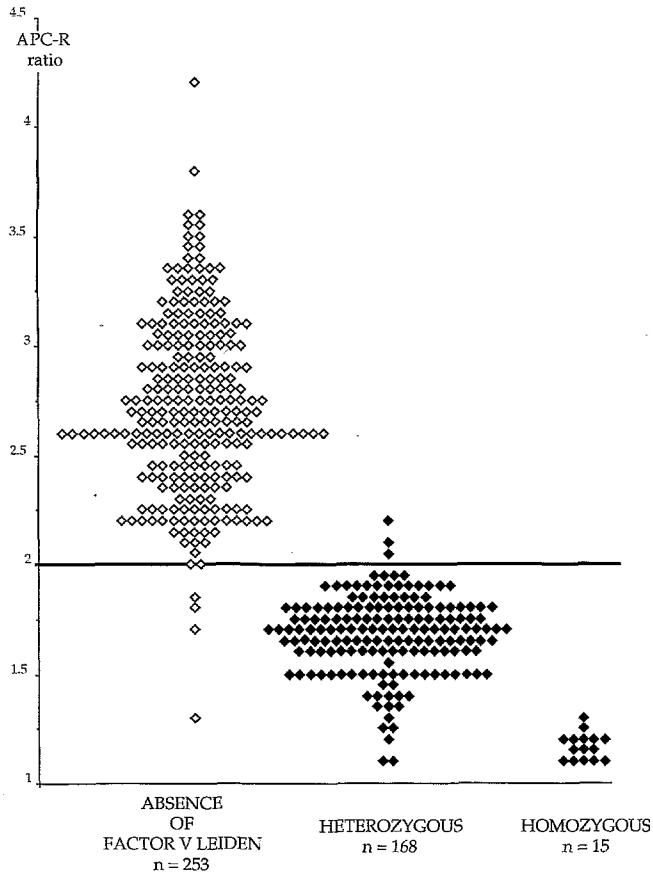

Fig. 1 Results of modified APC-R assay in 436 patients investigated for factor V Leiden (253 without factor V Leiden mutation, 168 heterozygous and 15 homozygous for the mutation)

sion of the data and there was no outlier (± 3 SD). In the remaining 183 patients, factor V Leiden was found (168 heterozygous, 15 homozygous). The mean values of heterozygous and homozygous patients were respectively: 1.68 ± 0.173 (1.08 to 2.18) and 1.17 ± 0.06 (1.09 to 1.27). The results of these patients and of those without factor V Leiden are shown in the figure. The positive (PPV) and negative predictive values (NPV) of the modified test were calculated: they were 97.3 and 98.8% respectively. They can be compared to those mentioned by Dahlbäck for the original APC-R assay: PPV = 69%, NPV = 99% (7). However, the use of home-made reagents has been reported as more reliable for screening than the commercial ones (6, 7).

Our results demonstrate that the modified test is a better screening test for the diagnosis of factor V Leiden than the original APC-R assay using the commercially available kit. It may be used not only for patients under oral anticoagulants but also in any patient. It appears to be very useful for the screening of factor V Leiden and to confirm molecular analysis. Furthermore, it may theoretically detect any abnormality of factor V responsible for APC-R, and possibly different from factor Leiden. However, the original APC-R assay might remain important in the biological investigation of thromboembolic diseases, independently of factor V Leiden screening, as a global functional test of hypercoagulability affecting the protein C pathway (1, 7).

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References

1. Zöller B, Svensson PJ, He X, Dahlbäck B. Identification of the same factor V gene mutation in 47 of 50 thrombosis-prone families with inherited resistance to activated protein C. J Clin Invest 1994; 94: 2521-4.
2. Jorquera JI, Montoro JM, Fernandez MA, Aznar JA, Aznar J. Modified test for activated protein C resistance, Lancet 1994; 344: 1162-3.
3. Trossaërt M, Conard J, Horellou MH, Samama MM, Ireland H, Bayston TA, Lane D. Modified APC resistance assay for patients on oral anticoagulants. Lancet, 1994; 344: 1709.
4. Tosetto A, Rodeghiero F. Diagnosis of APC resistance in patients on oral anticoagulant therapy. Thromb Haemost 1995; 73: 732-3.
5. Cadroy Y, Sié P, Alhenc-Gelas M, Aiach M. Evaluation of APC resistance in the plasma of patients with Q506 mutation of factor V (factor V Leiden) and treated by oral anticoagulants. Thromb Haemost 1995; 73: 734-5.
6. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PE, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64-7.
7. Dahlbäck B. Resistance to activated protein C, the Arg^{506} to Gln mutation in the factor V gene, and venous thrombosis. Thromb Haemost 1995; 73: 739-42.

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Lack of Activated Protein C Resistance in Healthy Hong Kong Chinese Blood Donors – Correlation with Absence of Arg^{506}-Gln Mutation of Factor V Gene

Dear Sir,

Recent advances in the understanding of the anticoagulant pathways have revealed deficiencies of antithrombin, Protein C, Protein S and activated protein C (APC) resistance as major risk factors for thromboembolism in the Caucasian population (1, 2). Of these factors, APC resistance due to a mutation Arg 506-Gln of the Factor V gene is now recognised as the most common abnormality found in thrombotic patients and may itself account for more than one fifth of all cases of thrombophilia (3). In the general population, the reported prevalence rate of APC resistance is 5-7% (3, 4). Given the relatively low incidence of thromboembolism in the Chinese compared to the West (5, 6) which may reflect genetic or environmental factors e.g. diet or exercise, we were interested in the APC resistance prevalence rate in

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the local blood donor population. The results were compared with the blood donor population of Caucasians in the UK.

Two hundred and ninety three healthy Hong Kong Chinese blood donors were tested for APC resistance. There were 177 males and 116 females, age 16-58 years (mean 26.5 years). The APC resistance test was performed using the Coatest APC resistance kit (Chromogenix AB, Sweden) on the ACL 3000R (Instrumentation Laboratory, Milan, Italy) or, in some cases, the Cobas Fibro. There was good correlation between the two instruments (correlation coefficient = 0.94). The APC sensitivity ratio was calculated by dividing the activated partial thromboplastin time (APTT) obtained with APC in the calcium chloride solution by that obtained using calcium chloride alone.

All Hong Kong Chinese blood donors had APC ratios above 2. The range was 2.2-5.1 (mean 3.2, SD 0.51). The lower limit of normal assay in a study of 301 healthy controls by Koster et al. (3) has been reported to be 2.17 (mean minus 1.96 SD).

In addition to the functional coagulation assay for APC resistance, analysis for the Arg 506-Gln mutation was performed on DNA extracted from peripheral blood buffy coat samples. A sequence of 267 bp of the factor V gene at Arg^{506} was amplified using primers as previously described (7). Following amplification, the DNA was restricted with 2 μ MnI I (Strategene) and subjected to agarose gel electrophoresis. The presence of the G to A mutation (nucleotide 1691) in the codon G Arg^{506} would result in loss of this restriction site. Samples were analysed in pools of 10, each sample supplying 50 ng genomic DNA to the pool. A duplicate pool which included 50 ng of a known heterozygote for the Arg 506-Gln mutation of factor V was run in parallel as a positive control. Mutation analysis was also performed on DNA extracted from 150 healthy Caucasian blood donors (age range 18-65 years).

The results from the PCR studies revealed, no patients in the Chinese group had the Arg 506-Gln mutation in Factor V. Six out of fifteen pools were positive for the Arg 506-Gln mutation in the Caucasian blood donor group. Previous studies in the UK population have revealed a prevalence of 3.5-5.6% (8, 9).

Our finding of a lack of APC resistance in Hong Kong Chinese blood donors may be the single most important factor to account for the low incidence of thromboembolism in the Chinese population. The recent observation that APC resistance is rare in the Japanese population (10) is of interest. Whether the gene defect is absent or rare in the Oriental population per se can be answered by similar studies in different geographical settings.

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References

1. Allart CF, Briët E. Familial venous thrombophilia. In: Haemostasis and Thrombosis. Bloom AL, Forbes CD, Thomas DP, Tuddenham EGD, eds. 3rd Ed. Churchill Livingstone 1994; 1349-60.
2. Dahlbäck B. New molecular insights into the genetics of thrombophilia. Thromb Haemost 1995; 74: 139-48.
3. Koster T, Rosendaal FR, de Ronde H, Briët F, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden thrombophilia study. Lancet 1993; 342: 1503-6.
4. Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994; 330: 517-22.
5. Duthie SJ, Ghosh A, Ma HK. Maternal mortality in Hong Kong, 1961-1985. Br J Obstet Gynaecol 1989; 96: 4-8.
6. Tso SC, Wong V, Chan V, Chan TK, Ma HK, Todd D. Deep vein thrombosis and changes in coagulation and fibrinolysis after gynaecological operations in Chinese: the effect of oral contraceptives and malignant disease. Br J Haematol 1980; 46: 603-12.
7. Bertina RM, Koeleman BCP, 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 1994; 369: 64-7.
8. Catto A, Carter A, Ireland H, Bayston TA, Philippou H, Barrett J, Lane DA, Grant PJ. Factor V Leiden gene mutation and thrombin generation in relation to the development of acute stroke. Arterioscler Thromb Vasc Biol 1995; 15: 783-5.
9. Beauchamp NJ, Daly ME, Hampton KK, Cooper PC, Preston FE, Peake IR. High prevalence of a mutation in the factor V gene within the UK population: relationship to activated protein C resistance and familial thrombosis. Brit J Haemat 1994; 88: 219-22.
10. Takamiya O, Ishida F, Kodaira H, Kitano K. APC-resistance and Mnl I Genotype (Gln 506) of coagulation factor V are rare in Japanese population. Thromb Haemost 1995; 74: 996.

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Warfarin Induced Skin Necrosis Associated with Activated Protein C Resistance

Dear Sir,

Warfarin induced skin necrosis (WISN) is a rare condition that has been described following warfarin treatment especially of patients with familial thrombophilia, such as deficiencies of protein C (1) and protein S (2). For most cases reported however no such association has been found (3). We describe a case of WISN in a patient with activated protein C resistance (APCR), the commonest cause of familial thrombophilia (4).

The 21 year old patient presented at the 12th week of pregnancy with a painful right leg and a clinical diagnosis of a DVT was made. No venogram was performed and she was treated with subcutaneous

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