The polymorphism nt 76 in exon 2 of SC is more frequent in Whites than in Blacks

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The Scianna blood group system comprises seven antigens encoded by alternative forms of SC. The SC gene also has two polymorphisms in the leader sequence, at nucleotides 54 (C/T, silent) and 76 (C/T, 26His/Tyr) in exon 2, which are not involved in expression of blood group antigens. The nucleotide change at position 76 has an NlaIII restriction enzyme site; thus, DNA samples from 100 Caucasians and 100 African Americans were analyzed for the SC nucleotide 76 change. DNA from Caucasian and African American donors was tested by polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) using the restriction enzyme NlaIII. In selected samples, sequencing of exon 2 was performed. PCR-RFLP results for samples from 100 donors (mostly Caucasian) and 100 African American donors (400 alleles) showed the nucleotide 76T variant had a prevalence of 25 percent in Whites and 5 percent in African Americans. In 11 samples (2 C/C, 3 C/T, and 6 T/T) sequencing of exon 2 confirmed the presence of the expected nucleotides at position 76. The allele frequency in Caucasians was 0.75 for nt76C and 0.25 for nt76T. In African Americans, the frequencies were, respectively, 0.95 and 0.05.

Key Words: alloantibodies, blood groups, ERMAP, ethnic diversity, human polymorphisms, Scianna

The Scianna (Sc) blood group system (ISBT 013) comprises seven antigens that are encoded by alternative forms of SC (or ERMAP). Sc1 and Sc2 have an antithetical relationship and are, respectively, high- and low-prevalence antigens. Sc3 is expressed on all RBCs except those from individuals with the Scnull phenotype. Sc4 (Rd) is a low-prevalence antigen, and Sc5 (STAR), Sc6 (SCER), and Sc7 (SCAN) are high-prevalence antigens whose antithetical relationship are not reported. The molecular bases associated with the antigens are known. In addition, two nucleotide (nt) polymorphisms (nt54C>T, a silent change; and nt76C>T, predicted to encode His26Tyr) are in the sequence of nucleotides that encode the leader sequence of the SC glycoprotein, which is not present on the RBCs. We report the analysis of DNA samples from 100 donors (mostly Caucasians) and 100 African Americans for the prevalence of the SC nt76 change.

Materials and Methods

Blood samples

Samples from 100 random (mostly Caucasian) donors and 100 donors who had either the homozygous mutated FY-GATA box (n = 20) or self-identified as being African American (n = 80) were obtained under Institutional Review Board–approved protocols and tested.

Sequence analysis of genomic DNA for SC

Genomic DNA was isolated from whole blood (QIAamp DNA Blood Mini Kit, QIAGEN, Inc., Valencia, CA). The regions of SC that included and flanked exons 2, 3, and 4 were amplified separately using the oligonucleotide primers listed in Table 1. The primers were synthesized by Life Technologies, Inc. (Gaithersburg, MD). Five microliters of DNA per reaction were amplified by 5 U of Taq DNA polymerase (HotStarTaq, QIAGEN Inc.) in a 50-μL reaction mixture containing 2.5 mM MgCl2, 1× PCR buffer, 0.2 mM dNTPs, and 100 ng of forward and reverse primer. Amplification was achieved during 35 cycles with a final extension time of 10 minutes. The PCR was performed using 62°C as the annealing temperature. The PCR products were sequenced in the Laboratory of Microchemistry at the New York Blood Center using an ABI 373XL sequencer (Applied Biosystems, Inc., Foster City, CA), and ABI Big Dye reagents with BD Half-Term (GenPak, Stony Brook, NY). The PCR products were directly sequenced using the forward and reverse primers that were used for PCR amplification.

Table 1. Primer sequences and expected products*

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequences 5’ to 3’</th>
<th>Region amplified (amplicon size)</th>
<th>RFLP using NlaIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc12F</td>
<td>caccccgcttggtagtgttc</td>
<td>Exon 2 (161 bp)</td>
<td>Wild type: 121 bp, 40bp</td>
</tr>
<tr>
<td>Sc12R</td>
<td>gacaccgacaggaaaaaggg</td>
<td>Variant: 161 bp</td>
<td></td>
</tr>
<tr>
<td>Sc3F</td>
<td>cccctccagtggctgtgctc</td>
<td>Exon 3 (149 bp)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Sc3R</td>
<td>cacactgacacgggacacc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sc4F</td>
<td>tctctgagtgctgtggttg</td>
<td>Exon 4 (188 bp)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Sc4R</td>
<td>tttccacagctgtggtcctc</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Primer sequences are written in lowercase letters because they are located in the introns.

PCR-RFLP analysis

One hundred DNA samples mostly from Caucasians and 100 from African Americans were amplified for the region of SC that included and flanked exon 2, using the Sc12F and Sc12R oligonucleotide primer pair (Table 1). The PCR products (161 bp) were digested using the restriction enzyme NlaIII. The restriction enzyme site of NlaIII is present in the wild-type DNA with nt 76C and ablated in the variant nt 76T. The expected sizes for bands in the wild type were 121 bp, 40bp.
and 40 bp, and for the variant, 161 bp (uncut). There is no restriction site to distinguish the wild-type nt54C from the variant nt54T.

**Results**

_**PCR-RFLP analysis on random samples**_

PCR-RFLP analysis for nt76C/T in the 100 random (mostly Caucasian) donors resulted in 62 samples that were consistent with the expected digestion pattern of the wild type (76C), 11 samples that were consistent with the expected digestion pattern for homozygosity for the variant (76T), and 27 that were consistent with the expected digestion pattern for heterozygosity for the variant (76C/T). For the 100 samples from African American donors, 91 were consistent with the expected digestion pattern of the wild type (76C), 1 was consistent with the expected digestion pattern for homozygosity for the variant (76T), and 8 were consistent with the expected digestion pattern for heterozygosity for the variant (76C/T). These results are summarized in Table 2.

_**Sequence analysis of the random samples**_

Eleven of the random donor samples, which demonstrated either a homozygous change (n = 6), heterozygous change (n = 3), or wild type (n = 2), were sent for direct sequencing. Sequence analysis of the six samples homozygous for the variant were confirmed to be nt76T and also shown to be homozygous for the variant nt54T. Sequence analysis of the three samples heterozygous for the variant selected on the basis of the PCR-RFLP assay were confirmed to be heterozygous nt76C/T and also shown to be heterozygous for nt54C/T. Sequence analysis of the two wild-type samples selected on the basis of the PCR-RFLP assay were confirmed to be homozygous wild type for nt76C and shown to be also homozygous wild type for nt54C.

**Discussion**

The SC nt76C>T change (His26Try) is located in the sequence of nucleotides that encode the leader sequence, which is cleaved and not present in the RBC-bound ERMAP. The SC nt54C>T silent change is also in this sequence but does not give rise to or ablate a restriction enzyme site. In the 11 samples analyzed by sequencing, either both nt54 and nt76 changes were present or neither was present. Whether these are linked polymorphisms in the heterozygotes requires plasmid cloning of the PCR products to evaluate single sequences. In our study, the allele frequency in Caucasians was 0.75 for 76C and 0.25 for nt76T. In African Americans, the frequencies were 0.95 and 0.05, respectively.

### Table 2. Results of PCR-RFLP using NlaIII

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>nt 76C (W/W)</th>
<th>nt 76 C/T (W/V)</th>
<th>nt 76T (V/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random donors (n = 100)</td>
<td>62</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>African Americans (n = 100)</td>
<td>91</td>
<td>8</td>
<td>1</td>
</tr>
</tbody>
</table>

V = variant; W = wild type.

**Acknowledgment**

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**References**

1. Wagner FF, Poole J, Flegel WA. The Scianna antigens including Rα are expressed by ERMAP. Blood 2003;101:752–7.

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