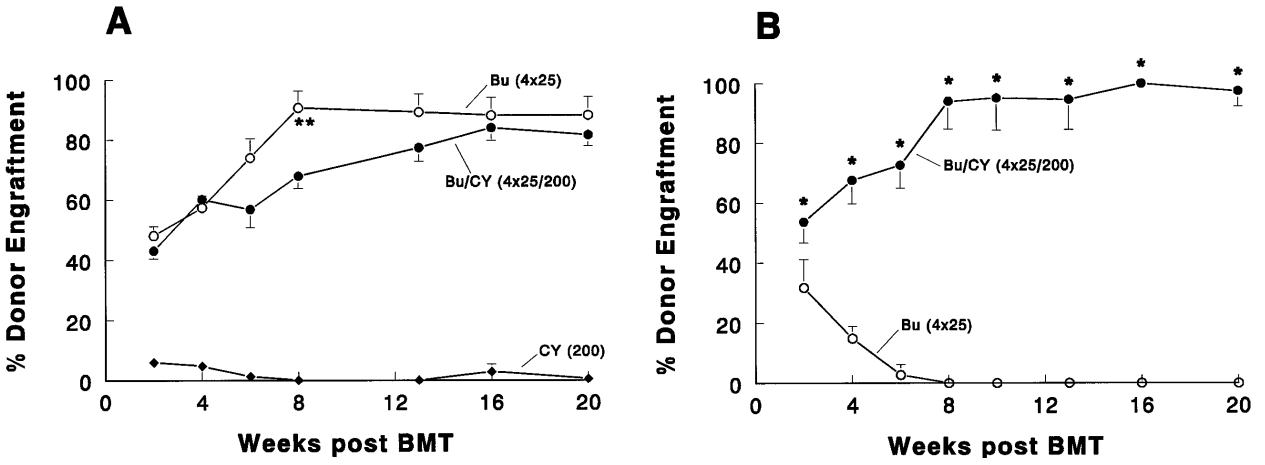

Fig 1. Syngeneic (A) and allogeneic (B) bone marrow engraftment after pretreatment with BU with or without CY. Male C57BL/6JIco (B6-*Gpi-1*^{b}/*Gpi-1*^{b}) mice (Iffa Credo, L'Arbresle, France), 12 to 16 weeks old and weighing 25 to 30 g, were used as recipients. Congenic C57BL/6J-*Gpi-1*^{a}/*Gpi-1*^{a} (B6-*Gpi-1*^{a}) and BALB.B10 mice (Jackson Laboratory, Bar Harbour, ME) were used as the source of syngeneic and H-2 compatible allogeneic donor bone marrow, respectively. Busulfan was injected intraperitoneally (IP) as a suspension in corn oil as fractionated doses (4 × 25 mg/kg) on 4 consecutive days. CY was administered (IP in phosphate-buffered saline, 200 mg/kg) 24 hours after (the last dose of) BU. BMT (10^6 bone marrow cells) was performed 24 hours after the last drug treatment or 48 hours after BU when CY was not in the regimen. Shown are the means (±SD, 4 to 6 mice per group) as percentages of Gpi-1^a type erythroid chimerism up to 5 months after BMT. Asterisks indicate significance in Mann-Whitney-U test (*$P < .01$; **$P < .05$).

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REFERENCES

1. O'Brien SG, Goldman JM: Busulfan alone as cytoreduction before autografting for chronic myelogenous leukemia. Blood 91:1091, 1998 (letter)

2. Down JD, Ploemacher RE: Transient and permanent engraftment potential of murine hematopoietic stem cell subsets: Differential effects of host conditioning with gamma radiation and cytotoxic drugs. Exp Hematol 21:913, 1993

3. Down JD, Boudewijn A, van Os R, Thames HD, Ploemacher RE: Variations in radiation sensitivity and repair among different bone marrow hemopoietic stem cell subsets following fractionated irradiation. Blood 86:122, 1995

4. Down JD, Westerhof GR, Boudewijn A, Setroikromo R, Ploemacher RE: Thiotepa improves allogeneic bone marrow engraftment without enhancing stem cell depletion in irradiated mice. Bone Marrow Transplant 21:327, 1998

5. Tutschka PJ, Santos GW: Bone marrow transplantation in the busulfan-treated rat. I. Effect of cyclophosphamide and rabbit antirat thymocyte serum as immunosuppression. Transplantation 20:101, 1975

6. Santos GW: The development of busulfan/cyclophosphamide preparative regimens. Semin Oncol 20:12, 1993

A Novel Factor V Null Mutation Detected in a Thrombophilic Patient With Pseudo-Homozygous APC Resistance and in an Asymptomatic Unrelated Subject

To The Editor:

Pseudo-homozygous APC resistance[1] is defined by finding the heterozygous factor V (FV) R506Q substitution (Leiden mutation,[2] 1691 G/A in the coagulation FV gene) in the presence of APC resistance ratios[3] similar to those of FV Leiden homozygotes. Although partial FV deficiency, invariably present in this condition, could compensate for the thrombophilic defect, all of the few cases reported so far are thrombophilic patients. The nature of the mutations responsible for FV deficiency in pseudo-homozygous APC resistance is still elusive, and their identification would make possible a more accurate diagnosis than that based on coagulation assays.

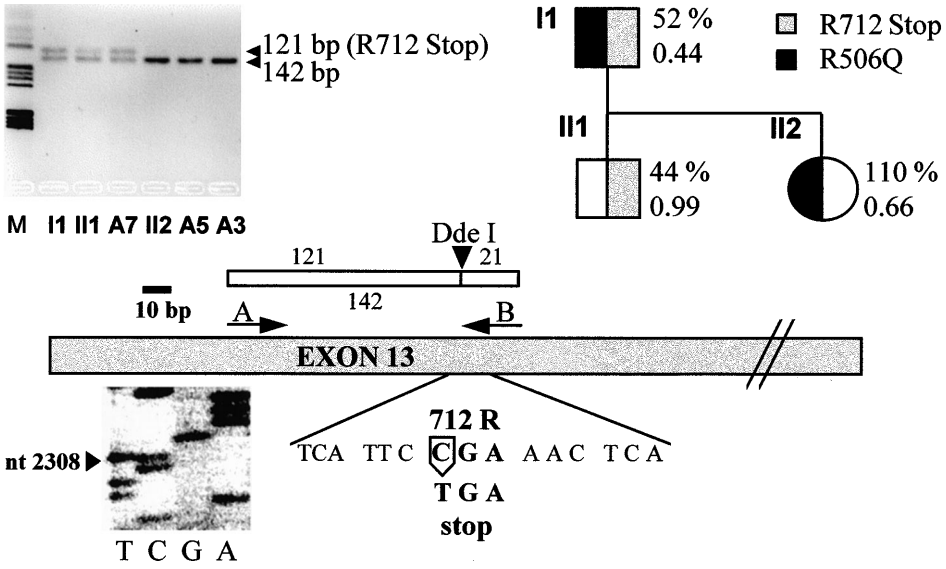

Fig 1. Family pedigree with plasmatic values (percentage of FV:c levels and APC ratio) and mutation detection by direct sequencing (bottom) or *Dde* I restriction (top). A3, A5, and A7 are asymptomatic subjects.

We report here the case of a 57-year-old man with pseudo-homozygous APC resistance who has experienced four thrombotic episodes in the lower limbs since the age of 30: three deep vein thrombosis episodes, two spontaneous and one following knee surgery and immobilization, as well as a spontaneous superficial thrombophlebitis. His 24-year-old daughter, who has inherited the FV Leiden allele (Fig 1), also developed a superficial thrombophlebitis at 17 years of age.

Direct DNA sequencing of FV exons and splicing junctions was used to search the whole FV gene for mutations. In the large exon 13 a heterozygous C to T transition at nucleotide 2308 was found (Fig 1) that affected the codon for Arg 712 (CGA), producing a stop codon (TGA) and premature termination of translation. The resulting truncated protein would lack the complete light chain (domains A3, C1, and C2). This nonsense mutation, inherited by propositus' son (Fig 1), also caused the virtual absence of the non-Leiden mRNA, as demonstrated by the homozygosity for FV Leiden after sequencing the FV cDNA obtained from platelet RNA. These findings explain both FV deficiency and marked APC resistance, because impaired expression of the non-Leiden gene results in the only presence of FV Leiden molecules in plasma.

Several recurrent mutations have been found in CpG sites within the coagulation FVIII gene, which is very similar in size and structure to the FV gene. Because the FV 2308 C to T transition is located in a CpG site, the screening of 18 unrelated subjects selected[4] for reduced FV levels (FV:C <70%) and potential carriers of FV deficiency was performed. A mutagenic (T to C, bold italic) reverse primer B (5′TCTTCCTG-GTTCAATGATGAGT***C***TC3′, nt 2334-2309), which introduces a *Dde* I restriction site (Fig 1) in the FV allele carrying the mutation, was used in polymerase chain reaction amplification with primer A (5′CCTC-CAGAATCTACAGTCATGGCT3′, nt 2182-2206).

One subject (A7 in Fig 1; FV activity, 51%; FV antigen, 44%) turned out to be heterozygous for the 2308 C to T transition. Eight-point FV gene haplotypes[5] were constructed and compared in the propositus and in subject A7. The same unfrequent haplotype was found to underlie the mutation in both unrelated subjects, which suggests identity by descent.

The present report provides an insight into the molecular mechanism underlying marked APC resistance that results from the combination of a frequent gain-of-function mutation (FV R506Q) and a novel FV mutation (Stop at codon 712). This null mutation is not peculiar to pseudo-homozygous APC resistance but belongs to the pool of FV gene mutations present in the normal population.

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REFERENCES

1. Greengard JS, Alhenc-Gelas M, Gandrille S, Emmerich J, Aiach M, Griffin J: Pseudo-homozygous protein C resistance due to coinheritance of heterozygous factor V-R506Q and type I factor V deficiency associated with thrombosis. Thromb Haemost 73:1361, 1995 (abstr)

2. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH: Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369:64, 1994

3. Dahlback B, Carlsson M, Svensson PJ: Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: Prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 90:1004, 1993

4. Lunghi B, Iacoviello L, Gemmati D, Di Iasio MG, Castoldi E, Pinotti M, Castaman G, Redaelli R, Mariani G, Marchetti G, Bernardi F: Detection of new polymorphic markers in the FV gene: Association with FV levels in plasma. Thromb Haemost 75:45, 1996

5. Bernardi F, Faioni EM, Castoldi E, Lunghi B, Castaman G, Sacchi E, Mannucci PM: A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. Blood 90:1552, 1997