died, the mutation was detected in an obligate carrier who showed levels of FIX: C= 32 IU/dL and FIX: Ag= 30 IU/dL, hence there are not discrepancies between both levels. This case was classified as severe HB, agreeing with the clinical history. Finally, the G396E (31308G→A) mutation was identified in obligate carriers, since the hemophiliacs have died (family 19). Other severe HB patients with the same mutated codon, listed in the worldwide mutation database, show extremely low levels of coagulant activity, and normal levels of antigen. All of the six newly described mutations cause severe HB, except the I298L change which produces a mild phenotype.

Finally, segregation studies, together with sequence analyses, indicate that approximately 2/3 of *de novo* mutations occur in germinal cells from the maternal grandfather. Moreover, the pathologic mutation was detected in all patients' mothers except in one case. In this kind of sporadic cases, a possible maternal somatic/germinal mosaicism may be considered, mainly in order to carry out accurate genetic counselling.[5,6]

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References

1. Yoshitake S, Shach BG, Foster DC, Davie EW, Kurachi K. Nucleotide sequence of the gene for human factor IX (antihemophiliac factor B). Biochemistry 1985;24:3736-50.
2. Giannelli F, Green PM, Sommer SS, Poon MC, Ludwing M, Schwaab R, et al. Haemophilia B: database of point mutations and short additions and deletions – eighth edition. Nucleic Acids Res 1998;26:265-8.
3. Lorenzo JI, Casaña P, Espinós C, Ferrer R, Aznar JA. Diagnosis of two related carriers of severe haemophilia B with no history. Haemophilia 2000;6:195-7.
4. Espinós C, Lorenzo JI, Casaña P, Martínez F, Aznar JA. Hemophilia B in a female caused by skewed inactivation of the normal X-chromosome. Haematologica 2000;85: 1092-5.
5. Costa JM, Vidaud D, Laurendeau I, Vidaud M, Fressinaud E, Moisan JP, et al. Somatic mosaicism and compound heterozygosity in female hemophilia B. Blood 2000;96: 1585-6.
6. Sommer SS, Scaringe WA, Hill KA. Human germline mutation in the factor IX gene. Mut Res 2001;487:1-17.

Evaluation of the factor V HR2 haplotype as a risk factor for ischemic cerebrovascular disease

The HR2 haplotype of the factor V (FV) gene has been identified as a cause of resistance to activated protein C, specially in the presence of FV Leiden. We studied the prevalence of the HR2 haplotype in 115 patients with a first episode of ischemic cerebrovascular disease (CVD) and 115 age- and sex-matched healthy controls. Our results show that the HR2 haplotype is not associated with an increased risk of CVD.

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Activated protein C resistance (APCR), is a biological condition related with venous and to a lesser extent arterial thrombosis. A single point mutation in the factor V gene, G1691A, known as FV Leiden, is the most frequent cause of APCR in the Caucasian population. APCR has been associated with an increased risk of CVD, independently of FV Leiden mutation.[1] On the other hand, the contribution of FV Leiden to the risk of CVD seems to be very low, if any.[2] Recently, a specific factor V gene haplotype known as HR2, which includes at least six polymorphisms in exons 13 and 16, has been described and associated with APCR, particularly in compound heterozygous FV Leiden/HR2 carriers.[3,4] Carriers of the HR2 haplotype have an increased ratio of a hyperglycosylated FV isoform in plasma. This variant could be more resistant to degradation by activated protein C than is the FV in HR2-free controls.[5]

We studied 115 consecutive patients, 56 males and 59 females, with a first episode of CVD (ischemic stroke or transient ischemic attack) before the age of 65 years (mean age 47 years, range 19-64) and without a previous history of venous or arterial thrombosis. Patients were recruited at two Spanish university hospitals, located in Pamplona and Salamanca. CVD was diagnosed by clinical evaluation and computed tomography or magnetic resonance imaging of the brain. Controls (n = 115; mean age 47 years, range 24 – 64) were selected from among hospital staff and people admitted to hospital without a history of venous or arterial thrombosis. Controls were 1:1 matched with patients for age (± 5 years), sex and recruitment area. Genomic DNA was obtained by standard procedures. FV Leiden and the HR2 haplotype (determined by the A4070G polymorphism, which identifies with certainty the entire haplotype) were analyzed by polymerase chain reaction (PCR) amplification and digestion of the PCR product with restriction enzymes MnlI and RsaI respectively, as previously described.[6,7]

Statistical analysis was performed by applying the McNemar 1:1 matched patients-controls χ^2 test, using odds ratios (OR) with corresponding 95% confidence interval (CI) as the measure of association. Results are summarized in Table 1. Briefly, the HR2 alelle was identified in fourteen patients (12.17%) and twenty (17.39%) controls, one of whom was homozygous (OR: 0.57 [95% CI: 0.22–1.45] $p = 0.29$). In our population FV Leiden did not significantly increase the risk of CVD (OR: 4.00 [95% CI: 0.79–27.33] $p = 0.11$). Separate evaluation of patients with ischemic stroke and with transient ischemic attack did not reveal differences between the two groups. The association between FV Leiden and HR2 haplotype could not be assessed as no compound heterozygotes were found in the control group. Regarding gene-enviroment interactions, no clear association between the HR2 alelle and acquired risk factors such as hypertension, hypercholesterolemia and smoking habit was found.

The role of the HR2 haplotype as a risk factor for venous

Table 1. Alelle frequencies of HR2 haplotype and factor V Leiden in patients and control subjects.

	Patients n (%)	*Controls n (%)*	*OR (95% CI)*
HR2	14 (12.17)	20 (17.39)	0.57 (0.22–1.45)
FV Leiden	8 (6.96)	2 (1.74)	4.00 (0.79–27.33)
HR2 + FV Leiden	1 (0.87)	0	–

thromboembolism (VTE) has been previously studied. Although the results are controversial, double heterozygotes for HR2 and FV Leiden seem to have an increased risk of VTE due to a synergistic action between both polymorphisms.[8] In another previous report no relationship between the HR2 haplotype and the risk for myocardial infarction was found.[9] To our knowledge, no study aimed at analyzing the HR2 haplotype as a predisposing factor for CVD has been published so far.

According to our results, the HR2 haplotype does not seem to play any role as a risk factor for CVD. Although the number of subjects included in this study is relatively small, both groups, patients and controls, were homogeneous, which reduces the probability of bias.

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References

1. van der Bom JG, Bots ML, Haverkate F, Slagboom PE, Meijer P, de Jong PT, et al. Reduced response to activated protein C is associated with increased risk for cerebrovascular disease. Ann Intern Med 1996;125:265-9.
2. Juul K, Tybjaerg-Hansen A, Steffensen R, Kofoed S, Jensen G, Nordestgaard BG. Factor V Leiden: the Copenhagen City Heart Study and 2 meta-analyses. Blood 2002;100:3–10.
3. Bernardi F, Faioni EM, Castoldi E, Lunghi B, Castaman G, Sacchi E, et al. A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. Blood 1997;90:1552-7.
4. de Visser MC, Guasch JF, Kamphuisen PW, Vos HL, Rosendaal FR, Bertina RM. The HR2 haplotype of factor V: effects on factor V levels, normalized activated protein C sensitivity ratios and the risk of venous thrombosis. Thromb Haemost 2000;83:577-82.
5. Castoldi E, Rosing J, Girelli D, Hoekema L, Lunghi B, Mingozzi F, et al. Mutations in the R2 FV gene affect the ratio between the two FV isoforms in plasma. Thromb Haemost 2000; 83: 362-5.
6. Koeleman BP, Reitsma PH, Allart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. Blood 1994;84:1031-5.
7. Lunghi B, Iacovello L, Gemmati D, Dilasio MG, Castoldi E, Pinotti M, et al. Detection of new polymorphic markers in the factor V gene: association with factor V levels in plasma. Thromb Haemost 1996;75:45-8.
8. Folsom AR, Cushman M, Tsai MY, Aleksic N, Heckbert SR, Boland LL, et al. A prospective study of venous thromboembolism in relation to factor V Leiden and related factors. Blood 2002;99:2720-5.
9. Doggen CJM, de Visser MCH, Vos HL, Bertina RM, Cats VM, Rosendaal FR. The HR2 haplotype of factor V is not associated with the risk of myocardial infarction. Thromb Haemost 2000;84:815-8.

Safety and effectiveness of low-dose, oral vitamin K1 administration in asymptomatic out-patients on warfarin or acenocoumarol with excessive anticoagulation

The management of asymptomatic overanticoagulation with low dose vitamin K1 is recommended in patients taking oral anticoagulants, independently of the type of coumarin being administered. To evaluate the safety and effectiveness of this practice we observed 127 patients on warfarin and 14 on acenocoumarol, who received 2 mg oral vitamin K1 on at least one occasion. At the first measurement, international normalized ratio (INR) mean values were significantly lower in patients on acenocoumarol than in patients on warfarin (p=0.0001) and all patients on acenocoumarol showed INR values below the therapeutic range. Vitamin K1 should be used cautiously in patients being anticoagulated with acenocoumarol.

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The major adverse event of oral anticoagulant therapy (OAT) is bleeding and its risk increases with INR levels. The optimal management of outpatients with asymptomatic elevation of INR remains debatable. Coumarin withdrawal with or without administration of a low dose of oral vitamin K1 is commonly suggested. Previous *ad hoc* studies reported rapid reversal of asymptomatic overanticoagulation with low dose vitamin K1 administration, without subsequent coumarin resistance in patients treated with warfarin.[1] On the basis of these results Scientific Societies recommended this practice for OAT patients independently of the drug administered.[2-3] However, few data are available on patients treated with acenocoumarol.[4]

In our Anticoagulation Clinic the routine treatment of asymptomatic overanticoagulation is temporary suspension of OAT (for one day) and low dose (2 mg) vitamin K1 oral administration. The aim of our study was to evaluate the safety and effectiveness of this protocol in patients treated with warfarin and acenocoumarol.

From June 1995 to December 2001 in Florence University Hospital Anticoagulation Clinic, we prospectively followed up 1068 patients for a total period of 2329 patient/years: 1021 were receiving warfarin (2254 patient/years) and 47 were receiving acenocoumarol (75 patient/years). The indications for OAT were: heart valve prostheses 23%, venous thromboembolism 27%, atrial fibrillation 28%, ischemic heart disease 8.5%, arterial vascular disease 7%, heart valve disease 6%, other 0.5%. In all patients with asymptomatic overanti-