# A new *RHD* variant allele (*RHD* Gly339Val) shows weakened D expression compared to *RHD* Gly339Glu and Gly339Arg mutants

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We report a new *RHD* variant allele (*RHD* 1016G>T, Gly339Val, GenBank Accession No. MF737523, ISBT nomenclature *RHD*DEL49*.) that was discovered in a Korean male hepatocellular carcinoma patient. We have performed epitope mapping of the D antigen and analyzed the expected effect of this variation with comparison to two other D weak–expressing variants with an amino acid change in the same position.

Pretransfusion testing of D blood group was performed: two commercial anti-D reagents (Ortho Clinical Diagnostics and Millipore) were used for D and weak-D typing, and results indicated no reactions in both routine D and weak-D testing. Further testing with multiple clones of anti-D reagents (D-screen, Diagast) showed no agglutination with weak-D tests, but positive with adsorption and elution test to Epitopes 2.1 through 6.4 (Fig. 1C). Serotyping for RhC/c and RhE/e antigens using an immediate-spin tube test with monoclonal anti-C, -c, -E, and -e (Ortho Clinical Diagnostics) in saline-filled test tubes; results showed as Ccee. Genotyping of all exons and surrounding intron regions of the *RHD* gene was done by sequencing methods established in our laboratory.[1] Although zygosity testing was not performed, hemizygosity was presumed as no observation of heterozygous single-nucleotide variants observed in the extent of the sequenced *RHD* gene was found, and hemizygous background is the most likely cause in this kind of situation. We observed a hemizygous single-nucleotide change in Exon 7 (*RHD* 1016G>T) compared to the reference cDNA sequence NM_016124.4, which translated to amino acid change of glycine to valine at p.339. The amino acid change is located in the transmembrane region between the fifth and sixth exofacial loops of the RHD protein (Figs. 1A and 1B; analysis performed with open-source online software Protter[2]). We speculate that this change resulted in the loss of Epitope 6.4 and beyond in our epitope mapping analysis (Fig. 1C). This is in concordance with reports of *RHD-CE-D* hybrids that show loss of Epitope 6 is often found with the substitution of Exon 7 of the *RHD* gene.[3]

Review of previously reported *RHD* variants at p.339 showed two known weak D types: weak D Type 7 (*RHD* 1016G>A, Gly339Glu) and Type 39 (*RHD* 1015G>A, Gly339Arg), both found in the Rhesus site.[4] To understand the effect of the amino acid change of *RHD* at p.339, we compared the results for each variant with the PROVEAN algorithm.[5] Results suggested that Gly339Val would be the most damaging effect on protein structure compared to the reference protein, followed by Gly339Arg and Gly339Glu. We suspect that the intensity of D antigen was weakest in the new allele found in this report, followed by Type 39 and Type 7. The literature indicated that the antigen strength of Type 39 and Type 7 as "weak D." In comparison, D antigen was only detected by adsorption and elution, suggesting Del phenotype in Gly339Val. Thus, we conclude that in silico analysis is in concordance with phenotypic results.

In conclusion, we have discovered a novel variation with amino acid change within the transmembrane region of the D protein. This resulted in extremely weakened D expression, along with loss of epitopes expression in the downstream location of the variation. Partial D expression can potentially cause alloimmunization when transfused with D+ blood, so we suggest that this phenotype is be treated as D– in RBC transfusion. Identification of the location of the single-nucleotide variation can be helpful in predicting the characteristics of the changed D protein. Furthermore, we utilized in silico tools in

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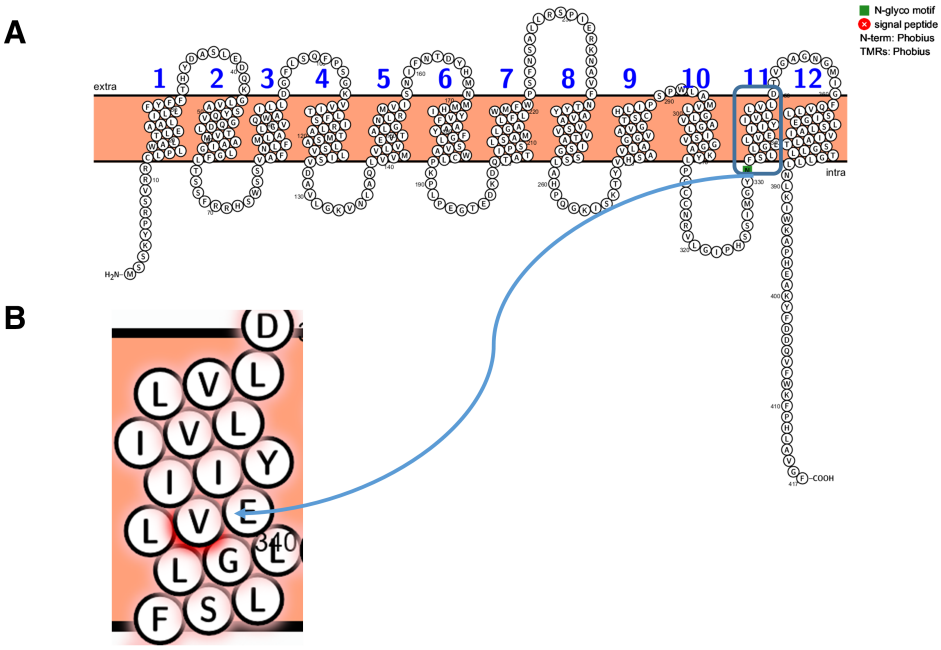


C

| D-epitope | anti-D clone | weak D test | Elution |
|---|---|---|---|
| | Ortho Bioclone | **(-)** | (+) |
| 2.1 | P3x249 | **(-)** | (+) |
| 3.1 | P3x290 | **(-)** | (+) |
| 5.4 | P3x35 | **(-)** | (+) |
| 5.4 | P3x241 | **(-)** | (+) |
| 6.4 | HM16 | **(-)** | (+) |
| 6.4 | P3x61 | **(-)** | **(-)** |
| 6.6 | HM10 | **(-)** | **(-)** |
| 8.2 | P3x21211F1 | **(-)** | **(-)** |
| 9.1 | P3x21223B10 | **(-)** | **(-)** |

**Fig. 1. Two-dimensional topology of D protein and weak-D test results on various anti-D reagents. Two-dimensional topology shows that the nucleotide change results in an amino acid change inside the 11th intermembrane region (A). Amino Acid Sequence 339 was changed from the reference glycine to valine (B). Weak-D tests, including adsorption and elution tests, showed that this variant shows negative to routine indirect anti-human globulin testing and only shows positive by adsorption and elution tests (described in C). Furthermore, Epitopes 6.6 and beyond are not expressed in this variant.**

predicting the impact of the amino acid change and associated D antigen expression.

### CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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