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ORIGINAL ARTICLE

The Polymorphism of *SMIM1* Gene in Chinese Dividuals

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Abstract The *SMIM1* gene, which encodes the high-frequency blood group antigen Vel, has not been systematically analyzed at the molecular level in Chinese individuals. To better understand the SMIM1 genetic polymorphism, we assessed mutations among healthy Chinese individuals, patients with red blood cell autoantibodies and hematological disease. A total of 130 patients with hematological disease (case I group), 50 patients with red blood cell autoantibodies (case II group), and 500 healthy controls (control group) were enrolled. Exons 3 and 4 in the *SMIM1* gene were sequenced to identify genetic variants or mutations. A polyclonal anti-Vel antibody was used to evaluate the expression of the Vel antigenon red blood cells in patients with novel alleles. The novel alleles of the SMIM1 gene were intron 3 position 193 (TT, CT, CC), 194 (GG, AG), 3′ untranslated region positions 81 (CC, CA) and 87 (AA, CA). The single nucleotide polymorphism (SNP) frequencies of intron 3 position 193 TT, CT, CC were 13.1, 39.2, 47.7% in case group I, 6.7, 33.3, 60.0% in case group II and 8.5, 35.6, 56.2% in the control group, respectively. Other minor allele frequencies were all greater than 10% and all SNPs in Chinese showed Vel antigen expression on RBC membranes. The allele at intron 3 position 193 was the most frequent mutant allele found in the Chinese population and Vel antigen deficiency may not cause problems in Chinese patients with hematological diseases and RBC autoantibodies.

Keywords *SMIM1* gene · Polymorphism · Vel antigen · Autoantibody

Introduction

By way of red blood cell (RBC) transfusion, anti-Vel alloantibodies target high-frequency Velantigen and permit alloimmunization, as these antibodies do not usually occur naturally [1, 2]. Anti-Vel antibodies are usually IgM antibodies, but have also been identified as IgM plus IgG antibodies, and are associated with autoimmune hemolytic diseases [3, 4]. Although anti-Vel was first identified in 1952, Vel antigen produced by the *SMIM1* gene has only recently been discovered [5–7]. Three mutations in the *SMIM1* gene have been shown to lead to Vel antigen deficiency on the RBC membrane in Europeans and Americans [8].

Polyclonal anti-Vel reacts weakly with the Vel antigen; thus, a two-step anti-agglutinin test should be used to enhance agglutinin, making it difficult to investigate the epidemiology of Vel [3, 9]. Some large-scale studies of blood donors have reported that the frequencies of the Vel^{-} phenotype through anti-globulin tests are 0.0004 in American individuals, 0.0003 in British individuals, and 0.0006 in Swedish individuals [4, 5, 10–12]. However, only two studies have investigated the frequency of Vel antigen in Chinese individuals. Wang et al. [13] used human-resource polyclonal anti-Vel to screen for Vel antigen in blood donors from Shanghai and showed that the frequency

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of Vel$^-$ individuals was 0.03% (2/6153). In a very recent report, Liu et al. [14] reported that the frequency of Vel$^-$ in Jiangsu blood donors was 0.01% (1/9122) through Vel genotyping. One study described a multiplex polymerase chain reaction (PCR) genotyping method for the screening of rare red cell groups [15]. Thus, only serology and genotyping have been used to screen Vel antigen in China, and sequencing results of the *SMIM1* gene to identify polymorphismhave not been conducted on Chinese individuals.

Because anti-Vel tends to be expressed as IgM autoantibodies, in this study, we conducted a systemic molecular analysis of healthy participants, patients with hematological conditions, and patients with RBC autoantibodies to examine the molecular sequence of the *SMIM1* gene fragment in Chinese individuals.

Materials and Methods

Study Groups and Sample Collection

A total of 130 patients with hematological conditions (case group 1) enrolled in this study from September to December 2016, including 45 patients with aplastic anemia, 47 patients with myelodysplastic syndromes, and 38 patients with chronic myelocytic leukemia; antibody screening tests were negative (Table 1). Fifty stored blood specimens (case group 2) were assessed, including 43 patients with RBC autoantibodies positive for anti-IgG and anti-C3d and seven patients with RBC autoantibodies positive for anti-IgG and negative for anti-C3d. An additional 500 healthy individuals who attended our hospital for health checkups were also enrolled in this study from December 2016 to January 2017; antibody screening tests in all individuals were negative, except for one case, which was positive for cold agglutinin.

Peripheral ethylenediaminetetraacetic acid (EDTA)-treated anticoagulated blood was stored at 4 °C. Serological tests and DNA extraction were performed within 12 h of blood acquisition. DNA was purified from buffy coat using micromagnetic technology from a commercial kit (Tiangen Biotech [Beijing] Co., Ltd., Beijing, China). All DNA samples were stored at − 80 °C until required for molecular analysis.

Serological Vel Typing

Serological testing for the Vel antigen was performed using indirect antiglobulin tests by micro-column method (Bio-Rad Cressier, Switzerland and Libiotech, Jiangsu, China) with polyclonal anti-Vel serum from immunized patients. To enhance the reactions, bi-thermal incubation (15 min at 37 °C and an additional 15 min at room temperature) was used, and the gel cards were centrifuged for 10 min at $85\times g$ [3].

Molecular Analysis of the SMIM1 Gene

Commercial kits were used to examine exons 3 and 4 of the *SMIM1* gene (Vel blood group sequence kit; Libiotech). Briefly, the PCR tubes contained 36 μL PCR matrix buffer, 0.32 μL Taq polymerase (5U/μL; Promega, USA), 4 μL genomic DNA(concentration: 20–100 ng/μL) and 2 μL primers(10 μM/μL) [16]. The cycling conditions were as follows: 2 min initial denaturation at 96 °C; five cycles of 20 s at 96 °C and 1 min at 68 °C; 10 cycles of 20 s at 96 °C, 50 s at 65 °C, and 45 s at 72 °C; 17 cycles of 20 s at 96 °C, 50 s at 62 °C, and 45 s at 72 °C; and a final 2 min at 72 °C. Amplification products were purified on 2% agarose gels containing 4S Green Plus Nucleic Acid Stain (Sangon Biotech, Shanghai, China), and results were documented using a UV gel documentation device. Sequencing of PCR purified products was performed by Sangon Biotech (Beijing, China)

Sequence Interpretation

The sequence results were analyzed using sequence analysis software (Geneious R9, New Zealand). The *SMIM1*

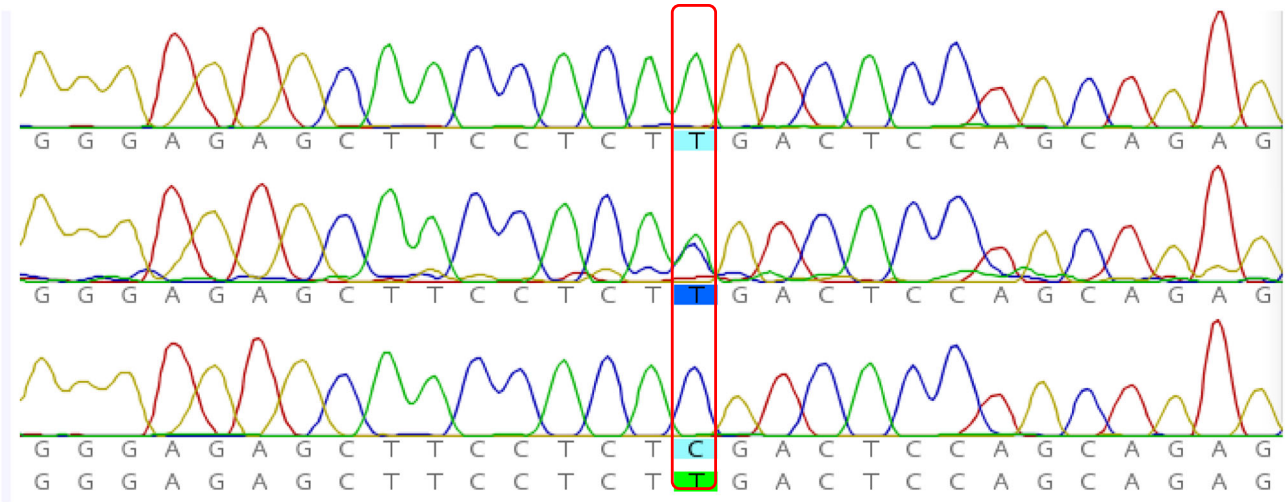
Table 1 The demographics and baseline characteristics in the case and control groups

	Case I (n = 130)	Case II (n = 50)	Control (n = 500)
Age (mean ± SD)	54. ± 8.4	49.7 ± 7.8	31 ± 10.6%
Sex-male (%)	52 (40.0%)	22 (44.4%)	135 (47.0%)
Clinical condition			
Myelodysplastic syndromes	47		
Chronic myelocytic leukemia	38		
Aplastic anemia	45		
Antibody screening test-positive	0 (100%)	50 (100.0%)	–
DAT: anti-IgG + C3d+	–	43 (86.0%)	–
DAT: anti-IgG + C3d−	–	7 (14.0%)	–

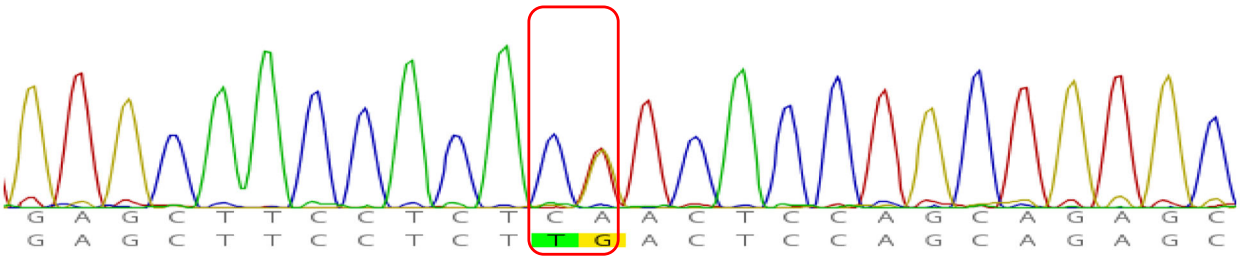
Table 2 Allele and SNP distributions and Hardy–Weinberg equilibrium testing among case and control groups

SNP	Case I group (n = 130)				Case II group (n = 30)				Control groups (n = 500)			
	Observed Value-n (%)	Expected Value- n (%)	X^2	p	Observed Value-n (%)	Expected Value-n (%)	X^2	p	Observed Value-n (%)	Expected Value-n (%)	X^2	p
Intron 3 193												
TT	17 (13.1%)	13.89 (10.7%)	0.70	0.705	2 (6.7%)	1.63 (5.4%)	0.03	0.985	41 (8.2%)	33.8 (6.8%)	1.27	0.530
CT	51 (39.2%)	57.21 (44.0%)			10 (33.3%)	10.73 (35.8%)			178 (35.6%)	192.4 (38.5%)		
CC	62 (47.7%)	58.89 (45.3%)			18 (60.0%)	17.63 (58.8%)			281 (56.2%)	273.8 (54.7%)		
Intron 3 194												
GG	128 (98.5%)	128.01 (98.5%)	0.00	1.000	30 (100.0%)	30 (100.0%)	0.00	1.000	487 (97.4%)	487.08 (97.4%)	0.00	1.000
AG	2 (1.5%)	1.98 (1.5%)			0 (0.0%)	0 (0.0%)			13 (2.6%)	12.83 (2.6%)		
AA	0 (0.0%)	0.01 (0.0%)			0 (0.0%)	0 (0.0%)			0 (0.0%)	0.08 (0.0%)		
3′ untranslated region 81												
CC	128 (98.5%)	128 (98.6%)	0.00	1.000	30 (100.0%)	30 (100.0%)	0.00	1.000	493 (98.6%)	493.02 (98.6%)	0.00	1.000
AC	2 (1.5%)	0.5 (0.3%)			0 (0.0%)	0 (0.0%)			7 (1.4%)	6.95 (1.4%)		
AA	0 (0.0%)	0.01 (0.0%)			0 (0.0%)	0 (0.0%)			0 (0.0%)	0.03 (0.0%)		
3′ untranslated region 87												
AA	129 (99.2%)	129 (99.2%)	0.00	1.000	30 (100.0%)	30 (100.0%)	0.00	1.000	494 (98.8%)	494.02 (98.8%)	0.00	1.000
AC	1 (0.7%)	1 (0.7%)			0 (0.0%)	0 (0.0%)			6 (1.2%)	5.96 (1.2%)		
CC	0 (0.0%)	0 (0.0%)			0 (0.0%)	0 (0.0%)			0 (0.0%)	0.02 (0.0%)		

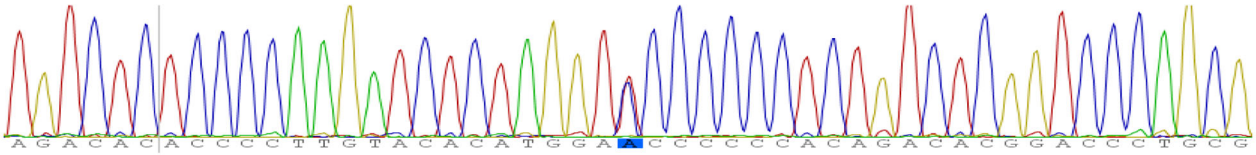
Panel A. Intron 3 position 193



Panel B. Intron 3 position194 G > A



Panel C. 3' untranslated region 81 C>A



Panel D. 3' untranslated region position 87 A>C

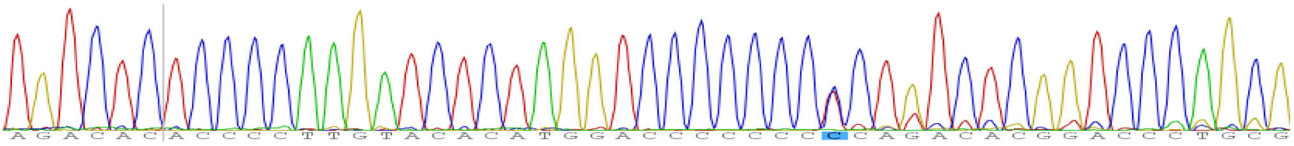


Fig. 1 SNPs of four novel alleles in the *SMIM1* gene. *a* Shows intron 3 position 193. The red square indicates SNPs, including TT (wide-type), CT (homozygous), and CC. *b* Shows intron 3 position 193 and 194. The red square indicates SNPs. *c* Shows 3′ untranslated region position 81 C > A. The blue background indicates SNPs. *d* 3′ untranslated region position 87 A > C. The blue background indicates SNPs (color figure online)

gene (GenBank No. NM_001163724) template was used as a reference for analysis and to mark the mutations.

Statistical Analysis

Hardy–Weinberg equilibrium testing (HWET) was performed using Chi square tests to check the allele and genotype frequencies in the study population. Continuous variables were assessed using t tests or analysis of variance (ANOVA), and categorical variables were assessed using Chi square tests or Fisher extract tests. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were determined to assess risks, and two-sided p values of less than 0.05 were considered statistically significant. All statistical analyses were conducted using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA).

Results

The Vel 3 and Vel 4 exons were successfully sequenced in 680 DNA samples from Chinese individuals, including 130 patients with hematological conditions, 50 patients with RBC autoantibodies, and 500 healthy individuals. In total, three new alleles were identified in the Vel exon 3 and exon 4 sequences, including one novel allele in intron 3 and two novel alleles in untranslated region (Table 2). The polymorphism of intron 3 position 193 has been published in Swedish blood donors (NC_018912.2) [17].

Four Novel Alleles

Polymorphisms were observed in intron 3 position 193 in all patients, and the major allele was 193C homozygous (47.7% in case group 1, 60% in case group 2, and 56.2% in controls), followed by 193 CT heterozygous (39.2% in case group 1, 33.3% in case group 2, and 35.6% in controls), followed by 193 TT homozygous (47.7% in case group 1, 60.0% in case group 2, and 56.2% in controls). Other SNPs were detected less frequently, with minor allele frequencies of not more than 1%. Three other novel SNPs were identified at intron 3 194 G > A, 3′ untranslated region 81 C > A, and 3′ untranslated region 87 A > C. The four novel genotype distributions of Vel corresponded well with the Hardy–Weinberg equilibrium in case group 1, case group 2, and controls (all $p > 0.05$). Other details are shown in Table 2 and Fig. 1.

Expression of Vel Antigens in Patients with Four Alleles

Poly-anti-Vel from an immunized patient reacted with patients showing novel alleles, including intron 3 position 193 (CC, CT, TT), intron 3 position 194 (GG, AG), 3′ untranslated region position 81 (CC, AC), and 87 (AA, AC). The enhanced anti-agglutinin test demonstrated that all novel alleles were positive, indicating that Vel antigen was expressed on the surface of the RBC membrane (Table 3).

Table 3 Serotypes of Vel antigen in patients with novel alleles

Allele	Group (n)	Serotype
Intron 3 193 TT (wide type)	Control (10)	+
	Case I (10)	+
Intron 3 193 TC	Control (10)	+
	Case I (10)	+
Intron 3 193 CC	Control (10)	+
	Case I (10)	+
Intron 3 194 GG	Control (10)	+
	Case I (10)	+
Intron 3 194 GA	Control (2)	+
	Case I (2)	+
3′ untranslated region 81 CC	Control (10)	+
	Case I (10)	+
3′ untranslated region 81 CA	Control (2)	+
	Case I (2)	+
3′ untranslated region 87 AA	Control (10)	+
	Case I (10)	+
3′ untranslated region 87 CA	Control (1)	+
	Case I (1)	+

Associations Between SMIM1 Polymorphisms in Intron 3 Position 193 and Patients with Hematological Diseases or RBC Alloantibodies

The allele and genotype frequencies of SNPs in the *SMIM1* gene did not differ significantly between the case groups and controls (all $p > 0.05$). However, the frequencies of the mutant genotype (CA + AA) at intron 3 position 193 were lower in patients with hematological conditions than those in controls ($p = 0.047$, 95% CI 0.28–1.00; Table 4).

Discussion

In this study, we investigated the frequencies of mutations in the *SMIM1* gene in Chinese patients with hematological diseases or RBC alloantibodies and in healthy controls. Our analysis identified four novel alleles in the *SMIM1* gene, including positions 193 and 194 in intron 3 and positions 81 and 87 in 3′ untranslated region; position 193 in intron 3 of the *SMIM1* gene was confirmed to harbor a significant polymorphism. We also showed that patients with

Table 4 Association between Vel intron 3 position 193 polymorphisms and patients

Genotype/allele	Control 500 n (%)		Case I 130 n (%)		OR	(95% CI)	*p*	Case II 30 n (%)		OR	(95% CI)	*p*
Intron 3 193												
TT (wide type)	41	8.2	17	13.1	Ref	–	–	2	6.7	Ref	–	–
CT	178	35.6	51	39.2	0.69	0.36–1.32	0.260	10	33.3	1.15	0.24–5.46	0.859
CC	281	56.2	62	47.7	0.53	0.28–1.00	***0.047***	18	60.0	1.31	0.29–5.87	0.721
CC + CT	459	91.8	113	86.9	0.59	0.33–1.08	0.087	28	93.3	1.25	0.29–5.44	0.765
Allele C	740	74.0	175	67.3	0.57	0.32–1.03	0.059	46	76.7	1.27	0.30–5.43	0.743

Bold indicates significant ($p < 0.05$)

hematological diseases exhibited lower heterozygote frequencies than controls, and enhanced anti-agglutinin tests demonstrated that Vel antigen was expressed on the surface of the RBC membrane in patients with all four novel alleles.

To the best of our knowledge, the sequence of the *SMIM1* gene in Chinese individuals has not been previously been reported. Only three studies in Chinese individuals have reported genotyping methods to screen for Vel^{-} genotype, and few studies have reported the sequence of the gene or polymorphisms in the gene in Chinese individuals [13–15]. Because of the characteristics of Vel isoimmunization, the high frequency of Vel antigens in the Chinese (higher than 99.99%), and the lack of a monoclonal anti-Vel antibody, screening for the Vel antigen using molecular techniques and sequencing of the *SMIM1* gene should be carried out routinely [7, 16, 18, 19].

Initially, we attempted to sequence *SMIM1*exons using the Vel antigen among controls and high-risk groups to produce RBC autoantibodies or alloantibodies. We assumed that some SNPs, even deletions, would be observed in high-risk groups. In comparison with healthy controls, patients in case group 1 showed lower expressions of the gene when the polymorphism at intron 3 position 193 (CA) heterozygous was present, although no differences were found in the Vel antigen expression on the RBC surface between these groups. Our study provided data in a standard controlled study, showing that four novel alleles expressed normal Vel antigens in our Chinese cohort, and that Vel antigen deficiency is not likely to cause problems in Chinese patients with hematological disease and RBC autoantibodies.

In our study, we limited sequencing to exons 3 and 4 in the *SMIM1* gene because these two exons encode Vel antigens. Whole-gene sequencing may provide additional information on polymorphisms in Chinese individuals.

In summary, our results identified four novel SNPs in the *SMIM1* gene among the Chinese population, including intron 3 position 193 (TT, CT, CC), intron 3 position 194 (GG, AG), 3′ untranslated region position 81 (CC, CA), and 3′ untranslated region position 87 (AA, CA). Our study also indicated that four SNPs normally produce the Vel antigen in Chinese individuals.

Compliance With Ethical Standards

Conflict of interest Ying Yu and Huaxi Xu declares that she has no conflict of interest. Hejiong Wang Linchao Zhu, Yushiang Lin and Haochun Chang declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with animals performed by any of the authors.

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