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Correlation between Activated Protein C-Resistance and Factor V Leiden Mutation

H. Köppel, W. Renner, M. Brodmann, E. Pabst, E. Pilger

Factor V Leiden has been shown to be one important genetic risk factor for venous thrombosis due to poor response to activated protein C. However, several publications suggest that genetic mutation might constitute only one factor causing resistance towards activated protein C (APC-R) and that other risk factors, such as alcohol consumption, cholesterol levels or acute phase reactants could lead to APC-R, too. This, in turn, would mean that existence of factor V Leiden mutation could only be detected with a genetic test, while resistance towards APC can be found with a biochemical test, the so-called "COATEST". In our paper we demonstrate the correlation of APC-R and existence of factor V Leiden mutation in all investigated individuals. Our results clearly point out the crucial role of factor V Leiden mutation in the development of APC-R. The performance of both the genetic and the biochemical test may thus be redundant. *J Clin Basic Cardiol* 2001; 4: 73–74.

Key words: factor V Leiden, APC-resistance, COATEST, venous thrombosis

During the last decade several variant alleles of genes regulating blood coagulation have been identified as crucial risk factors in the development of venous thrombosis. One of these, which then turned out to be the most important, was the mutation of factor V Arg 506 to Gln (= Factor V Leiden, F.V 506 Q) [1–3]. Result of the mutation in this gene is poor response to activated protein C, thus promoting thrombophilia. This mutation has meanwhile been found to be the most common inherited risk factor for venous thrombosis. In several international studies the prevalence in the general population lies around 4 % and around 20 % in patients with DVT [2, 4–6]. Carriers of this particular gene are supposed to have an approximately 3.5–5 fold increased risk for the development of deep vein thrombosis [7, 8]. Especially in combination with oral contraceptives, longhaul flights, pregnancy or other genetic disorders, such as existence of prothrombin 20210G to A (= F2 20210A) knowledge about the existence of this mutation is crucial.

On the one hand there is a functional test which was developed for the determination of the *in vitro* response of plasma on addition of APC in an aPTT-based assay system (COATEST®, [9]) in order to identify resistance towards APC. APC resistance results in a low to moderate prolongation of clotting time after addition of APC and CaCl₂ as compared to addition of CaCl₂ alone. On the other hand there is the genetic test, where the existence of heterozygosity or homozygosity of the factor V Leiden mutation or the existence of the wild type can be identified using polymerase chain reaction.

It has been suggested in the literature that existence of factor V Leiden is only one factor causing resistance towards APC and other influences like oral contraceptives [10], cholesterol levels, alcohol consumption or acute phase reactants [11] could, at least temporarily, be responsible for resistance towards APC. We have therefore looked at correspondence of the COATEST with the genetic test in order to investigate the importance of factor V Leiden in the development of APC-R.

Methods

Subjects

In our study we have investigated 427 patients admitted to the Division of Angiology of the Department of Internal Medicine, University Hospital Graz, between December 1997 and November 1999. 100 of them had a positive history

for DVT, 327 served as controls. The study was performed according to the Austrian Gene Technology Act and to the guidelines of the Ethical Committee of the Universitätsklinik Graz, written informed consent was obtained from all participating subjects, all subjects were Austrian.

Measurement of APC sensitivity

APC sensitivity has been investigated using the so-called COATEST® [9] which was developed by Chromogenix AB, Mölndal, Sweden. Briefly, plasma was incubated with APTT reagent at 37 °C for 5 minutes, followed by addition of CaCl₂. Immediately after addition, CaCl₂ was replaced by a mixture of CaCl₂ and APC. The APC ratio was calculated by dividing APTT + APC by APTT – APC.

Genetic analysis

Venous blood was collected in 5 ml EDTA tubes, genomic DNA was isolated using a Nucleospin Blood kit (Macherey-Nagel) and stored at 4 °C. Genotyping for factor V Leiden was by allele specific digestion of PCR products as described previously [2]. As quality control of the method, 10 randomly chosen subjects were additionally genotyped by sequencing of PCR products using an ABI 310 capillary sequencer (Perkin Elmer).

Results

In our study homozygosity for the wild type factor V (GG) was found in 370 subjects. The corresponding APC ratios ranged from 2.0–3.3 (2.6 ± 0.2). Among patients with recorded history of DVT homozygosity of the wild-type was found in 75 subjects. APC ratios in these patients ranged from 2.2–3.3 (2.6 ± 0.2). 295 controls without DVT were homozygous for the wild type with APC ratios from 2.0–3.0 (2.6 ± 0.2). Heterozygosity for factor V Leiden (GA) was found in 55 subjects with a range of APC ratios from 1.4–1.8 (1.6 ± 0.1). 23 patients with DVT were heterozygous for factor V Leiden. In these patients APC ratios were 1.4–1.8 (1.6 ± 0.1). 32 controls without DVT were heterozygous for factor V Leiden. APC ratios in these subjects ranged from 1.4–1.8 (1.6 ± 0.1). In our study only 2 subjects were homozygous for factor V Leiden (AA), both of them were patients with a history of DVT. The corresponding APC ratios ranged from 1.1–1.3 (1.2 ± 0.1); see also Figure 1. The criti-

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cal value of 1.9, where a clear answer about APC-R cannot be given, was never reached. Homozygous individuals lay within a range of 1.1–1.3, heterozygous individuals unanimously between 1.4–1.8 and carriers of the wild-type unanimously within the range of 2.0 to 3.3. Correspondence concerning APC-R between the functional test and the genetic test therefore was reached in all cases. All patients suffering resistance towards APC had the factor V Leiden mutation.

Discussion

Resistance towards activated protein C due to mutation in the factor V of the coagulation system has been identified as one of the most common heritable risk factors for venous thromboembolism [1–3]. In our study we compared correspondence of the widely used functional test (COATEST®), which measures APC-R to the genetic test, which identifies the F.V 506Q mutation.

Kiechl et al. report in a recent publication that fewer than 50 % of 826 individuals in Bruneck, Italy showing resistance towards APC were carriers of factor V Leiden [11]. These results are somewhat in contrast to other data in the literature [2, 8, 12, 13, 14]. However, Kiechl and co-workers conclude that other factors like estrogens, high cholesterol levels, alcohol consumption, age or infections could, at least temporarily affect sensitivity to APC-R. Their findings seem to be supported by a paper by Henkens and co-workers [10] who showed significantly lower APC-ratios in women taking oral contraceptives compared to men or women who were not taking oral contraceptives. Unfortunately Henkens and co-workers did not take factor V Leiden mutation into account at all. In contrast, in a study carried out in 187 individuals in Ireland, only two subjects who did not have the factor V Leiden mutation were APC resistant and another two subjects with the factor V Leiden mutation did not demonstrate APC resistance [15]. Our own findings demonstrate that all individuals who showed resistance towards APC were carriers of the factor V Leiden mutation, thus indicating that other factors apart from the genetic mutation play, if any, only a negligible role. Our data therefore are in conflict with the results of Kiechl et al., where only approximately 50 % of the patients with APC-R were carriers of the factor V Leiden mutation. It is well known, however, that some factors like anticoagulant therapy, coagulation disorders like for example lupus anticoagulants or low levels of vitamin K-dependent proteins due to severe liver-damage render the functional test unreliable [16]. In such cases there may be a discrepancy between functional and genetic test. This could be one possible explanation for the different results of our own study compared to the study of Kiechl and co-workers. In our study we have excluded patients with positive lupus anticoagulants or patients who were

on oral anticoagulation. This could be an explanation for the high correspondence between functional test and genetic test in our study.

Another finding in our study is quite remarkable: individuals who were homozygous for the wild-type of factor V had APC ratios of 2.0–3.3, heterozygous individuals lay within the range of 1.4–1.8 and the individuals who were homozygous for factor V Leiden had APC ratios of 1.1 to 1.3. So in all cases the value of the APC ratio revealed whether an individual was carrier of the mutation and whether he was heterozygous or homozygous for factor V Leiden. Within the different groups there was no difference in the risk for venous thrombosis, individuals with an APC-R of 1.4 for example had the same risk as individuals with an APC-R of 1.8. This finding also argues in favour of the fact that other influences apart from the genetic mutation may slightly affect the APC-ratio, but they only play a negligible role because within the groups the risk for VTE does not change.

Considering the results of our study, one might also raise the question whether it is necessary to perform both the genetic test and measurement of the APC ratio, presupposed that the laboratory meets high standards. The functional COATEST® is cheap, reliable and easy to perform. This makes it appropriate for routine genetic screening but it is not applicable for patients on anticoagulant therapy and those with disorders in the coagulation system, for example patients with lupus anticoagulants. The genetic test should furthermore be recommended in patients with borderline APC ratios and in small children because they naturally still have low levels of vitamin-K-dependent coagulation proteins and thus higher APC-ratios. Performing the functional test, one should always look at the value of the APTT in the absence of APC because this may give further information about low protein S levels and reduced levels of factors V, VIII and IX. The disadvantage of the genetic test is, of course, that it requires experience in DNA technologies. All DNA technologies harbour the risk of contamination in which case the results are not reliable.

References:

- Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by a poor anticoagulant response to activated protein C. *Proc Natl Acad Sci USA* 1993; 72: 477–80.
- Bertina RM, Koelman 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* 1994; 169: 64–7.
- Greengard JS, Sun X, Xu X, Fernandez JA, Griffin JH, Evatt B. Activated protein C resistance caused by Arg506Gln mutation in factor Va. *Lancet* 1994; 343: 1361–2.
- Koster T, Rosendaal FR, de Ronde H, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden thrombophilia study. *Lancet* 1993; 342: 1503–6.
- de Ronde H, Bertina RM. Laboratory diagnosis of APC resistance: A critical evaluation of the test and the development of diagnostic criteria. *Thromb Haemost* 1994; 72: 880–6.
- Rosendaal FR, Bertina RM, Reitsma PH. Evaluation of activated protein C resistance in stored plasma. *Lancet* 1994; 343: 1289–90.
- Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med* 1994; 330: 517–21.
- Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of venous thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). *Blood* 1995; 85: 1504–8.
- Johansson K, Lindberg K, Menschik M, Rosen S. Evaluation of a kit for detection of resistance in plasma towards activated protein C activity in a modified aPTT assay. *Thromb Haemost* 1993; 69: 1120.
- Henkens CMA, Born VJJ, Seinen AJ, van der Meer J. Sensitivity to activated protein C; influence of oral contraceptives and sex. *Thromb Haemost* 1995; 73:402–4.
- Kiechl S, Muigg A, Santer P, Mitterer M, Egger G, Oberhollenzer M, Oberhollenzer F, Mayr A, Gasperi A, Poewe W, Willeit J. Poor response to activated protein C as a prominent risk predictor of advanced atherosclerosis and arterial disease. *Circ* 1999; 99: 614–9.
- Zöller B, Dahlbäck B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. *Lancet* 1994; 343: 1536–8.
- Zöller B, Svensson PJ, He X, Dahlbäck B. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. *J Clin Invest* 1994; 94: 2521–4.
- Majerus PW. Bad blood by mutation. *Nature* 1994; 369: 14–5.
- Vaughan J, Power C, Nolan C, Mc Carthy D, Shirley I. The incidence of factor V Leiden in a normal Irish population and its relationship to the laboratory diagnosis of APC resistance. *Thromb Haemost* 1999; 81: 661–3.
- Dahlbäck B. Resistance to activated protein C, the Arg506 to Gln mutation in the factor V gene, and venous thrombosis. Functional tests and DNA-based assays, pros and cons. *Thromb Haemost* 1995; 73: 739–42.

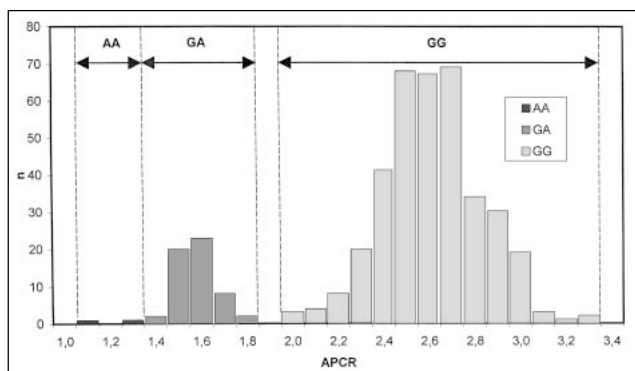


Figure 1. Distribution of APC ratios in 427 individuals with the wild type factor V (GG), heterozygous (GA) and homozygous (AA) factor V Leiden mutation

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