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REPORT OF NEW ALLELES OR ANTIGENS

TRANSFUSION

# Novel *RHD* allele with c.333C>G change predicted to encode p.Phe111Leu

**Gauthier Alluin**[1] | **Elisabeth Durieux-Roussel**[1] | **Pierre Fayoux**[2] | **France Pirenne**[3,4] | **Christophe Tournamille**[3,4] | **Aline Floch**[3,4]

[1]Laboratoire d'immunohématologie, Etablissement français du sang Hauts-de-France-Normandie, Lille, France

[2]ORL et Chirurgie Cervico-faciale Pédiatrique, CHU Lille, Lille, France

[3]Laboratoire de Biologie Médicale de Référence en Immunohématologie Moléculaire Henri Mondor, Etablissement français du sang Ile de France, Créteil, France

[4]Université Paris Est Créteil, INSERM U955, Equipe Transfusion et maladies du globule rouge, IMRB, Créteil, France

**Correspondence**
Aline Floch, Établissement français du sang, LBMR IHM, 5 rue Gustave Eiffel batiment UITC, 94000 Créteil, France.
Email: aline.floch@efs.sante.fr

## 1 | INTRODUCTION

The D (RH1) antigen is a major antigen in immunohematology, both from a transfusion point of view due to its immunogenicity and polymorphism, but also from an obstetrical point of view. It may be responsible for hemolytic transfusion reactions and hemolytic disease of the fetus and newborn.[1,2]

We investigated the sample of a 17-year-old French female of Congolese ancestry, hospitalized for an otolaryngology intervention, with no known history of transfusion or pregnancies.

## 2 | BRIEF METHODS

Serologic typing was performed using multiple clones of monoclonal anti-D (Table 1) by automation with Qwalys 3 EVO (Diagast, Loos, France), IH-500 (BioRad, Hercules, CA), and Innova (Ortho Clinical Diagnostics, Raritan, NJ) instruments and manually by gel-test with ID-partial RhD typing set, ID-Diaclon anti-D (BioRad), and clone HM16 (Diagast).

**Abbreviation:** RBC, red blood cells.

**TABLE 1** Testing with anti-D clones

| Reagent and specific clones | Reactivity |
|---|---|
| Automation | |
| Qwalys (Diagast) | |
| P3X61 (IgM) | 4+ (IS) |
| Innova (Ortho Clinical Diagnostic) | |
| Anti-D well: D7B8 (IgM) | 4+ (IS) |
| Anti-CDE well: MAD2 (IgM) | 2+ (IS) |
| IH-500 (BioRad) | |
| LHM59/20 (LDM3)+175-2 (IgM) | 3+ (IS) |
| Manual testing | |
| Diagast | |
| HM16 (IgG) | 3+ (IAT) |
| Biorad | |
| ID-Diaclon anti-D (ESD1) (IgG) | 3+ (IAT) |
| ID-partial RhD typing Set (BioRad) | |
| LHM 76/55 (IgG) | 3+ (IAT) |
| LHM 77/64 (IgG) | 3+ (IAT) |
| LHM 70/45 (IgG) | 0 (IAT) |
| LHM 59/19 (IgG) | $1+^{w}$ (IAT) |
| LHM 169/80 (IgG) | 3+ (IAT) |
| LDM1 (IgM) | 3+ (IS) |

Abbreviations: IAT, indirect antiglobulin test; IS, immediate spin.

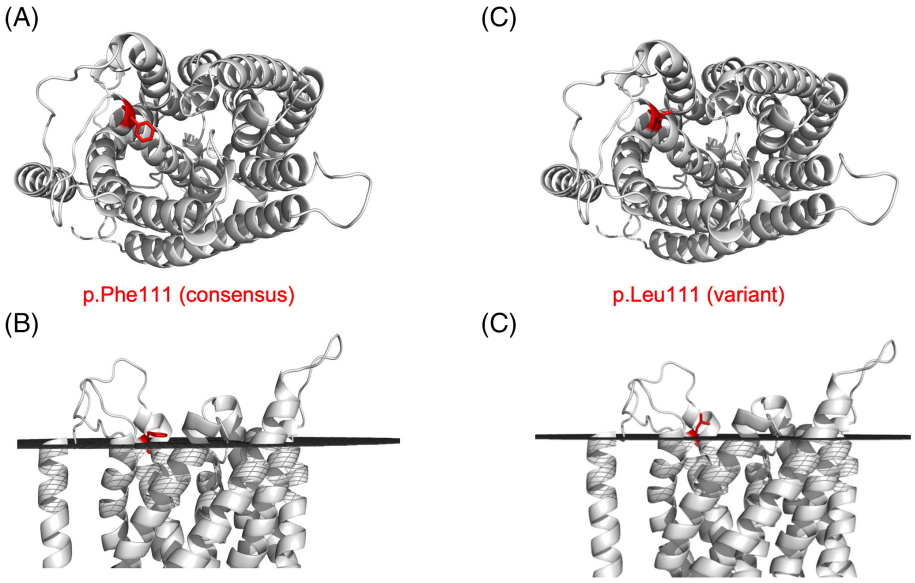


**FIGURE 1** Position of residue p.111 of the RhD protein according to the 3D model by Floch et al.[3] The consensus p.Phe111 (panels A and B) and the substituted p.Leu111 (panels C and D) are colored red and viewed from the extracellular perspective (A and C) and from the lateral perspective with the lipid bilayer limit as mesh (B and D).

Genomic DNA was extracted from white blood cells (NucloMag, Macherey-Nagel, Hoerdt, France) and RHD Beadchip (Immucor, Warren, NJ) and Sanger sequencing of *RHD* exons 1–10 and flanking intron regions was performed. To determine the associated *RHCE* allele, RHCE Beadchip (Immucor) was performed. For additional insight into the impact of the variant, 3D protein modeling was performed as previously described.[3]

## 3 | RESULTS

By automated techniques, the patient's red blood cells (RBC) typed D positive, with weakened reactivity in the CDE well of the Ortho gel (2+) and on IH-500 (3+). By the Biorad ID partial RhD typing set, the RBCs were nonreactive with LHM70/45, weakly reactive ($1+^{w}$) with LHM59/19, and positive with all other clones (3+). This pattern was inconclusive according to the interpretation of the manufacturer. Non-ambiguous typing by serologic means was not possible, justifying further exploration by molecular testing. The patient's RBCs also typed C–E–c+e+ with no reduced reactivity noted.

Molecular analysis by *RHD* Beadchip found no changes from conventional, but *RHD* sequencing found a homozygous c.333C>G change in exon 2, predicted to encode p.Phe111Leu. No changes were found in *RHD* exons 1 and 3–10. The sequence was deposited in Genbank with the accession number ON564310. The RHCE Beadchip showed *RHCE*ce* (*RHCE*01*) and *RHCE*ce733G* (*RHCE*01.20.01*).

By 3D protein modeling, residue p.111 is predicted to be in the second extracellular loop as shown in Figure 1. Intraprotein interaction analysis predicted a single hydrophobic interaction for the consensus p.Phe111 residue, while the change to p.Leu111 was predicted to form one hydrophobic interaction with p.Leu115 and hydrogen bonds between main chains and p.Thr109, p.Ile113, p.Arg114, and p.Leu115.

## 4 | BRIEF SUMMARY

We report a novel *RHD* allele with a c.333 C>G change predicted to be responsible for the amino acid substitution p.Phe111Leu, presumed to be in the second extracellular domain.[3] Although zygosity testing was not performed, the patient is most likely hemizygous for the new allele. Serologic testing suggests the lack of one or more epitopes. Differences were predicted in intraprotein interactions and could participate in additional alteration of D epitopes. Consequently, even in the absence of clinical evidence of anti-D, decision was made to consider this female of childbearing age as carrying a partial D. She will be provided D– RBCs for transfusion and considered a candidate for RhIG for future pregnancies.

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## CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

## ORCID

*Elisabeth Durieux-Roussel* https://orcid.org/0000-0002-9451-4277
*France Pirenne* https://orcid.org/0000-0003-3547-3994
*Christophe Tournamille* https://orcid.org/0000-0001-7889-5404
*Aline Floch* https://orcid.org/0000-0003-3238-4566

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