


Identification of a novel single-nucleotide mutation in *SMIMI* gene that results in low Vel antigen expression

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NOVEL ALLELE REPORT

Vel-negative individuals may become alloimmunized after blood transfusion with Vel-positive blood or through pregnancy after carrying a Vel-positive child, potentially leading to transfusion reactions or to hemolytic disease of the newborn in subsequent pregnancies. Previously it was found that the single-pass transmembrane protein *SMIMI* harbors the Vel blood group system, ISBT34.¹⁻³ Vel-negative individuals have a homozygous 17-bp deletion (c.64-80del) in *SMIMI*, but specific single-nucleotide polymorphisms in *SMIMI* additionally regulate Vel expression. For instance, weak Vel expression is correlated with single-nucleotide polymorphism rs1175550 in Intron 2, with the minor G allele associated with higher *SMIMI* transcript levels compared to the major A allele,^{1,4} and previously described single heterozygous missense mutations in Exon 4 of VEL*01 W.01 (c.152T>A, p.Met51Lys) and of VEL*01 W.02 (c.152T>G, p.Met51Arg) are shown to significantly decrease Vel expression.⁴ To facilitate appropriately matched transfusions it is important to identify mutations in *SMIMI* that lead to very weak Vel expression that are difficult to phenotype when needed.

Nine putative Vel-negative donors were identified by automated serologic screening using a human anti-Vel serum (BN60) in a period of 3 weeks (1488 donors tested). Genotyping of the *SMIMI* gene confirmed that one donor was homozygote for the c.64_80del in *SMIMI* and thus indeed Vel negative. In seven donors heterozygosity was revealed for c64_80del and AA for rs1175550 (see Fig. 1A). In one donor, who was homozygous AA for rs1175550, a novel mutation was detected: *SMIMI**c.161T>C (p.Leu54Pro; GenBank Accession Number MK783942; see Fig. 1B). This missense mutation resulted in an amino acid change in the transmembrane region of *SMIMI*. The Leu54 is a conserved hydrophobic amino acid, and the change into proline is deleterious according to in silico predictive methods PolyPhen-2, PROVEAN, and mutation tasting (*SMIMI* NM_001163724.2 c.161T>C; Fig. 1C). Manual serology analysis of the red blood cells (RBCs) carrying the *SMIMI**c.161T>C mutation showed that Vel expression was not detected by human anti-Vel serum (MB46) by column cards (see Fig. 1D) or tube testing, but was only detected by

agglutination with anti-Vel monoclonal antibody M5F3S (data shown in van der Rijst et al.⁵). Flow cytometry on the RBCs of this *SMIMI* missense mutation donor showed that the Vel antigen could be detected but was very low. The RBCs of this donor had lower Vel antigen expression compared to RBCs from donors with a heterozygous 17-bp deletion in *SMIMI* (see Fig. 1E). Ectopic expression of wild-type *SMIMI* and *SMIMI**c.161T>C in HEK293T cells showed that *SMIMI**c.161T>C resulted in lower Vel expression compared to wild-type (Fig. 1F) but did not show the same low Vel expression as was observed in RBCs.

Here we identified a novel *SMIMI* mutation, p.Leu54Pro, resulting in very weak Vel expression on RBCs. As the expression levels were lower than in donors with a heterozygous deletion, as was the case for two previously described *SMIMI* mutations VEL*01 W.01 and VEL*01 W.02,⁴ this weak Vel expression suggests a dominant negative effect of the mutated *SMIMI* protein. This missense mutation is not present in the gnomAD database containing more than 125,000 exomes (<https://gnomad.broadinstitute.org/gene/ENSG00000235169>, accessed April 10, 2019).

In conclusion, to date all reported heterozygous *SMIMI* missense mutations in donors with very weak Vel expression

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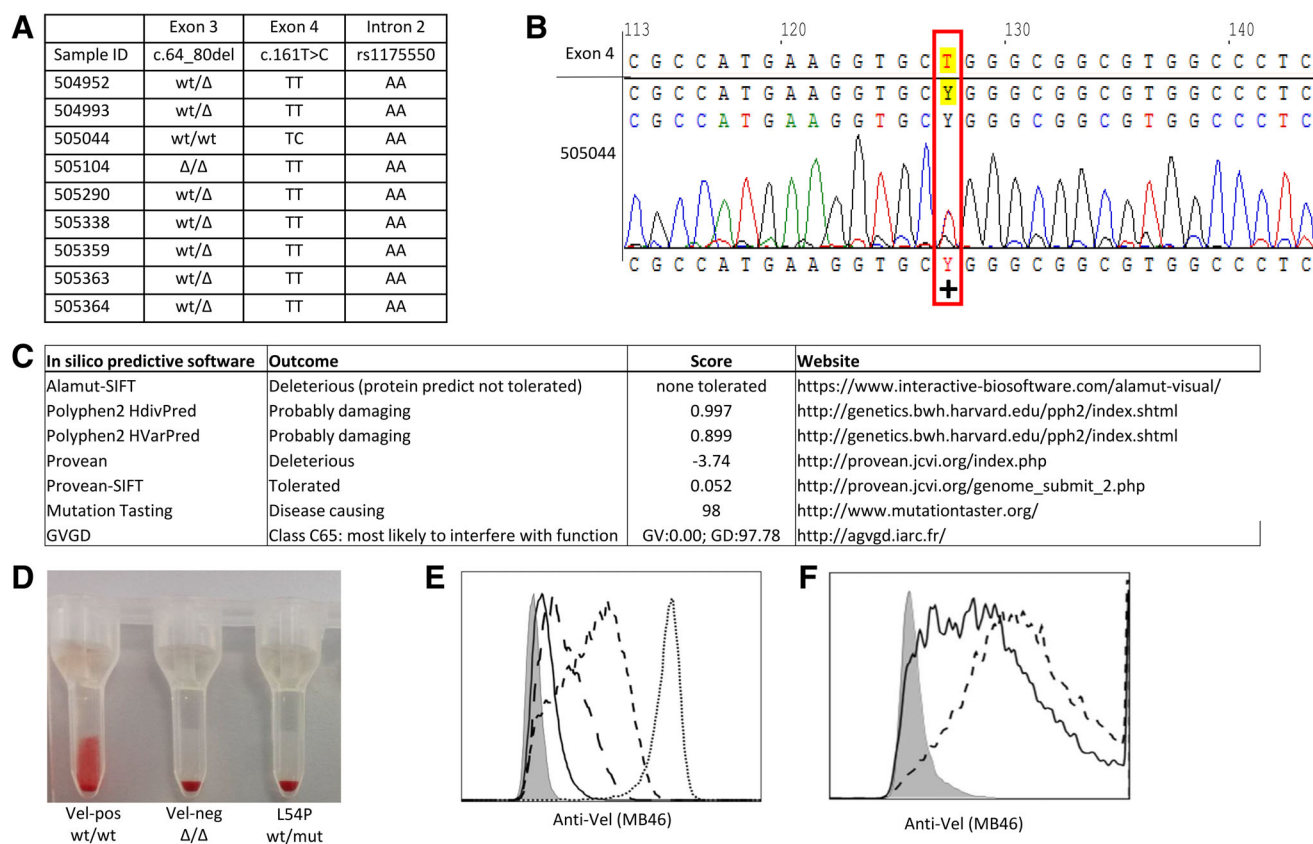


Fig. 1. (A) Genotyping analysis of the *SMIM1* gene of nine putative Vel-negative donors. (B) Sequence analysis of a novel heterozygote mutation in exon 4 of the *SMIM1* gene: *SMIM1161T>C (p.Leu54Pro). (C) Scores and outcome of the used in silico predictive methods. (D) Serology analysis with serum of MB46 of p.Leu54Pro in column cards with a Vel-positive donor as positive control (Vel-pos wt/wt) and a homozygote deletion donor as a negative control (Vel-neg Δ / Δ). (E) Flow cytometry Vel expression analysis of *SMIM1* genotyped RBCs: Vel-negative (Δ / Δ ; filled), *SMIM1**161T>C (p.Leu54Pro; solid), heterozygote donor (wt/ Δ ; long-dashed), and Vel-positive (wt/wt + rs1175550 AA; dashed, wt/wt + rs1175550 GG; dotted). (F) Flow cytometry analysis of transfected HEK293T cell line with pcDNA3.1+ empty vector (filled), wild-type *SMIM1* (dashed), and *SMIM1**161T>C (p.Leu54Pro); solid. Mut = mutation; wt = wild-type; Δ = c.del64_80.**

show to exert the same dominant negative effect on wild-type *SMIM1* resulting in very weak Vel expression. The origin of this dominant effect remains however unknown. From a clinical point of view, it is important to genotype for the c.64_80del mutation to identify Vel-negative RBC donors, as this case again demonstrates that serology might fail to identify donors carrying a missense mutation as Vel very weak.

STUDY DESIGN AND METHODS

Donor material

Dutch donors were serologically screened for the Vel blood group by automated screening using a very strong human anti-Vel serum (BN60 titer 1:32) on the Magister Blood Grouping System (Sanquin Reagents). Screened Vel-negative RBC were cryopreserved.⁴ Informed consent was in accordance with the Declaration of Helsinki and Dutch national and Sanquin internal ethics boards.

Flow cytometry

Flow cytometry analysis was performed as described previously.⁵ HEK293T cells were transfected with either empty pcDNA3.1+ vector (Invitrogen), wild-type *SMIM1*, or *SMIM1**c.161T>C (double-stranded DNA ordered at gBlocks IDT) as described previously.⁵ Antibodies used were as follows: immunopurified anti-Vel (MB46) as primary antibody and Alexa Fluor-647 anti-human IgG (Invitrogen) as secondary antibody. Results were acquired on a flow cytometer (BD CantoII/LSRII/Fortessa, BD Bioscience, Breda, the Netherlands) and analyzed using computer software (FloJo, Treestar).

Agglutination assays

Donor blood samples were serologically tested with anti-Vel serum of immunized patients. Automated screening on the Magister was performed by adding 20 μ L anti-Vel serum (BN60) with 40 μ L 0.6% (vol/vol) RBCs in low-ionic-strength saline (LISS) in screen column cards (both Cellbind, Sanquin

Reagents), for 15 minutes at 37°C; subsequently the cards were centrifuged with increasing speed: 120 sec at 75 g, 60 sec at 200 g, and 420 sec at 1790 g. After centrifugation the reactions in the column cards were photographed and thereupon interpreted by the computer software (Magister). Manual testing was performed as described previously,⁵ with anti-Vel serum MB46, using standard LISS/antiglobulin test column cards (BIO-RAD) or tubes.

Genetic analysis

All donor samples were genotyped for the *SMIM1* gene using a Taqman assay specific for the c.64_80del, Exons 3 and 4, and Intron 2 as described previously.^{4,5} Sequence reactions were performed on a thermocycler (Veriti, Thermo Fisher Scientific), and sequence products were analyzed on a genetic analyzer (3130, Applied Biosystems) with use of computer software (ContigExpress module of Vector NTI Advance 11.5.4 software, Invitrogen) and Vel-reference sequence (ENST000004444870.2).

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AUTHOR CONTRIBUTIONS

LV and JJ performed the automated screening. MvdR performed the other experiments. MvdR, EvdA, and EvdS designed the experiments, analyzed the data, and wrote the manuscript. The manuscript was critically revised by all authors. The authors declare no competing financial interest.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest

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