haematologica
the hematology journal

s2

ISSN 0390-6078
Official Organ of the European Hematology Association
Published by the Ferrata-Storti Foundation, Pavia, Italy
Volume 91, supplement no. 2, September 2006

www.haematologica-thj.org
www.ehaweb.org
cme.haematologica.org

XIX Congress of the Società Italiana per lo Studio dell'Emostasi e della Trombosi (SISET)

(Italian Society for Studies on Hemostasis and Thrombosis)

Milan, Italy, September 14-17, 2006

ABSTRACT BOOK

lies, 14 presented with double heterozygous PS defect and A3 haplotype, 52 with PS defect alone, 17 with A3 haplotype alone and 51 were non-carriers of either defect. VTE had occurred in 7.1%, 23%, 5.9% and 3.9% of family members, respectively. In the upper quartile of sEPCR levels, individuals with PS defects presented a risk 3-fold higher to develop VTE as compared to those without PS defects. In the lower quartile of sEPCR levels, the risk for VTE was about 2-fold higher in carriers as compared to non-carriers of PS defects. Among 103 individuals from AT deficient families, 8 presented with double heterozygous AT defects and A3 haplotype, 43 with AT defects alone, 9 with A3 haplotype alone and 43 with no defect. VTE had occurred in 25%, 33%, 5.5% and 1.2% of family members, respectively. The risk of VTE in carriers of AT was 5-fold higher as compared to non-carriers both in the upper and in the lower quartile of sEPCR levels. In conclusion, the presence of A3 haplotype and sEPCR plasma levels might modulate the clinical expression of thrombophilia in clotting inhibitor deficient patients. Prospective studies are needed to confirm these findings.

**PREMIO SISET 2006*

C043

EFFECT OF APC RESISTANCE ASSOCIATED WITH FACTOR V MUTATIONS ON PLASMA FIBRINOLYSIS

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Factor V is endowed with both procoagulant and anticoagulant properties. APC resistance caused by homozygous FV Leiden mutation has been shown to inhibit fibrinolysis through the enhancement of thrombin-mediated activation of TAFI (thrombin activatable fibrinolysis inhibitor). It is unclear, however, whether heterozygous carriers of Leiden mutation or carriers of other FV genotypes associated with APC resistance have a defective fibrinolytic activity. We studied 107 subjects with different FV genotypes (table). Sixty-seven were unrelated asymptomatic subjects with or without Leiden mutation while 40 belonged to 6 families with combined FV mutations. Plasma fibrinolytic capacity was studied by evaluating the lysis time of tissue factor-induced clots exposed to 25 ng/ml exogenous t-PA. The assay was performed in the absence and in the presence of APC (1 microg/ml) and the fibrinolytic response was calculated by the ratio of the two lysis times (APC lysis ratio). Heterozygous Leiden mutation was associated with a significantly reduced APC lysis ratio both in unrelated subjects and in members of thrombophilic families. Moreover, the combination of Leiden mutation with type I FV deficiency (pseudohomozygous APC resistance) made the plasma totally unresponsive to the fibrinolytic effect of APC, mimicking the homozygous Leiden mutation. Neither the HR2 haplotype nor type I FV deficiency, when present in heterozygous form, influenced the response to APC. Interestingly, the two patients with Leiden plus HR2 and the patient with HR2 plus type I deficiency were also refractory to the fibrinolytic activity of APC. These data indicate that heterozygous carriers of the Leiden mutation are resistant to the fibrinolytic effect of APC. This resistance, however, is attenuated by the presence of normal factor V as indicated by strong refractoriness to APC of the combinations of Leiden with either type I deficiency or HR2 haplotype.

Table 1. APC lysis ratio in patients with different factor V genotypes

Unrelated subjects		*Members of thrombophilic families*						
Normal	Leiden Het	Normal	Leiden Het	HR2 Het	Type I def Het	Leiden+ Type I def	Leiden+ HR2	HR2+ Type I def
n=33	n=34	n=13	n=10	n=2	n=5	n=7	n=2	n=1
+1.75 ± 0.26	1.57* ± 0.26	1.57 ± 0.29	1.21* ± 0.22	1.44 ± 0.24	1.54 ± 0.30	1.10*† ± 0.09	1.08 ± 0.02	1.11

** p<0.05 as compared to normal; † p<0.05 as compared to Leiden Het.*

C044

TISSUE FACTOR A-603G GENOTYPE ASSOCIATES WITH CAROTID INTIMA-MEDIA THICKNESS IN SUBJECTS UNDERGOING CARDIOVASCULAR RISK PREVENTION

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Tissue factor (TF), key initiator of coagulation, is also ascribed a non-haemostatic function in inflammation, cell migration and proliferation, suggesting a role of TF not only in thrombosis but also in atherosclerosis development. Polymorphisms in the TF gene promoter have been shown to modulate the expression of TF, and thereby potentially also its involvement in atherosclerosis and individual predisposition to atherosclerotic disease. Hence, this study was aimed at investigating associations between TF promoter polymorphisms and carotid intima-media thickness (IMT), a well-established surrogate marker of atherosclerotic disease. To this end, the TF A-603G polymorphism was analysed in 316 subjects enrolled in a primary and secondary cardiovascular risk prevention programme, with measurements of carotid IMT by B-mode ultrasound. Also, the TF Ins-1208Del polymorphism was investigated in a limited number of subjects, which confirmed the previously reported complete concordance between the -603A and -1208Del alleles. The subjects were aged 60.2±8.4 years, 80% were male, and 78% were undergoing secondary prevention with a history of coronary, cerebrovascular, or peripheral atherosclerotic disease. Both mean and maximum carotid IMT (measured at the common carotid artery, carotid bifurcation, and internal carotid artery) differed significantly according to A-603G genotype, being highest in -603A/A (n=93), intermediate in A/G (n=161) and lowest in G/G (n=62) (mean IMT: A/A 1.31±0.36 mm, A/G 1.27±0.33 mm, G/G 1.19±0.32 mm; max IMT: A/A 2.36±0.88 mm, A/G 2.26±0.85 mm, 2.05±0.88 mm; both $p<0.05$; adjusted for age, gender, and statin treatment). In summary, a significant association between TF promoter genotype and carotid IMT was observed, perhaps mediated via alterations of TF expression levels in the circulation or within the carotid vessel wall. These findings support the hypothesis that TF plays a role in the atherosclerotic process, beyond its well-known role in haemostasis and thrombosis, thus further implicating TF not only in thrombotic complications of atherosclerotic disease, but also in plaque progression.

C045

HIGH-THROUGHPUT MULTIPLEX SINGLE-NUCLEOTIDE POLYMORPHISM (SNP) ANALYSIS IN GENES INVOLVED IN METHIONINE METABOLISM

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Hyperhomocysteinemia is a well known independent risk factor for atherothrombotic diseases. Mild hyperhomocysteinemia may result from both acquired and genetic influences. Several polymorphisms are suspected to be associated with hyperhomocysteinemia, but often date are limited and inconsistent due to the lack of robust studied populations and/or of data on the possible interaction among SNPs in genes coding molecules influencing methionine metabolism. High-throughput genotyping technologies, such as that provided by the GenomeLab SNPStream from Beckman Coulter (4,600-890,000 genotypes/day) are now available and an appropriate definition of SNPs to be analysed could represent a strong resource in order to quickly and definitively define the role of genetic risk factors on diseases. We developed a multiplex PCR-oligonucledotide extension assay with the GenomeLab SNPStream platform to detect SNPs in genes coding molecules involved in the methionine metabolism. We selected SNPs based on their putative function and frequency in candidate genes extracted from PubMed resources. The annotation of each SNP and its frequency in Caucasian populations was assessed in several databases. We selected 72 SNPs: 7 in MTHFD1, 5 in NNMT, 3 in PON1, 2 in PON2, 5 in TCNII, 4 in AHCY, 7 in MTRR, 5 in BHMT, 3 in GCP2, 5 in MTHFR, 6 in MTR, 3 in TYMS, 3 in cSHMT1, 5 in RFC1, 7 in CBS, 2 in BHMT2 gene. They were analyzed in 6 panels of 12 SNPs each according to their nucleotide substitution (1 AT, 1 CA, 1 GC, 1 GA and 2 CT panels). Among the 6 panels analyzed, in 3 panels 12/12 and in 3 panels 11/12 designed SNPs worked: therefore, we could analyze 69 out of 72 (96%) SNPs. As concerns 3 SNPs, C677T and A1298C in MTHFR gene and A2756G in MTR gene, we compared data obtained with an electronic microchip technology (Nanogen) in 288 subjects. We showed a 98.5% concordance with the two technologies. The