vel** donors with the SMIM1*84 del in heterozygosis was also confirmed. Donors who presented the deletion of 17 nucleotides (c.64_80del) in heterozygosis and the rs1175550 SNP in homozygosis for the major A allele showed variable intensity of Vel expression, as shown in Table 1. One heterozygous A/G donor for rs1175550 SNP was found in this small group of Vel** as reported for a larger group of 88 subjects also heterozygous for the c.64_80del (Haer-Wigman et al., 2015). Although this study has shown specific differences in the Vel antigen expression in donors heterozygous for the 64–80del allele ranging from 1+ to 4+, the phenotyping is very dependent on the quality of anti-Vel serum and on the sensitivity of the test technique that can be variable in accordance with commercial technique (Wieckhusen et al., 2015).

The data obtained in the present study greatly contribute to characterising the Vel phenotype in a population in Brazil, but further studies with a larger number of samples are required to determine the frequency in other states, mainly of the Vel− phenotype, which were not found in this study. These studies are very important for the supply of blood units compatible to rare phenotype patients. These patients require units of blood phenotype compatible once the anti-Vel, developed against the Vel antigen, has clinical importance and is regularly responsible for acute severe reactions or delayed haemolytic transfusion (HTR) that may induce life-threatening kidney failure and be associated with haemolytic disease of the foetus and newborn (Van Gaggeren et al., 2008; Daniels, 2013). Patients with antibodies directed to the Vel antigen and who are in need of a blood transfusion have a transfusion hazard in an emergency situation. When a patient with anti-Vel is transfused with Vel red blood cells (RBCs), a severe immediate haemolytic transfusion reaction can occur due to intravascular haemolysis of Vel RBCs (Haer-Wigman et al., 2015).

The difficulties encountered are that such patients are characterised by their phenotype after prior contact with Vel antigen and development of associated antibody to the high-prevalence Vel antigen. Co-authors of our study lived this experience, in this case with a happy ending. Due to the patient’s favourable clinical conditions, it was possible to receive autologous transfusion. When this is not possible, intense efforts are necessary because the supply of blood Vel must be managed with help from national or international rare blood banks (Nance, 2009).

These problems can be foreseen or prevented with the introduction of donor screening blood bank services for rare phenotypes as well as the study of potential families identified as heterozygous donors, using molecular tools. The knowledge of the molecular mechanisms of the Vel blood group system will enable this type of studies, which were previously limited as serological assays did not yield reliable results due to variations in the expression intensity of the Vel antigen and to the unavailability of commercial reagents.

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CONFLICTS OF INTEREST

The authors have no competing interests.

REFERENCES


