The Gly331Ser mutation in factor VII in Europe and the Middle East

The Gly331Ser mutation in factor VII (FVII) was found in the homozygous condition in several unrelated FVII deficient subjects from Southern Italy and from Germany, one of Turkish origin. Genotyping for several polymorphisms revealed that the mutation, occurring at a CpG site, is associated with two haplotypes, suggesting multiple occurrence.

haematologica 2003; 88:1434-1436
(*http://www.haematologica.org/2003_12/1434.htm*)

Following vascular injury, factor VII (FVII) binds to tissue factor and triggers blood coagulation. FVII deficiency,[1] a rare inherited clotting factor deficiency, produces a wide range of hemorrhagic phenotypes and is caused by a variety of molecular lesions.[2] The association between phenotype and the underlying clotting defect, and the threshold FVII levels able to guarantee coagulation are still debated.

We present here the study of the distribution and the haplotyping of the Gly331Ser mutation in several FVII deficient patients. Ten subjects from Southern Italy (group A) with FVII coagulant activity (FVIIc) ≤4% and normal FVII antigen (FVIIAg) were initially studied. Subjects A1-5 have been previously described[3] as PFVII33-37. Subjects A6 and A7 were asymptomatic. Patient A8 experienced gum-bleeding and was treated with recombinant activated FVII (rFVIIa) (NovoNordisk, Bagsvaed, Denmark), A9 had a central nervous system bleed at the age of six years and A10 was treated with rFVIIa after post-trauma hemorrhage.

Mutations were searched by MspI restriction analysis for the Gly331Ser change[3] and sequencing. Five subjects (A1-5) were homozygous for the Gly331Ser mutation and one (A6) was doubly heterozygous for the Gly331Ser and Cys310Phe changes (Table 1). Three patients (A7-9) were homozygous for the Cys310Phe, Ala294Val and Arg304Gln mutations in the FVII catalytic domain. All these mutations are responsible for dysfunctional FVII molecules.[4-6] No mutation in coding regions was detected in subject A10. The frequency of Gly331Ser among unrelated subjects from Southern Italy prompted us to investigate 22 additional subjects with mild to asymptomatic FVII deficiency (group B, mean FVIIc 31%, range 10-63%) from this geographical area. The Gly331Ser mutation was present in four heterozygotes (Table 1).

Moreover, five unrelated carriers of this change were identified among 189 German subjects (group C) included in the Greifswald Research Study on FVII Deficiency[7] (Table 1).

The Gly 331Ser-FVII variant is associated with a cross-reacting material positive (CRM+) FVII deficiency, as indicated by

Table 1. Geographical, coagulation and clinical features of the Italian (I) and German (G) carriers of the FVII Gly331Ser mutation.

Patient (G-PIN)	*Center*	*FVIIc (FVIIAg) %*	*Genotype (second mutation)*	*Clinical phenotype*	*Haplotype*
A1	Bari (I)	2	Homozygous	Asymptomatic	A1-G1-H1-b-M1
A2	Naples (I)	0.7 (85)	Homozygous	Asymptomatic	A1-G1-H1-b-M1
A3	Salerno (I)	0.7 (100)	Homozygous	Asymptomatic	A1-G1-H1-b-M1
A4	Napoli (I)	0.6 (100)	Homozygous	Mild	A1-G1-H1-b-M1
A5	Palermo (I)	1.1	Homozygous	Mild	A1-G1-H1-b-M1
A6	Naples (I)	4 (95)	D. het. (C310F)	Asymptomatic	A1-G1-H1-b-M1
B1	Naples (I)	25 (85)	Heterozygous	Asymptomatic	A1-G1-H1-b-M1
B2	Naples (I)	37 (85)	Heterozygous	Asymptomatic	A1-G1-H1-b-M1
B3	Naples (I)	41 (80)	Heterozygous	Asymptomatic	A1-G1-H1-b-M1
B4	Napoli (I)	47 (100)	Heterozygous	Asymptomatic	A1-G1-H1-b-M1
C1 (9490)	Hamburg (G)	6 (100)	D. het. (A294V)	Mild	A1-G1-H1-b-M1
C2 (9254)	Frankfurt (G)*	5.6	D. het. (A294V)	Asymptomatic	A2-G2-H2-a-M2
C3 (9291)	Kaiserslautern (G)	21	Heterozygous	Asymptomatic	#G1/2-H1/2-M1/2
C4 (9496)	Halle (G)	45	Heterozygous	Asymptomatic	#G1/2-H1/2-M1/2
C5 (9933)	Greifswald (G)	35	Heterozygous	Asymptomatic	A1-G1-H1-b-M1

*G-PIN: Identification Number of German Patients, enrolled in the Greifswald Research Study on FVII Deficiency. D. het.: doubly heterozygous #Family members were not available for this study. *Turkish origin.*

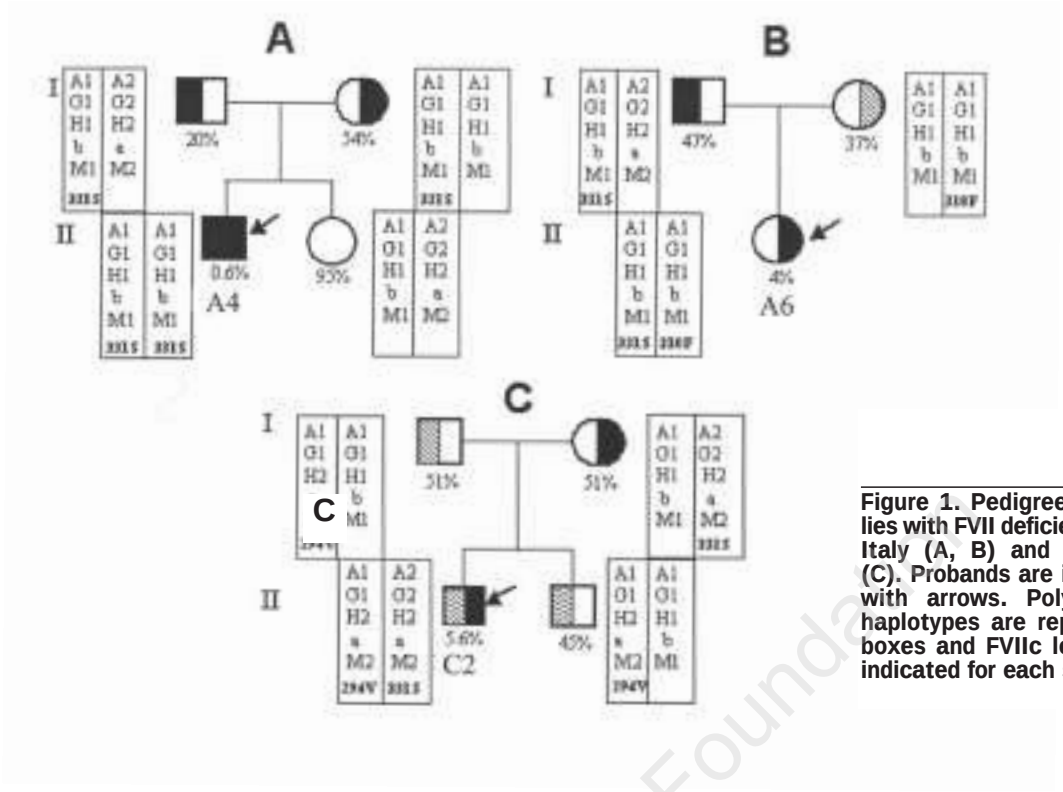

Figure 1. Pedigree of families with FVII deficiency from Italy (A, B) and Germany (C). Probands are indicated with arrows. Polymorphic haplotypes are reported in boxes and FVIIc levels are indicated for each subject.

the decreased FVIIc and the normal FVIIAg levels observed in patients' plasma (Table 1) and confirmed by previous and present expression studies performed in our laboratory. Expression of the FVII variant and functional assays were conducted as reported elsewhere.[3] FXa generation activity in patients' plasma was 0.7±0.2% of that observed in pooled normal plasma and, in reconstituted systems, the Gly 331Ser-FVII activity was 2.7±1.1% that of wild type FVII. These findings predict that this variant is able to trigger coagulation thus explaining the mild to asymptomatic phenotype observed in homozygotes, and the spread of the Gly331Ser mutation in Europe. One British heterozygous carrier of the Gly331Ser mutation and one French compound heterozygote for the Gly331Ser and the Gln100Arg mutations have been described (*http://europium.csc.mrc.ac.uk*).

The slightly different FVIIc levels observed among the Gly331Ser homozygotes (Table 1) could be explained by the different thromboplastins used in routine clotting assays. Carriers of the Gly331Ser mutation were further characterized for five FVII gene polymorphisms:[8-9] a 10bp insertion at -323 (A1/A2 alleles), 73G to A transition (G1/G2), 7880C to T transition at His115 (H1/H2), intron 7 repeat polymorphism (a/b/c/d) and Arg353Gln substitution (M1/M2).

The availability of three families allowed segregation studies to be performed. In the A4 pedigree (Figure 1A) the M2 allele, present in the father who was heterozygous for the Gly331Ser mutation, contributed to further reduction of FVII level.[10] Pedigree analysis of A6 (Figure 1B) revealed segregation of the Ser331 and Phe310 alleles. Segregation of the Ser331 and Val294 alleles was observed in family C2 (Figure 1C) of Turkish origin.

Genotyping for polymorphisms showed that the Gly331Ser mutation is associated with the same genetic background, A1-G1-H1-b-M1, in the Italian and German subjects, but it was associated with the A2-G2-H2-a-M2 haplotype in the Turkish family (Table 1). These findings support the independent origin of the mutation in Europe and in Turkey.

The recurrence of this transition may be considered in the light of its position (nt 10908), in a CpG dinucleotide, one of the several *hot spots* in the FVII gene prone to recurrent mutations.

The high incidence of the Gly331Ser mutation among unrelated subjects from Southern Italy, and its association with the same haplotype, led to a scenario of a founder effect in the past, likely followed by consanguineous spread. We suggest screening for this mutation in FVII deficient patients from this geographical area, through a simple Msp I restriction analysis. This strategy also reveals the frequent M1/M2 polymorphism able to predict reduced FVII levels. Analysis for the presence of the Gly 331Ser-FVII variant, whose residual activity prevents life-threatening bleeding symptoms in FVII deficient patients with very reduced FVIIc levels might also have implications for prevention and treatment of this disease.

Daniela Etro, Mirko Pinotti,* Karin Wulff,°*
Giovanna Marchetti, Angiola Rocino,#*
*Falko H. Herrmann,° Francesco Bernardi**

Dipartimento di Biochimica e Biologia Molecolare, Università di Ferrara, Italy; Institute of Human Genetics, University of Greifswald, Greifswald, Germany;° #Centro Emofilia e Trombosi, Ospedale Nuovo Pellegrini, Naples, Italy*

Key words: mutation, CRM+ FVII deficiency, founder effect.

Acknowledgments: the authors thank F. Capazzo, N. Ciavarella, G. Mariani, W. Kreuz, T. Bernig, B. Eifrig, R.M. Loret and H.H. Wolff for providing blood samples and clinical data.

Funding: the financial support of Telethon Italy (grant no. GGP02182) is gratefully acknowledged.

Correspondence: Professor Francesco Bernardi, Dipartimento di Biochimica e Biologia Molecolare, Università degli Studi

di Ferrara, via L. Borsari 46, 44100 Ferrara, Italy.
Phone: international +39.0532.291443.
Fax: international +39.0532.202723. E-mail: ber@unife.it

Manuscript processing

This manuscript was peer-reviewed by two external referees and by an Associate Editor. The final decision to accept this paper for publication was taken by the Editors. Manuscript received June 3, 2003; accepted October 6, 2003.

References

1. Perry DJ. Factor VII deficiency. Br J Haematol 2002;118: 689-700.
2. Cooper DN, Millar DS, Wacey A, Banner DW, Tuddenham EGD. Inherited factor VII deficiency: molecular genetics and pathophysiology. Thromb Haemost 1997;78:151-60.
3. Pinotti M, Etro D, Bindini D, Papa ML, Rodorigo G, Rocino A, et al. Residual factor VII activity and different hemorrhagic phenotypes in CRM(+) factor VII deficiencies (Gly331Ser and Gly283Ser). Blood 2002;99:1495-7.
4. Marchetti G, Patracchini P, Gemmati D, DeRosa V, Pinotti M, Rodorigo G, et al. Detection of two missense mutations and characterization of a repeat polymorphism in the factor VII gene (F7). Hum Genet 1992;89:497-502.
5. Bernardi F, Castman G, Redaelli R, Pinotti M, Lunghi B, Rodighiero, et al. Topologically equivalent mutations causing dysfunctional coagulation factors VII (Ala294Val) and X (Ser334Pro). Hum Mol Gen 1994;3:1175-7.
6. O'Brien DP, Gale KM, Anderson JS, McVey JH, Meade T, Miller G, et al. Purification and characterization of FVII-304 Gln: a variant molecule with reduced activity isolated from a clinically unaffected male. Blood 1991;78:132-40.
7. Wulff K, Herrmann FH and study group. Greifswald study on congenital factor VII deficiency. Gene diagnosis of inherited bleeding disorders. Pabst Science Publishers; Lengrich, Berlin, Riga, Rom, Wien, Zagreb. 2002. p. 127-34.
8. Bernardi F, Marchetti G, Pinotti M, Arcieri P, Baroncini C, Papacchini M, et al. Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma. Arterioscler Thromb Vasc Biol 1996;16:72-6.
9. Peyvandi F, Mannucci PM, Bucciarelli P, Zeinali S, Akhavan S, Sacchi E, et al. A novel polymorphism in intron 1a of the human factor VII gene (G73A): study of a healthy Italian population and of 190 young survivors of myocardial infarction. Br J Haematol 2000;108:247-53.
10. Green F, Kelleher C, Wilkes H, Temple A, Meade T, Humphries S. A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. Arterioscler Thromb 1991;11:540-6.

Genetic determinants of iron metabolism plasma phenotypes and their relationship with risk of thrombosis

This study evaluates the relative contributions of genetic and environmental factors to 6 iron-related phenotypes and the relationship between these phenotypes and the risk of thrombosis. All of these phenotypes were influenced significantly by genetics, with heritability ranging between 18% for transferrin saturation to 55% for soluble transferrin receptor (sTfR). Only sTfR exhibited a significant genetic correlation with thrombosis.

haematologica 2003; 88:1436-1438
(*http://www.haematologica.org/2003_12/1436.htm*)

The physiologic and biochemical pathways involved in iron metabolism are very complex. Given the spectrum of iron metabolism-related phenotypes, it is likely that there are a number of interacting genetic and environmental factors that jointly determine the variable expression. The primary objective of the present investigation was to examine the relative roles of genetic and environmental factors in determining iron-related phenotypes. To this end, we studied a series of extended Spanish kindreds, half of which were enrolled through individuals with thrombophilia. They were recruited in a project called GAIT (Genetic Analysis of Idiopathic Thrombophilia) designed to look for new genetic risk factors for thrombosis through the analysis of intermediate phenotypes.[1-3] Recent data on a possible association between the hemochromatosis gene mutation HFE Cys282Tyr and cardiovascular diseases[4-6] motivated us to include iron metabolism-related phenotypes in the GAIT Project. In 1981, Sullivan proposed a possible relationship between iron and ischemic heart disease. He hypothesized that iron depletion protects against myocardial infarction and could explain the sex differences in the rates of heart disease.[7] The ascertainment criteria for our families also allowed us to study the potential relationship of iron metabolism phenotypes with thromboembolic disease.

The details on the enrollment of families and statistical methods used in the GAIT Project can be found elsewhere.[1-3] Plasma iron, unsaturated iron binding capacity (UIBC) and soluble transferrin receptor (sTfR) were measured in a Hitachi 911 biochemical analyzer by colorimetric and immunoassay methods, respectively (Fe, UIBC and sTfR Tinaquant). Total iron binding capacity (TIBC) was obtained as the sum of plasma iron and UIBC. Circulating ferritin was evaluated using an electrochemiluminiscent immunoassay (Ferritina) in a biochemical analyzer (ELECSYS). All assays and the analyzer were from Roche Diagnostics (Mannheim, Germany). Transferrin saturation (SAT) was obtained by dividing iron values by TIBC values and was expressed as a percentage. Intra- and inter-assay coefficients of variation were less than 10% for all of the phenotypes. We logarithmically (ln) transformed the values of sTfR and ferritin to obtain normal distributions.

Table 1 presents the estimated components of the residual variance for the iron metabolism-related phenotypes. All of the traits had significant heritability (h^2), ranging between 18% and 55% of the residual phenotypic variability. The proportion of the residual phenotypic variability accounted for by shared *household effects* (χ^2) tended to be considerably smaller than that accounted for by genetic effects. Household effects were significant for only 2 traits: ferritin and SAT. The remaining variance (not accounted for in Table 1) was attributed to individual-specific random environmental influences and random error.

Table 2 shows the results of bivariate genetic analyses of thrombosis with each of the quantitative iron metabolism-