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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 (= F.202010A) 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-
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 unani-
mously between 1.4–1.8 and carriers of the wild-type unani-
mosly within the range of 2.0 to 3.3. Correspondence con-
cerning 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 throm-
boembolism [1–3]. In our study we compared correspond-
ence 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 to-
wards 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 consump-
tion, age or infections could, at least temporarily affect sensitiv-
ity 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 contracep-
tives compared to men or women who were not taking oral contracep-
tives. Unfortunately Henkens and co-workers did not take fac-
tor 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 dem-
strate that all individuals who showed resistance towards
APC were carriers of the factor V Leiden mutation, thus indic-
ating 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 anti-
coagulant 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 func-
tional 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 pa-
tients 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: indi-
viduals 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 ho-
mozygous 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
homozygous or homozygous for factor V Leiden. Within the
different groups there was no difference in the risk for ve-
nous thrombosis, individuals with an APC-R of 1.4 for ex-
ample had the same risk as individuals with an APC-R of 1.8.
This finding also argues in favour of the fact that other influ-
ces 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 ge-
netic 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 pa-
tients with lupus anticoagulants. The genetic test should fur-
thermore be recommended in patients with borderline APC
results 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 re-
quires experience in DNA technologies. All DNA technolo-
gies harbour the risk of contamination in which case the re-
results are not reliable.

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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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