Tissue factor +5466A>G polymorphism predicts plasma TF levels in subjects with cryptogenic ischaemic stroke

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Dear Sir,

Tissue factor (TF) is a key initiator of blood coagulation in vivo. Its –1812C>T (rs958587) polymorphism, completely concordant with the other three TF variants (–1322C>T, –1208D>1 and –603A>G) (5), was inconsistently associated with clinical outcomes of ischaemic heart disease (5,6). Another TF polymorphism, +5466A>G (rs3917643), was reported to be a predictor of cardiovascular death in patients with acute coronary syndrome (6). Recently, we have shown that the thrombin-lowering effects of simvastatin are at least partly determined by TF+5466A>G polymorphism (7).

Although data on the links between genetic thrombophilic factors and cryptogenic stroke are discordant (8–10), search for novel risk factors might have practical implications given the fact that cryptogenic stroke represents one third of all strokes (11). We sought to investigate the impact of TF –1812C>T and +5466A>G polymorphisms on TF regulation in subjects who survived cryptogenic ischaemic stroke.

We studied 94 patients (42.6 ± 1.4 years; 26 men; 6 diabetics; 33 subjects with arterial hypertension) following documented cryptogenic ischaemic stroke 2–5 years before enrollment and 33 subjects with arterial hypertension following documented arterial stroke (Fig. 1A), lower free TFPI (AA vs. AG+GG, 17.2 ± 0.3 vs. 15.0 ± 0.3), differences in plasma TFPI levels were controlled for. Three possible haplotypes (–1812T|+5466A, –1812C|+5466A and –1812C|+5466G) were distributed between patients (0.484, 0.436 and 0.080) and controls (0.484, 0.440 and 0.076) (all p>0.05). Three possible haplotypes (–1812T|+5466A, –1812C+5466A and –1812C+5466G), identical to those reported previously (6), were similar distributed between patients (0.484, 0.436 and 0.080) and controls (0.484, 0.440 and 0.076) (all p>0.05).

In patients, but not in controls, there were correlations between TF and TFPI (R=–0.22, p=0.03), and TFPI and high-density lipoprotein cholesterol (HDL-C) levels (R=0.23, p=0.02). Minor allele of +5466A>G polymorphism was associated with higher TF (Fig. 1A), lower free TFPI (AA vs. AG+GG, 17.2 ± 0.3 vs. 15.0 ± 0.3).

Qualitative data were compared by χ²-test. Quantitative data were presented as mean ± standard error of the mean (SEM) and compared by Mann-Whitney U-test or Kruskall-Wallis ANOVA. The post-hoc power analyses for the significant pairwise genotype-related differences in TF levels were conducted using log-transformed (normalised) values. Correlations were calculated using Spearman’s rank correlation coefficient. Distribution of haplotypes was estimated using Haplovie_4.0 software (http://www.broad.mit.edu/mpg/haplovie). Haplotypic effects on TF levels were assessed by Thesias_3.1 program (http://genecanvas.ecgene.net/news.php). Multiple linear regression analysis to examine the effect of +5466A>G genotype (ordered form AA to AG+GG) on TF or TFPI levels was controlled for age, sex, glucose, lipids, smoking, diabetes, arterial hypertension and TFPI or TF.

Distributions of +5466A>G and –1812C>T polymorphisms were in Hardy-Weinberg equilibrium. Distributions of genotypes in patients (–1812C, 26 CC, 45 CT, 23 TT; +5466A, 80 AA, 13 AG, 1 GG) and controls (–1812C, 32 CC, 65 CT, 28 TT; +5466A, 106 AA, 19 AG, 0 GG) did not differ in any comparison (all p>0.05).

TFPI levels were lower (16.8 ± 0.3 vs. 23.6 ± 0.5 ng/ml, p<0.000001), while TF levels were higher (119.2 ± 3.0 vs. 50.3±1.2 pg/ml, p=0.000001) in stroke subjects than in controls. In patients, but not in controls, there were correlations between TF and TFPI (R=–0.22, p=0.03), and TFPI and high-density lipoprotein cholesterol (HDL-C) levels (R=0.23, p=0.02). Minor allele of +5466A>G polymorphism was associated with higher TF (Fig. 1A), lower free TFPI (AA vs. AG+GG, 17.2 ± 0.3 vs. 15.0 ± 0.3).

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0.3 ng/ml, p=0.0002) and lower HDL-C (AA vs. AG+GG, 1.45 ± 0.04 vs. 1.22 ± 0.10 mM, p=0.02). Minor allele of –1812C>T polymorphism was associated only with lower TF levels (Fig. 1B). No genotype-related associations were observed in controls.

Haplotypic analysis of TF concentrations demonstrated that +5466A>G polymorphism was the major predictor of TF levels, while the contribution of –1812C>T variant was comparatively low (Fig. 1C). It might be assumed that an association between –1812C>T polymorphism and TF levels in stroke patients was largely determined by its haplotypic relationship with +5466A>G variant, which is consistent with previous findings (6). The observation that, under lipopolysaccharide stimulation, +5466G allele-carrying monocytes demonstrated higher increase in TF activity compared to cells with +5466AA genotype (6) might also explain our findings regarding higher TF levels in stroke patients carrying the +5466G allele. A weak “own” positive effect of –1812C allele on TF levels in the current study might be supported by observations that, being associated with higher TF expression, –1208D (completely concordant with –1812C) allele demonstrated variant transcription factor binding (6) might also explain our findings regarding higher TF levels in patients carrying the +5466G allele. A weak “own” positive effect of –1812C allele on TF levels in the current study might be supported by observations that, being associated with higher TF expression, –1208D (completely concordant with –1812C) allele demonstrated variant transcription factor binding to –1208D TF promoter region (12). Subsequent confirmatory multiple regression models analyzing the effects of +5466A>G polymorphism on TF or TFPI showed that the presence of +5466G allele-carrying monocytes demonstrated higher in-stent –1812C>T|+5466A>G haplotypes. Values are expected haplotypic mean (representing a half of the expected diplotypic/phenotypic mean) of TF levels [95% CI]. Haplotypic analysis conducted using Thesias_3.1 program (http://genecanvas.ecgene.net/news.php) based on the Stochastic-EM (SEM) algorithm.

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