Profiling the mutational landscape of coagulation factor V deficiency

Elvezia Maria Paraboschi,[1] Marzia Menegatti,[2] Valeria Rimoldi,[1] Munira Borhany,[3] Magy Abdelwahab,[4] Donato Gemmati,[5,6] Flora Peyvandi,[2,7] Stefano Duga[1,8] and Rosanna Asselta[1,8]

[1]Department of Biomedical Sciences, Humanitas University, Milan, Italy; [2]Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, and Luigi Villa Foundation, Milan, Italy; [3]National Institute of Blood Disease and Bone Marrow Transplantation (NIBD), Karachi, Pakistan; [4]General Pediatric and Pediatric Hematology Departments, Cairo University Pediatric Hospital and Social and Preventive Medicine Center, Medical Center, Cairo, Egypt; [5]Department of Biomedical & Specialty Surgical Sciences and Centre Haemostasis & Thrombosis, University of Ferrara, Ferrara, Italy; [6]University Center for Studies on Gender Medicine, University of Ferrara, Ferrara, Italy; [7]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy and [8]Humanitas Clinical and Research Center, IRCCS , Rozzano, Italy.

Correspondence: ROSANNA ASSELTA - rosanna.asselta@hunimed.eu

doi:10.3324/haematol.2019.232587

SUPPLEMENTARY MATERIAL
Profiling the mutational landscape of coagulation factor V deficiency

Elvezia Maria Paraboschi[1], Marzia Menegatti[2], Valeria Rimoldi[1], Munira Borhany[3], Magy Abdelwahab[4], Donato Gemmati[5,6], Flora Peyvandi[2,7], Stefano Duga[1,8], Rosanna Asselta[1,8,*]

[1] Department of Biomedical Sciences, Humanitas University, Via Rita Levi Montalcini, 20090 Pieve Emanuele, Milan, Italy;
[2] Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, and Luigi Villa Foundation, Milan, Italy;
[3] National Institute of Blood Disease and Bone Marrow Transplantation (NIBD), Karachi, Pakistan;
[4] General Pediatric and Pediatric Hematology Departments, Cairo University Pediatric Hospital and Social and Preventive medicine center;
[5] Department of Biomedical & Specialty Surgical Sciences and Centre Haemostasis & Thrombosis, University of Ferrara;
[6] University Center for Studies on Gender Medicine, University of Ferrara;
[7] Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milano, Milan, Italy;
[8] Humanitas Clinical and Research Center – IRCCS –, Via Manzoni 56, 20089 Rozzano, Milan, Italy.

[*] **Corresponding author**:
Prof. Rosanna Asselta, PhD
Department of Biomedical Sciences
Humanitas University
Via Rita Levi Montalcini - 20090, Pieve Emanuele (Milano), Italy
phone: +39 02 82245215; fax: +39 02 82245290
email: rosanna.asselta@hunimed.eu

List of contents:

Supplementary Table 1: List of previously reported mutations in the *F5* gene with relevant references.

*cDNA level **	*Native protein*	*Mature protein ***	*Reference*
c.692T>G	p.Met231Arg	p.Met203Arg	1
c.1321C>T	p.Arg441Cys	p.Arg413Cys	2
c.2218C>T	p.Arg740X	p.Arg712X	3
c.2862delT	p.Ser955AlafsX4	p.Ser927AlafsX4	4
c.3088C>T	p.Arg1030X	p.Arg1002X	3
c.3296delA	p.Asp1099AlafsX72	p.Asp1071AlafsX72	5
c.3481C>T	p.Arg1161X	p.Arg1133X	6
c.3924_3927delTCAG	p.Ser1308ArgfsX24	p.Ser1280ArgfsX24	7
c.4906G>A	p.Glu1636Lys	p.Glu1608Lys	8
c.5189A>G	p.Tyr1730Cys	p.Tyr1702Cys	9
c.6304C>T	p.Arg2102Cys	p.Arg2074Cys	10
c.6305G>A	p.Arg2102His	p.Arg2074His	11
c.6419G>A	p.Gly2140Asp	p.Gly2112Asp	12
c.6443T>C	p.Met2148Thr	p.Met2120Thr	13
The HR2 Haplotype: p.Met385Thr, p.His1299Arg, p.Met1736Val, p.Asp2194Gly			14

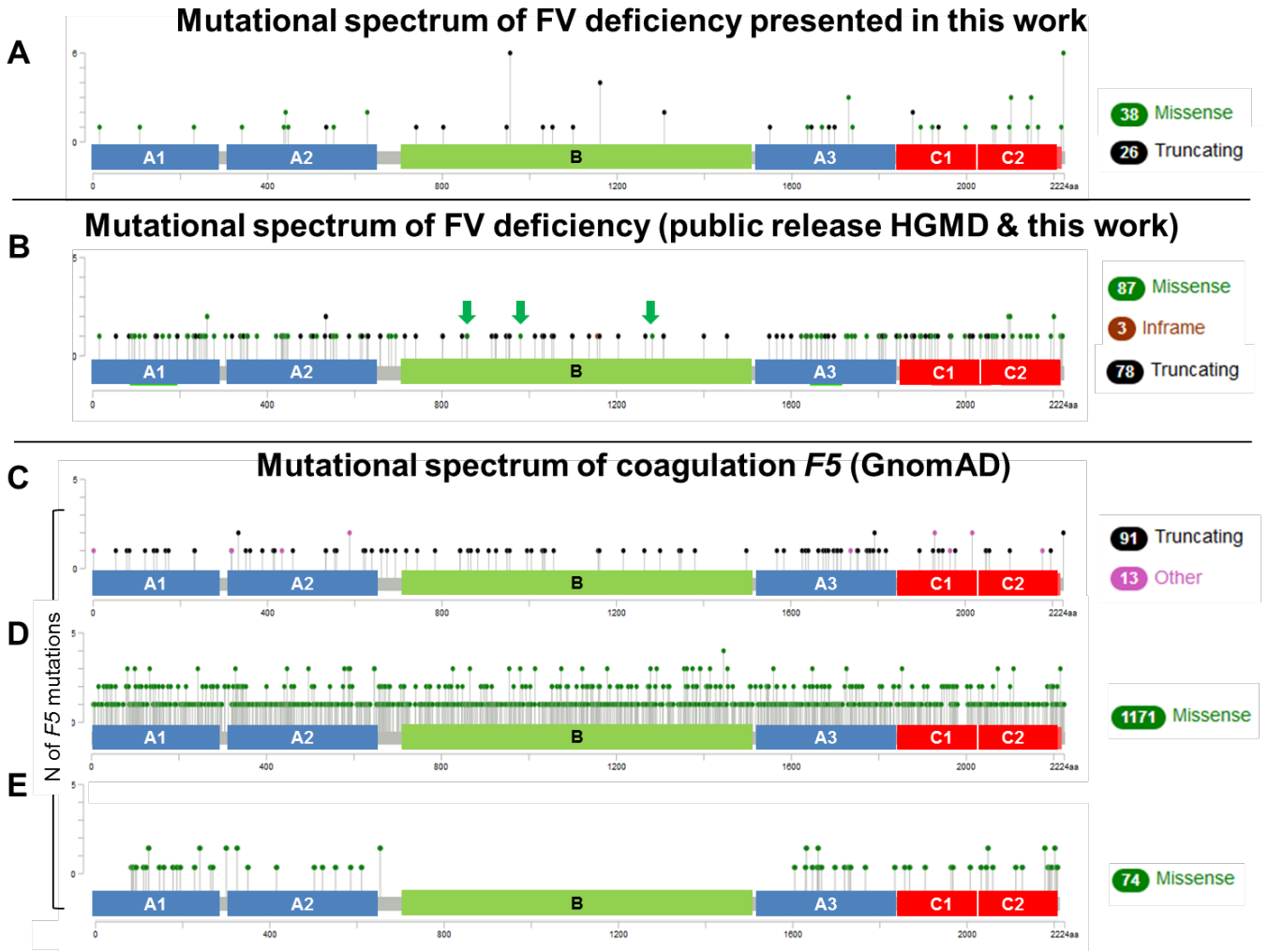
The table reports the previously reported mutations identified in our case series of 50 FV-deficient patients All mutations, are named following the official recommendations of the Human Genome Variation Society (HGVS, http://www.hgvs.org/mutnomen/recs-DNA.html). For mutation names at the protein level, the three-letter code annotation was adopted. In the case of the HR2 allele, the 4 major missense variants contributing to the haplotype are listed.

Nd, not done; Homo, homozygous; Hetero, heterozygous; Comp hetero, compound heterozygous.

* Numbering starting from ATG, according to NM_000130.4.

** Numbering omitting the signal peptide (28 amino-acid long).

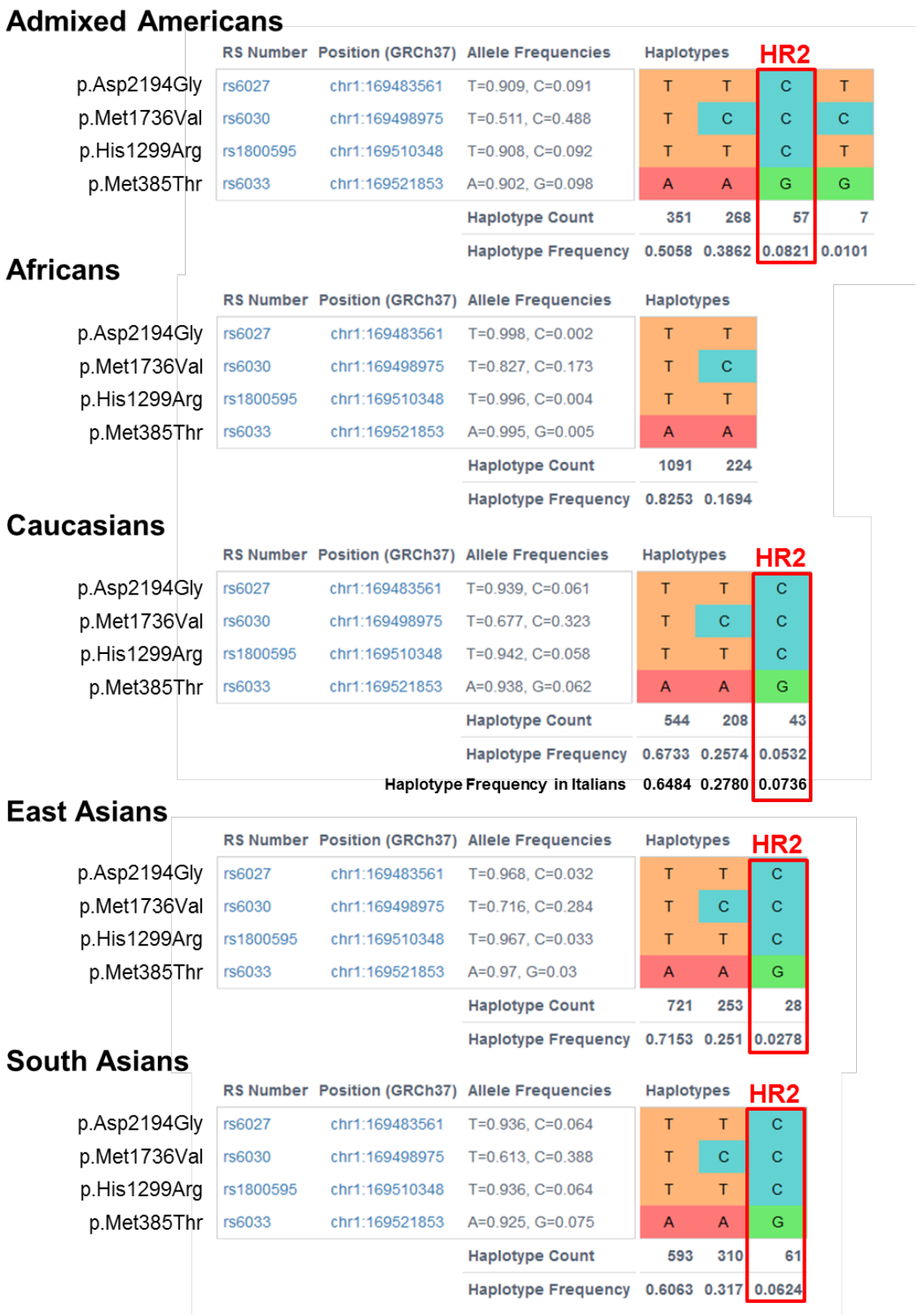
SUPPLEMENTARY FIGURE 1



Supplementary Figure 1: Mutational spectrum of the *F5* gene. The five lolliplots report *F5* mutations projected on the schematic A1-A2-B-A3-C1-C2 domain representation of the human FV protein. In all schemes, mutations are reported as circles (green for missense mutations; black for nonsense, nonstop, point frameshift deletion/insertion, and splice site mutations; brown for inframe deletions and insertions; violet for all other types

of mutations, such as long duplications). In all cases, except in panel B, the score on the left corresponds to the number of patients/individuals carrying the relevant mutation.
In particular:
A) Mutational spectrum emerging from the present work;
B) Mutational spectrum of FV deficiency merging the data from the present work together with those reported in the Human Gene Mutation Database (HGMD, public version, http://www.hgmd.cf.ac.uk/ac/index.php; accessed on April 05, 2019). The only three missense mutations identified in the B domain, are pointed by a green arrow;
C-E) Mutational spectrum of the *F5* gene emerging from the data present in the GnomAD repository (data on >125,000 exomes and >15,000 genomes from European, African, Asian, and Admixed American populations; https://gnomad.broadinstitute.org/; accessed on April 05, 2019; all populations were included in this representation). In panel C, all mutations except missense variants are reported. In panel D, only missense variants are present, independently from their pathogenicity. In panel E, all missense variants predicted ad damaging by three out of three predictions programs (MutationAssessor/SIFT/Polyphen-2) are reported. From the comparison between panels D and E, it emerges the high tolerance to missense substitutions of the unstructured B domain.
Lolliplots were produced using the NM_000130 RefSeq and the MutationMapper software, freely available through the Cbioportal (https://www.cbioportal.org/mutation_mapper). The the distribution of the different types of mutations in the *F5* genes in panel B is not significantly different from that reported in panels C+E (Pearson chi-square test =0.14).

SUPPLEMENTARY FIGURE 2



Supplementary Figure 2: Frequency distribution of the HR2 haplotype across different populations. The genotypes of the four main polymorphisms constituting the HR2 haplotype[14] were used to calculate the HR2 frequency distribution across different populations. Data on Admixed Americans, Africans, Caucasians, east Asians, and South Asians were retrieved from the 1000 Genomes Project reference panels (data from Phase 3, Version 5;

http://www.internationalgenome.org/). For the Italian population, we retrieved genotypes of the HR2 polymorphisms from our in-house cohort of 3541 individuals, for whom whole-exome sequencing (WES) data are available. WES was performed at the Broad Institute (Boston, MA, USA); exome capture methods, sequencing, variant annotation, and data processing of the samples have been described previously.[15]

Haplotypes were produced through the LDhap application web tool of the LDlink suite (https://ldlink.nci.nih.gov/?tab=ldhap).

SUPPEMENTARY REFERENCES

1. Chapla A, Jayandharan GR, Sumitha E, et al. Molecular basis of hereditary factor V deficiency in India: identification of four novel mutations and their genotype-phenotype correlation. Thromb Haemost. 2011;105(6):1120-1123.

2. Delev D, Pavlova A, Heinz S, Seifried E, Oldenburg J. Factor 5 mutation profile in German patients with homozygous and heterozygous factor V deficiency. Haemophilia. 2009;15(5):1143-1153.

3. Montefusco MC, Duga S, Asselta R, et al. Clinical and molecular characterization of 6 patients affected by severe deficiency of coagulation factor V: Broadening of the mutational spectrum of factor V gene and in vitro analysis of the newly identified missense mutations. Blood. 2003;102(9):3210-3216.

4. Ajzner E, Balogh I, Szabó T, Marosi A, Haramura G, Muszbek L. Severe coagulation factor V deficiency caused by 2 novel frameshift mutations: 2952delT in exon 13 and 5493insG in exon 16 of factor 5 gene. Blood. 2002;99(2):702-705.

5. Asselta R, Dall'Osso C, Duga S, Spreafico M, Saxena R, Tenchini ML. Coagulation factor V gene analysis in five Indian patients: identification of three novel small deletions. Haematologica. 2006;91(12):1724-1726.

6. van Wijk R, Montefusco MC, Duga S, et al. Coexistence of a novel homozygous nonsense mutation in exon 13 of the factor V gene with the homozygous Leiden mutation in two unrelated patients with severe factor V deficiency. Br J Haematol. 2001;114(4):871-874.

7. Guasch JF, Cannegieter S, Reitsma PH, van't Veer-Korthof ET, Bertina RM. Severe coagulation factor V deficiency caused by a 4 bp deletion in the factor V gene. Br J Haematol. 1998;101(1):32-39.

8. Peyvandi F, Duga S, Akhavan S, Mannucci PM. Rare coagulation deficiencies. Haemophilia. 2002;8(3):308-21.

9. Castoldi E, Simioni P, Kalafatis M, et al. Combinations of 4 mutations (FV R506Q, FV H1299R, FV Y1702C, PT 20210G/A) affecting the prothrombinase complex in a thrombophilic family. Blood. 2000;96(4):1443-1448.

10. Duga S, Montefusco MC, Asselta R, et al. Arg2074Cys missense mutation in the C2 domain of factor V causing moderately severe factor V deficiency: molecular characterization by expression of the recombinant protein. Blood. 2003;101(1):173-177.

11. Schrijver I, Houissa-Kastally R, Jones CD, Garcia KC, Zehnder JL. Novel factor V C2-domain mutation (R2074H) in two families with factor V deficiency and bleeding. Thromb Haemost. 2002;87(2):294-299.

12. Duckers C, Simioni P, Spiezia L, et al. Residual platelet factor V ensures thrombin generation in patients with severe congenital factor V deficiency and mild bleeding symptoms. Blood. 2010;115(4):879-886.

13. Scanavini D, Girelli D, Lunghi B, et al. Modulation of factor V levels in plasma by polymorphisms in the C2 domain. Arterioscler Thromb Vasc Biol. 2004;24(1):200-206.

14. Yamazaki T, Nicolaes GA, Sørensen KW, Dahlbäck B. Molecular basis of quantitative factor V deficiency associated with factor V R2 haplotype. Blood. 2002;100(7):2515-2521.

15. Do R, Stitziel NO, Won HH, et al. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. Nature. 2015;518(7537):102-106.