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Review

Molecular Basis and Clinical Research Progress of the Vel Blood Group System

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ABSTRACT: This article focuses on the major advances in the study of the Vel blood group, which was named after a patient who experienced an adverse transfusion reaction in 1952. The antigen of this blood group is encoded by the *SMIM1* gene and exhibits autosomal inheritance. The Vel blood group system currently contains only one antigen, and the anti-Vel antibody is clinically known to cause hemolytic transfusion reactions and hemolytic disease of the fetus or the newborn. The *SMIM1* gene is located at chromosome 1p36.32. Its specific mutations are strongly associated with the Vel blood group phenotype. Genetic screening technology has made significant progress in the Vel blood group research, and CRISPR/Cas9 technology provides a powerful tool. The relevant study and analysis suggested that SMIM1 was linked to diseases and could potentially serve as a biomarker for tumors. The SMIM1 protein may have a potential role in *Plasmodium* infection, and individuals carrying a homozygous deletion of the *SMIM1* gene are associated with obesity. In the future, it is expected to further reveal the molecular basis, antigenic structure and function, clinical significance and interrelationship with diseases of the Vel blood group system.

Keywords: Vel blood group; Vel antigen; SMIM1 protein; Vel antibody; *SMIM1* gene

1. Introduction

Erythrocyte blood group antigens play crucial roles in human cell structure, physiological metabolism, and immunoregulation. Advances in technology, including the widespread use of mass spectrometers and flow cytometers, have facilitated the discovery and thorough examination of erythrocyte antigens. Moreover, advancements in genotyping and sequencing technologies have deepened our molecular-level understanding of these antigens. Since 2010, significant strides have been made in the field, leading to the identification of several new blood group systems.

The Vel antigen, initially discovered by Sussman and Miller in 1952, was originally categorized as part of a "series" of antigens. Over time, research has progressively uncovered the gene loci, exon sequences, and molecular biological characteristics of the Vel antigen. A landmark moment came in 2014 when the International Society of Blood Transfusion (ISBT) officially recognized the Vel blood group system as a standalone entity, designated with the number 034, following comprehensive molecular analysis [1]. As of November 2024, this system is acknowledged to contain a single antigen referred to as Vel (number 034001).

The official symbol for the Vel blood group system is 'VEL', with the antigen denoted as 'Vel'. Genotypes are represented using the system symbol followed by an asterisk, with alleles or haplotypes separated by a slash and italicized (e.g., *VEL*01.01/VEL*01N.01*). Phenotypes are indicated by the system symbol followed by a colon and a list of antigens separated by commas (e.g., VEL:1).

The ongoing advancements in technology and molecular biology have significantly enhanced our knowledge of erythrocyte antigens, exemplified by the detailed characterization of the Vel blood group system. These developments

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not only contribute to the fundamental knowledge of human genetics but also have practical implications for transfusion medicine and clinical practice.

The Vel antigen, encoded by the *SMIM1* gene, is a prevalent antigen found in human blood, with Vel-negative (Vel−) status being a rare phenotype. Individuals with the Vel− phenotype can develop anti-Vel antibodies following immune stimuli such as pregnancy or blood transfusion. These antibodies can trigger severe hemolytic transfusion reactions, intravascular hemolysis, and hemolytic disease of the fetus and newborn (HDFN), underscoring their significant clinical implications.

Historically, the Vel blood group has posed unique challenges in immunohematology due to its variable antigenic strength among individuals, the potent lytic activity of anti-Vel against Vel-positive red blood cells (RBCs), and the difficulty in identifying sufficient numbers of compatible Vel-negative donors. Despite these challenges, advancements in screening methodologies have improved the identification of Vel-negative donors, although the exact physiological function of the SMIM1 protein remains elusive [2].

In clinical settings, while anti-Vel-induced HDFN is relatively uncommon, the potential for severe transfusion reactions necessitates vigilance and careful management. An in-depth understanding of the characteristics and underlying mechanisms of the Vel blood group is essential for enhancing transfusion safety, elucidating disease pathogenesis, and developing novel therapeutic strategies.

This review explores the genetic architecture of the Vel blood group, including the expression patterns and polymorphism distribution of the Vel antigen. It also delves into the physiological functions of the Vel antigen, its clinical significance, and its correlation with various diseases. Additionally, it highlights major advances in the Vel blood group research, providing comprehensive insights into the antigen's characteristics and population distribution. This information serves as an essential reference for both immunohematology research and clinical blood transfusion practice, aiming to facilitate safer and more effective patient care. Continued research into the Vel blood group not only aids in resolving longstanding mysteries but also supports the development of targeted interventions that can significantly improve outcomes for patients in need of blood transfusions.

2. Gene Structure, Antigen Expression and Polymorphism Distribution of the Vel Blood Group

2.1. Gene Structure of the Vel Blood Group

The *SMIM1* gene, which codes for the Vel blood group antigen, determines the expression and regulation of the Vel blood group [2]. The *SMIM1* gene, with GenBank number 388588, is located on band 6 of region 3 of the short arm of chromosome 1, 1p36.32. Its reference genome, NG_033869.1, has a sequence length of 10 222 base pairs (bp), and its mRNA sequence is NM_001163724.3. The *SMIM1* gene is comprised of four exons, totaling 559 bp in length, as illustrated in Figure 1. Specifically, the exons are distributed as follows: exon 1 measures 93 bp, exon 2 spans 119 bp, exon 3 extends to 185 bp, and exon 4 reaches 162 bp.

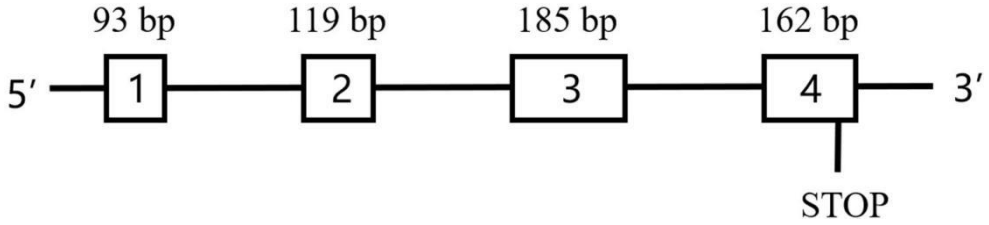

Figure 1. The four exons of the Vel blood group gene *SMIM1*.

2.2. Antigen Expression of the Vel Blood Group

The *SMIM1* gene encodes a 78-amino acid type II transmembrane glycoprotein, designated as small integral membrane protein 1 (SMIM1). This protein is commonly known as the Vel glycoprotein or Vel antigen. There exists a singular antigen in the VEL system, designated as number 034001. The molecular weight of the Vel antigen is about 18 kDa. The glycoprotein peptide chain of the Vel antigen consists of 78 amino acids [3]. SMIM1 is a tail-anchored transmembrane protein. The N-terminal region of the protein, comprising approximately 50 amino acids (positions 1 to 47), is located within the cytoplasm. The transmembrane domain (TMD), predicted to span amino acids 48 to 67, consists of about 20 amino acids. The short C-terminal region (amino acids 68 to 78) is exposed on the extracellular side of the cell membrane and presents the Vel antigen. This structural configuration is schematically illustrated in Figure 2.

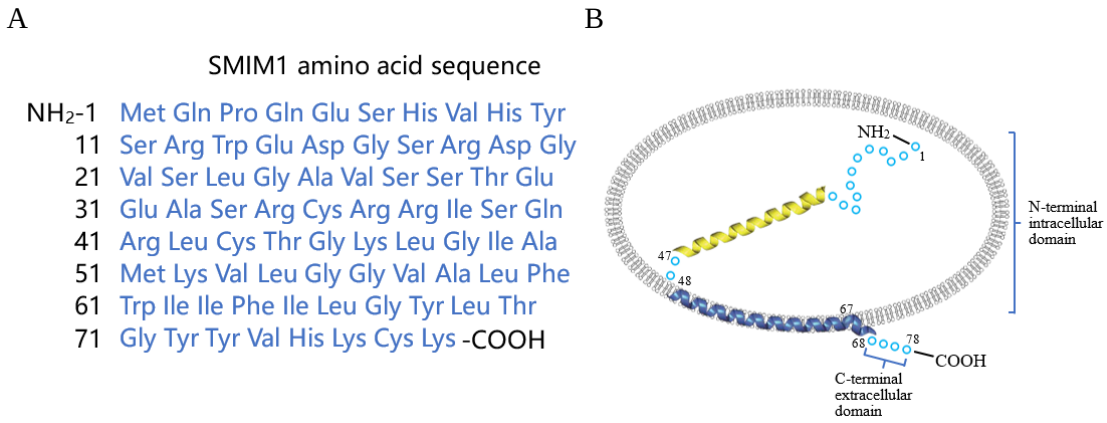

Figure 2. Schematic diagram of amino acid sequence and structure of the SMIM1 protein. (**A**): The amino acid sequence of the SMIM1 protein. (**B**): The relative positioning of the SMIM1 amino acid sequence with respect to red blood cells.

The three-dimensional structure of the SMIM1 protein can also be simulated using SWISS-MODLE [4], based on the AlphaFold DB model of SMIM1_HUMAN template B2RUZ4.1.A, as depicted in Figure 3 with a different transmembrane segment. The peptide chain traverses the membrane in a single pass, with the epitopes of the Vel antigen expressed exclusively in the extracellular portion of the peptide chain.

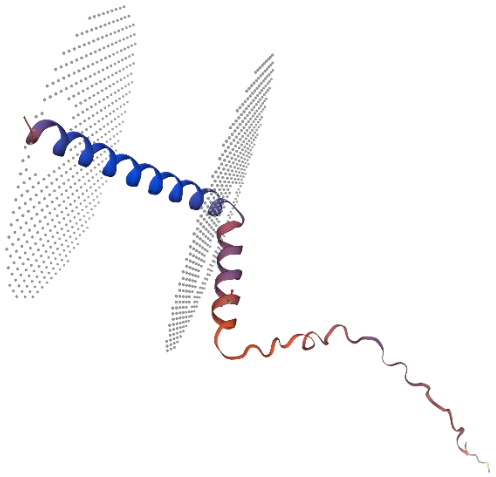

Figure 3. The three-dimensional structure of SMIM1 protein simulated by SWISS-MODLE. The SMIM1 protein is comprised of 78 amino acids. The shorter peptide segment with the carboxyl terminus which harbors Vel antigen is positioned on the exterior of red blood cell membrane. Conversely, the longer amino acid sequence, which includes the amino terminus, extends into the intracellular space within red blood cells.

The Vel antigen begins to be expressed at 12 weeks of fetal development; however, during the fetal and neonatal periods, its expression levels are lower than those observed in adults. In addition to being present in RBCs, the Vel antigen exhibits widespread distribution in numerous tissues, including the testis, bone marrow, kidney, and 14 additional tissues [2].

The expression of blood group antigens has complex mechanisms including the critical role of transcriptional, post-transcriptional, translational, and post-translational regulation [5]. The observed variability in antigen expression is related to its molecular basis. Firstly, the expression of the Vel antigen was associated with the purity of the c.64_80del deletion in *SMIM1*. In 2013, three independent research groups utilizing different approaches identified a novel small protein on erythrocytes called SMIM1 (small integral membrane protein 1) [6]. All three groups demonstrated that Vel-negative erythrocytes derive from a deletion of a fragment of the *SMIM1* gene. A 17-base homozygous deletion in exon 3 of *SMIM1* (c.64_80delAGCCTAGGGGGCTGTGTC) leads to premature termination of gene translation, resulting in the deletion of SMIM1 in Vel-negative individuals.

Subsequent research has revealed that variations in Vel antigenic strength can be attributed to polymorphisms within intron 2 of the *SMIM1* gene, a region critical for gene transcription [7]. Specifically, the single nucleotide polymorphisms (SNPs) rs1175550, rs6673829, and rs143702418 in *SMIM1* intron 2 independently regulate *SMIM1*

expression. For example, the A allele of rs1175550 is significantly associated with reduced Vel antigen expression, while the G allele of rs1175550 and the C allele of rs143702418 correlate with elevated levels of the Vel antigen.

The level of agglutination reactivity, which typically ranges from 2+ to 3+, is strongly linked to the base variants in intron 2 of *SMIM1*. The minor G allele at rs1175550 increases Vel antigen expression by reducing the binding affinity for an inhibitory factor, whereas the major A allele is associated with decreased expression. This relationship is confirmed by data showing that among samples with 2+ agglutination, 91% carried the major A allele, while 80% of 3+ samples carried the minor G allele.

Notably, individuals harboring both the *SMIM1*64_80del* allele and the major A allele of rs1175550 exhibit variable Vel antigen expression, ranging from absent (0) to weak (2+). Other studies have demonstrated that heterozygosity for the deletion allele, combined with the major A allele of rs1175550, results in negative or weak Vel expression, while the presence of the minor G allele on the non-deletion haplotype restores normal Vel expression [8].

The SNP rs6673829 shows a statistically significant distribution in samples with variable Vel expression. The minor A allele of rs6673829 was present in 60% of samples with 2+ reactivity and in 20% of samples with 3+ agglutination. Interestingly, two samples typed as Vel-negative carried the minor A allele of rs6673829, suggesting that it may contribute to reduced Vel expression. Further research is necessary to verify the role of rs6673829 in modulating Vel antigen expression.

These findings highlight the complex interplay between specific SNPs in *SMIM1* intron 2 and Vel antigen expression, emphasizing the importance of further investigation into the genetic determinants of this clinically significant blood group antigen. Beyond Vel expression variations explained by SNPs in *SMIM1* intron 2, experimental evidence demonstrates that missense mutations in *SMIM1* can lead to the deletion of Vel expression in cultured erythrocytes during in vitro denucleation and in vivo reticulocyte maturation [9]. The result showed that the *SMIM1*152A* allele (rs1182690110) was involved in reducing Vel expression due to its encoded amino acids being located very close to the transmembrane region of the SMIM1 protein. Despite this, the study observed changes in two individuals positive for the c.152T>A mutation, where the c.*58G>A mutation in the 3′UTR could potentially reduce Vel expression. However, there were discrepancies in three samples: two with 2+ reactivity and one with 3+ reactivity, which was not consistent with the study's findings. The proposed hypothesis suggests that alterations in Vel expression within these three discrepant samples may be influenced by genetic or environmental factors beyond those associated with *SMIM1*.

2.3. Distribution of Polymorphisms of the Vel Blood Group

The Vel antigen exhibits a high prevalence, being present in approximately 99.9% of individuals from diverse populations. Globally, the Vel-negative phenotype is considered rare, with frequencies of around 0.04% in North America and 0.56% in Africa. The frequency of Vel-negative individuals is approximately 1 in 4000 in Europe, 1 in 5000 among Caucasians, and 1 in 2000 in Germany [10]. Notably, the proportion of Vel-negative individuals is slightly higher in northern Sweden, with an incidence of about 1 in 1762.

Studies in Europe have reported a Vel-negative frequency of 0.025%, which is remarkably consistent with the 0.021% observed in the Brazilian population [11]. This similarity in frequencies may be indicative of historical migration patterns and genetic exchange between European and Brazilian populations, suggesting a common ancestral origin for the allele. Further research on the demographic history and genetic flow among these regions could provide valuable insights into the distribution of the Vel-negative phenotype.

Studies in China on the Vel blood group system have yielded interesting insights. For instance, a study conducted in the Ili region of Xinjiang, screening 3328 donors, did not identify any homozygous deletions but detected 14 heterozygous mutations involving a 17 bp deletion [12]. Similarly, in Jiangsu province, only one case of a 17 bp heterozygous mutation was found among 9122 donors. Another study in Shanghai identified two Vel-negative individuals out of 6153 blood donors [13].

Allelic frequency analysis for the *SMIM1* c.64_80del mutation, which is associated with the Vel-negative phenotype, has been performed using PCR-sequence-specific primers (PCR-SSP) [14]. Variations in global allele frequency of *SMIM1*64_80del* are evident through comparisons: European populations exhibit frequencies ranging from 1.46% to 2.9%, exemplified by a rate of 1.81% in southwestern Germany. Conversely, African and Asian populations show much lower frequencies, at 0.56% and 0.6%, respectively. In the Ili region, the frequency was approximately 0.21% [12].

These findings underscore the genetic diversity of the Vel blood group system and highlight the importance of thorough donor screening in transfusion medicine, particularly in regions where the Vel-negative phenotype may be more prevalent.

The reference allele for the Vel blood group (Vel+) is *VEL*01* [1]. Table 1 provides a comprehensive overview of the identified polymorphisms within the Vel blood group gene, including their genomic locations and the resulting phenotypic variations. For example, a single nucleotide mutation at position 152 in exon 4 leads to an amino acid substitution that results in diminished expression of the Vel antigen. Furthermore, a shifted deletion encompassing nucleotides 64–80 in exon 3 causes the loss of 17 amino acids from the Vel glycoprotein, leading to non-expression of the Vel antigen. This genetic alteration significantly impacts the structure and function of the Vel glycoprotein, effectively rendering it undetectable on the surface of RBCs. Such mutations play a crucial role in transfusion medicine by potentially influencing the compatibility of blood types between donors and recipients.

Table 1. Polymorphisms in *SMIM1* gene.

Phenotype	Allele name	Nucleotide change	Exon intron	Predicted amino acid change	rs number
Vel_{strong}	*VEL*01.01*	c.-75-335A>G	i2	none	rs1175550
Vel−	*VEL*01N.01*	c.64_80delAGCCTA GGGGCTGTGTC	3	p.Ser22Glnfs*	rs566629828
Vel_{weak}	*VEL*01W.01*	c.152T>A	4	p.Met51Lys	rs1182690110
Vel_{weak}/Vel−	*VEL*01W.02*	c.152T>G	4	p.Met51Arg	rs1182690110
Vel_{weak}	*VEL*01W.03*	c.161T>C	4	p.Leu54Pro	n.a.
Vel_{weak}	*VEL*01W.05*	c.122G>A	4	p.Arg41Lys	n.a.

n.a.: not available.

3. Physiological Functions of the Vel Blood Group Antigen

Erythrocyte blood group antigens have several physiological functions, e.g., blood group glycoprotein C (GYPC) is a minor salivary glycoprotein in the human erythrocyte membrane and plays an important role in regulating erythrocyte stability. The Rh-associated glycoprotein (RHAG) correlates with the expression of Rh blood group antigens and is involved in ammonia transport in the erythrocyte membrane. The function of Vel blood group antigens is highly dependent on their dimeric structure and can be further impacted by associated proteins.

3.1. Formation of Vel Protein Multimers

The *SMIM1* gene is composed of four exons and three introns, of which exons 3 and 4 encode the functional protein, and exons 1 and 2 are non-coding. The theoretical molecular weight of the SMIM1 protein is approximately 8.7 kDa. However, SMIM1 exhibits varying molecular weights under different conditions due to its ability to form oxidation-dependent multimers: approximately 18 kDa under reducing conditions and around 32 kDa under non-reducing conditions. Consequently, in the SDS-PAGE analysis, SMIM1 does not migrate at its theoretical molecular weight.

Biochemical analyses and flow cytometry studies on cells expressing both wild-type and mutant alleles of *SMIM1* have revealed that the protein undergoes dithiothreitol-sensitive dimerization [15]. The process of dimerization contributes to an increase in Vel antigen expression on the cell surface. Specifically, SMIM1 dimerization is facilitated by extracellular homodimeric disulfide bonds formed through cysteine residue Cys77 and by GxxxG motifs within the transmembrane domain, which mediate helix-helix interactions.

The structural and functional characteristics of SMIM1, including its multimerization properties and the impact of specific amino acid residues on Vel antigen expression, highlight the complex nature of this protein. These findings provide valuable insights into the mechanisms underlying Vel antigen presentation and have significant implications for transfusion medicine and immunohematology.

The Vel antigen exhibits notable chemical stability, maintaining its antigenicity despite exposure to acidic conditions that typically cause denaturation. However, exposure to a reducing agent such as 200 mmol/L dithiothreitol (DTT) can compromise its antigenic properties. Moreover, certain proteolytic enzymes such as ficin, papain, trypsin, and α-chymotrypsin have been shown to markedly potentiate the reactivity of the Vel antigen.

These findings are instrumental in elucidating the clinical serologic profiles observed in patients, providing insights into the varying intensities of anti-Vel antibody responses. Such understanding is critical for precise blood typing and crossmatching, ultimately enhancing patient safety in transfusion medicine.

3.2. Vel Proteins and Regulation of Erythropoiesis

The gene *SMIM1* (NG_033869.1) is located in a 10-kb haplotype block at band 6 of region 3 of the short arm of chromosome 1. The gene *SMIM1* for the Vel antigen is in close proximity to the locus for the RH antigen (the *RH* gene is also located at 1p36); however, the linkage between *SMIM1* and the RH antigen remains undetermined.

SMIM1 protein is highly expressed in hematopoietic cells, but the exact mechanism of its involvement in red lineage development is unknown. In zebrafish knockdown of *SMIM1* gene, SMIM1 protein deficiency was found to be associated with low hemoglobin levels, suggesting its role as an erythropoietic regulator [16]. The STRING database shows that the majority of the SMIM1-related proteins are associated with the regulation of erythropoiesis, as illustrated in Figure 4 [17].

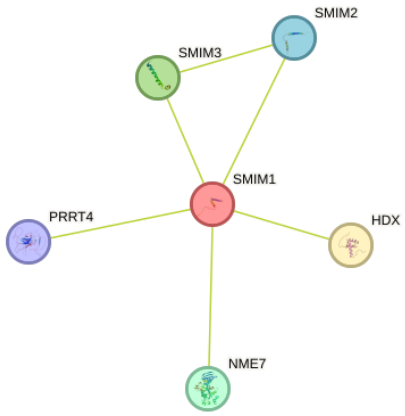

Figure 4. The proteins associated with SMIM1.

3.3. Vel Blood Group Antigens and Metabolism

The Vel blood group antigens may play a significant role in metabolic function, as evidenced by the metabolic characterization of individuals homozygous for the *SMIM1* deletion (homozygous Vel-negative). Studies utilizing plasma biochemistry, calorimetric chambers, and dual-energy X-ray absorptiometry (DXA) scans have revealed that these individuals exhibit a range of metabolic traits [18]. These include increased adiposity, signs of inflammation, altered liver function, and changes in triglyceride and lipoprotein metabolism. These metabolic alterations appear to be at least partially attributable to reduced energy expenditure, which is a key risk factor for obesity.

In severe cases, these metabolic changes can lead to impaired insulin sensitivity and an elevated risk of developing metabolic syndrome. This condition is frequently accompanied by heightened susceptibility to cardiovascular disease—a correlation supported by analyses of drug prescriptions and electronic medical records. The observed metabolic disturbances, increased cardiovascular risks, and patterns of *SMIM1* expression are consistent with findings indicative of mild hypothyroidism resulting from SMIM1 deficiency.

Future research should aim to elucidate the precise mechanisms by which this small transmembrane protein influences metabolic processes. Understanding these mechanisms could uncover new therapeutic targets and opportunities for intervention, potentially leading to innovative treatments for metabolic disorders and associated conditions. The direct effects of the Vel blood group antigen on metabolism suggest a broader physiological significance than previously recognized, highlighting the need for further investigation into its functional role and clinical implications.

4. Clinical Significance, Relevant Testing and Screening of the Vel Blood Group

4.1. Clinical Significance of the Vel Blood Group and Vel Antibodies

The Vel antigen is a high-frequency blood group antigen, with its alloantibodies implicated in hemolytic transfusion reactions [19]. Individuals who are Vel-negative can develop alloimmune antibodies against the Vel antigen. Most anti-Vel antibodies are a mixture of IgG and IgM and can sensitize RBCs, resulting in a strong positive direct antiglobulin test (DAT) reaction. This sensitization can lead to significant hemolytic reactions upon transfusion. Anti-Vel has also been shown to react positively in monocyte monolayer assays, indicating its potential for causing severe acute or delayed hemolytic transfusion reactions.

Homozygous immunization against the Vel antigen can result in adverse transfusion reactions or hemolytic disease in fetuses and newborns [20]. Given the clinical significance of anti-Vel, it is imperative to identify Vel-negative donors to ensure safe transfusions for patients who have developed homozygous immunization. Transfusing Vel-positive blood into a Vel-negative individual or during pregnancy with a Vel-positive fetus can trigger the production of alloantibodies, potentially leading to severe hemolytic reactions. Due to the rarity of the Vel-negative phenotype, it is crucial to screen recipient blood samples for relevant antibodies to mitigate the risks associated with blood transfusion.

To address these challenges, innovative methods such as machine learning have been applied to classify and determine RBC antigens from genotyping array data. This approach offers an effective tool for predicting and screening for antigen-negative blood donors [21]. In genetic prediction studies, the Vel blood group has served as a model for assessing viability and narrowing down candidate lists of donors with rare blood types [22]. Effective blood management is especially critical for patients requiring organ transplantation who possess anti-Vel antibodies, as it can significantly impact the success and safety of the procedure [23].

In summary, the identification and management of Vel-negative individuals are vital components of transfusion medicine, ensuring patient safety and optimal outcomes in cases where anti-Vel antibodies are present. The application of advanced technologies like machine learning in antigen prediction enhances our ability to provide tailored and safer transfusion practices for patients with rare blood types.

4.2. Serologic Testing and Screening for the Vel Blood Group

The complexity of phenotyping and characterizing the Vel blood group stems from significant individual variability in Vel antigen expression and the inherent instability of human polyclonal antibodies utilized for this purpose. For patients who develop anti-Vel alloantibodies, receiving Vel-negative blood is crucial to prevent adverse transfusion reactions; however, such individuals are uncommon, and identifying them is challenging due to the lack of commercially available serologic reagents designed for detecting the Vel antigen.

Ensuring a supply of Vel-negative blood necessitates the identification of compatible donors, yet this process is hampered by the difficulty in achieving precise Vel blood typing. The variable expression levels of the Vel antigen and the scarcity of reliable anti-Vel typing sera contribute to these challenges. Current serological studies on the Vel blood group rely primarily on antibodies sourced from Vel-negative individuals who have generated an immune response following exposure to Vel-positive cells through transfusion or pregnancy. These antibodies must undergo a series of processes, including adsorption, dispersion, and purification, before they can be applied to serological screening. However, the effectiveness and consistency of antisera derived from different individuals can vary widely, leading to inconsistencies and potential instability in screening outcomes. Moreover, the complex expression patterns of Vel antigen in heterozygous deletions may result in false-negative results during testing.

To address these issues, researchers have explored innovative approaches, such as isolating the variable region sequences from anti-Vel-specific B cells obtained from homozygous immunized patients. By cloning these sequences into an immunoglobulin expression vector, they were able to produce recombinant anti-Vel antibodies that exhibit enhanced sensitivity, capable of recognizing even weakly expressed Vel antigen [24]. In another study, CRISPR/Cas9 technology was utilized to knock down the *SMIM1* gene in K562 cells, which laid the foundation for the subsequent construction of a cellular model of Vel antigen phenotype-negative cells, and provided a new solution to the identification of antibodies in Vel-negative individuals [25]. The advancement represents a significant step toward improving the accuracy and reliability of Vel blood group typing.

4.3. Molecular Biology Testing and Screening for the Vel Blood Group

The *SMIM1* gene, encoding the Vel blood group antigen, features a 17-base pair deletion in exon 3 that leads to the absence of Vel antigen expression. Identifying Vel-negative donors is paramount for providing safe transfusion support to patients lacking this antigen but poses a significant challenge for immunohematology laboratories.

Prior to the elucidation of the molecular basis for the Vel antigen, the screening process for identifying Vel-negative donors relied exclusively on serological methods using human antisera. However, this approach is constrained by the limited availability and variability of reagents, complicating efforts to ensure a sufficient supply of compatible blood for Vel-negative patients. The discovery of the genetic foundation behind Vel− has opened new avenues for more precise and efficient donor identification strategies, moving beyond the limitations of traditional serologic testing. The molecular mechanisms underlying the generation of the Vel− phenotype are specific and well-defined, making the use of PCR-SSP targeting the c.64_80del allele for genotyping an effective way to overcome the limitations of classical serology.

Since the molecular basis for the Vel-negative phenotype was identified as a 17-base pair deletion in exon 3 of the *SMIM1* gene, genotyping has emerged as the preferred method for identifying individuals with this rare trait. Molecular biology techniques not only elucidate the variability in Vel antigen expression but also enable efficient high-throughput screening. Various molecular protocols have been proposed for identifying and screening Vel-negative individuals, including PCR-RFLP, high-resolution melting curve analysis, real-time PCR, and Sanger sequencing. For regions like Arabia countries and Iran, where there is an increased need to secure blood supplies due to migration patterns, advanced technologies such as MALDI-TOF Mass Spectrometry combined with PCR-SSP have been employed to screen for and detect blood group allele frequencies [14].

The SNaPshot multiplex assay stands out for its ability to simultaneously genotype multiple rare blood groups, making it a valuable tool for screening rare blood donors. Incorporating genotyping for the *SMIM1*64_80del* allele into the SNaPshot protocol can enhance the detection of Vel-negative donors, ensuring safe transfusions for patients with rare blood types [11].

Vel antigen expression in blood donor populations is influenced by genetic variations within intron 2 of *SMIM1*, particularly SNP rs1175550. Understanding these molecular mechanisms across diverse populations can inform the development of robust methods for identifying Vel-negative donors. Genotyping of blood donors has already proven instrumental in providing compatible blood for patients with alloantibodies and in generating rare reagent cells through serological detection [26]. Studies assessing the concordance between serological phenotypes and genotypes of common blood group antigens in Omani blood donors highlight the importance of combining both approaches [27].

Moreover, certain anomalies in Vel antigen expression necessitate molecular tools for accurate interpretation. Negative or weak serological responses are often linked to the 17 bp deletion in *SMIM1* when present in a heterozygous form. However, other nucleotide changes, such as missense mutations at position 152 (152T>A or 152T>G), can also impact Vel expression. The A allele of SNP rs1175550, located in the erythrocyte-specific regulatory region of intron 2 in *SMIM1*, is associated with diminished transcription of the gene, further contributing to variability in Vel antigen levels.

5. Correlation between the Vel Blood Group and Diseases

Research into the interplay between blood types and disease susceptibility is of paramount importance, offering insights that can lead to improved patient care and public health strategies. A recent study involving a large cohort of 650 156 Danish blood donors with diverse secretor statuses and blood groups, including Vel, has revealed a significant association between reduced susceptibility to SARS-CoV-2 and individuals with the Lewis type Le^a antigen or blood group O. This finding underscores the complex relationship between blood group antigens and infectious diseases, potentially guiding future epidemiological studies and therapeutic approaches [28].

Beyond its established role as the Vel blood group antigen, emerging evidence from bioinformatics analyses suggests that SMIM1 may serve as a novel biomarker for cancer. Studies indicate that this protein could play a critical role in the development and progression of various tumors [29]. The introduction of a new qPCR assay for detecting imported human *Plasmodium* species further highlights the versatility of molecular techniques in transfusion medicine and infectious disease diagnostics [30].

The structural similarity between SMIM1 and glycoproteins, along with observations of its phosphorylation in erythrocytes infected by *Plasmodium falciparum* (*P. falciparum*), has led researchers to hypothesize that SMIM1 might be implicated in malaria pathogenesis. This hypothesis opens up an intriguing avenue for investigating the potential involvement of SMIM1 in parasitic infections, which could have implications for understanding host-parasite interactions and developing targeted interventions against malaria.

These findings expand our knowledge regarding the impact of blood group antigens on disease susceptibility and broaden the scope of SMIM1's biological functions beyond transfusion medicine, suggesting its potential roles in oncology and infectious diseases. Continued research in these areas promises to uncover more about the multifaceted nature of SMIM1 and its relevance to human health.

5.1. SMIM1 Protein and Tumors

Research has progressively illuminated the potential link between the *SMIM1* gene and its protein product with various types of cancer. In 2014, a study reported an intriguing correlation: the expression of *SMIM1* was notably upregulated in leukocytes from colorectal cancer patients following oxaliplatin-based chemotherapy [31]. This observation suggests a possible association between SMIM1 and the development of oxaliplatin-induced peripheral

neuropathy, a common side effect of this treatment. These findings hint that SMIM1's role may extend beyond erythropoiesis to influence certain disease states.

Further evidence supporting *SMIM1*'s broader significance comes from erythrocyte protein profiling studies involving hepatocellular carcinoma (HCC), cirrhosis, and healthy controls [32]. The research revealed significant differences in *SMIM1* expression among these groups, particularly highlighting elevated levels in HCC patients. This differential expression suggests SMIM1 could serve as a biomarker for early diagnosis of hepatocellular carcinoma. Immunohistochemical analysis confirmed increased *SMIM1* expression in the cancerous tissues of HCC patients, indicating its involvement not only in erythropoiesis but also potentially in tumor-induced erythrocytosis associated with liver cancer.

The DepMap Portal database corroborates these observations by revealing differential expression of SMIM1 across several tumor cell lines, with notably high expression in myeloid tumors. This aligns with other studies showing elevated SMIM1 levels in bone marrow and erythroleukemia cell lines [33], reinforcing the gene's potential role in hematopoietic malignancies.

Moreover, *SMIM1*'s high expression in kidney and breast tissues points to its possible involvement in the development of cancers within these organs. This expression pattern suggests that SMIM1 holds promise as a biomarker for diagnosing and treating various tumors, expanding its relevance beyond its traditional role in RBC formation.

Accumulating evidence indicates that SMIM1 plays a multifaceted role in both normal erythropoiesis and pathological conditions, including cancer. Its potential as a biomarker for early diagnosis and therapeutic targeting in multiple cancer types warrants further investigation, opening new avenues for personalized medicine approaches in oncology.

5.2. SMIM1 and Plasmodium Infection

Plasmodium vivax (P. vivax) is a pathogenic agent that infects human erythrocytes, with its development and the disease's pathological processes being closely associated with blood group antigens. *Plasmodium* species influence the expression and sequence variation of erythrocyte surface receptor genes [34]. For instance, *P. vivax* requires the Duffy antigen for successful invasion of reticulocytes, while *P. falciparum* utilizes sialic acid residues on glycoproteins and other receptors such as complement receptor 1 (CR1) and band 3 proteins to enter host erythrocytes. Disease phenotypes, including rosette formation, are influenced by ABO blood type, with type O individuals exhibiting a lower risk of severe malaria, whereas type A individuals may be more prone to developing severe forms of the disease.

It was noted that SMIM1 shares biochemical properties with glycoproteins, particularly glycoprotein A (GPA), a type I transmembrane protein [6]. The SMIM1 protein could play a critical role in the pathogenesis of malignant malaria infections, suggesting it might serve as an invasion receptor for *P. falciparum*. SMIM1 has been characterized as a type II transmembrane protein with phosphorylation sites within its cytoplasmic region, indicating its potential involvement in the interaction between *P. falciparum* and human erythrocytes during infection. Phosphorylation of erythrocyte membrane proteins, such as band 3, induced by *P. falciparum* infection, is crucial for parasite migration, likely due to weakened membranes resulting from the dissociation of phosphorylated band 3 proteins from the cytoskeleton, which disrupts the organization of the erythrocyte cytoskeleton. Genome-wide and phosphoproteomics analyses of *P. falciparum*-infected erythrocytes have revealed that SMIM1 undergoes phosphorylation during the lysosomal stage of the parasite's life cycle [35].

Structurally, SMIM1 is capable of forming part of the junctional membrane complex alongside glycoprotein C, Rh antigens, and the Kel glycoprotein [36]. Although a study on Kenyan children did not show an association between SMIM1 polymorphisms and severe malaria, the potential relationship between SMIM1 and the structural organization of the erythrocyte membrane, coupled with its phosphorylation in *P. falciparum*-infected erythrocytes and similarities to glycoproteins, suggests that the role of SMIM1 in *P. falciparum* infections merits further investigation [34].

The multifaceted interactions between *Plasmodium* parasites and erythrocyte surface receptors highlight the complexity of malaria pathogenesis. The emerging evidence pointing to SMIM1's involvement in these processes underscores the need for continued research into this protein's function, potentially leading to new insights into malaria biology and therapeutic targets.

5.3. Association of SMIM1 with Obesity and Dyslipidemia

The underlying cause of obesity is an imbalance in energy expenditure, which is due to the interaction of lifestyle, environmental and genetic factors. In a study with 488 376 participants, 104 individuals with the *SMIM1*-/- genotype were found. A comparative study with the homozygous *SMIM1*-positive population revealed significant metabolic differences between individuals who were homozygous for the loss-of-function genetic variant of small integral

membrane protein 1 (SMIM1) and the general population. Compared with *SMIM1*+/+ individuals, *SMIM1*-/- individuals were heavier, had dyslipidemia, and may be more prone to cerebral hemorrhage and thrombotic stroke; they were more likely to be on statins, and exhibited characteristics similar to episodes of metabolic syndrome. SMIM1 is expected to have a direct effect on dyslipidemia and liver function. Reduced energy expenditure due tomild hypothyroidism may be the underlying cause [18].

6. Summary and Perspectives

The study of the Vel blood group has made remarkable multifaceted progress in recent years. By delving into the molecular mechanisms underlying Vel antigen generation and combining this with the design of specific primers, researchers have provided new approaches for large-scale screening of rare blood groups. These advancements can significantly reduce the time and cost associated with screening, enhance screening efficiency, and promote the application of the Vel blood group identification in both laboratory and clinical settings. This work also facilitates the identification of other rare blood groups with similar molecular mechanisms.

In the field of transfusion medicine, a deeper understanding of the Vel blood group system enables more accurate identification of Vel-negative phenotypes, thereby effectively preventing hemolytic transfusion reactions and ensuring transfusion safety. Additionally, research on the associations between the Vel blood group and conditions such as tumors, *P. vivax* infection, and obesity has opened up new avenues and potential applications in these areas.

Looking ahead, with the continuous innovation in technology and the deepening of interdisciplinary research, the study of the Vel blood group is poised to make further breakthroughs and contribute significantly to the advancement of medical science.

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Author Contributions

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Ethics Statement

Not applicable.

Informed Consent Statement

Not applicable.

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Declaration of Competing Interest

The authors declared no conflict of interests.

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