Triggered C-reactive protein (CRP) concentrations and the CRP gene −717A>G polymorphism in acute stroke or transient ischemic attack


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C-reactive protein (CRP) increases following an acute stroke/transient ischemic attack (TIA), but the increment level varies among patients. We analyzed CRP concentrations during an acute stroke/TIA in relation to the CRP gene −717A>G polymorphism. Six months following an acute ischemic stroke/TIA, basal concentrations of CRP were measured in 507 controls and 219 patients and were found to be unassociated with the CRP −717A>G polymorphism. However, during the acute phase of stroke/TIA, individuals with the AG/GG genotype had significantly elevated CRP concentrations as opposed to those with the AA genotype (2.02 ± 1.59 vs. 1.73 ± 1.69 mg/l, *P* = 0.027). In addition, significant 3.22-fold increments in CRP concentrations was noted in individuals carrying the −717G allele when comparing the acute phase with the basal state of each patient and averaging the results. CRP −717A>G polymorphism is associated with triggered CRP concentrations during acute stroke/TIA. These findings might shed more light on the mechanisms of CRP elevation in acute ischemic stroke/TIA.

**Introduction**

There are multiple lines of evidence to suggest a role of inflammation in acute ischemic conditions [1]. The onset of cerebral ischemia serves as a trigger for inflammation [2] which might have a detrimental effect on the brain tissue [2,3]. C-reactive protein (CRP) has been shown to be a valuable biomarker for this inflammation [4,5]. As with other proteins, the concentrations of CRP might be influenced by genetic variations [6–8]. In the present study, we investigated the association of a common polymorphism in the promoter region of the CRP gene (−717A>G) with the basal levels of CRP and its triggered concentrations during acute stroke/transient ischemic attack (TIA).

**Materials and methods**

**Subjects**

We evaluated a group of controls (group I) who were subjects recruited from the various clinics of our Medical Centre including the clinics for diabetes, hypertension and metabolic disorders and had no signs or symptoms of any clinically overt infection/inflammation or infarction during the 6 months prior to recruitment. The study group (group II) included consecutive patients with acute ischemic stroke/transient ischemic attack (TIA). Patients were excluded from the study if stroke resulted from trauma or any invasive procedure, cerebral hemorrhage, or if the patients history of malignant tumor, acute or chronic inflammatory disease.

Venous blood was obtained from all stroke/TIA patients within 24 h of hospital admission (mean time 25.6 ± 13.5 h from onset of symptoms), as well as 6 months thereafter.

A written informed consent was obtained from all patients and controls, signed by the participants or a first-degree relative in case of aphasic patients, as approved by the Institutional Ethics Committee. The stroke sub-types were classified according to the TOAST classification [9].

In group I we studied the association between the −717A>G polymorphism in the promoter region of the CRP gene and basal circulating CRP concentrations, whereas in group II the association between the CRP −717A>G polymorphism with triggered CRP concentrations following acute stroke/TIA was analyzed and compared with the patient’s basal CRP concentrations, 6 months following the insult.

**Biochemical analysis**

Serum high-sensitive CRP concentrations were determined using the Boering BN II Nephelometer (DADE
Enzymatic methods were used to assess the serum concentration of total, high-density lipoprotein (HDL)-, and low-density lipoprotein (LDL)-cholesterol and triglycerides (Roche, Mannheim, Germany).

**Gene polymorphism analysis**

The −717A > G polymorphism in the promoter region of the CRP gene (designated as rs2794521 on the NCBI SNP database) was determined by genomic polymerase chain reaction-allele specific restriction assay, as previously described [11].

**Statistical analysis**

All continuous data were summarized and displayed as mean ± SD. As the distribution of high sensitivity (hs)-CRP was irregular, a logarithmic transformation was employed, and all results expressed as hs-CRP were back-transformed geometric mean ± SD. Differences in the baseline characteristics and between genotypes were evaluated using the Student t-test and ANOVA. The paired t-test and Mann–Whitney test were used to evaluate the differences in CRP concentrations between baseline and the 6-month examination. Deviations of the genotype distributions were analyzed using the chi-squared test. The relationship between genotype and serum CRP concentrations was examined using multivariate stepwise linear regression models. P < 0.05 was considered statistically significant. SPSS/WIN (version 12.0, SPSS Inc.) software was used to carry out all statistical analyzes.

**Results**

**Participants**

Group I consisted of 534 subjects aged 66.8 ± 7.8 years (241 males), of which 507 were analyzed. The reasons for exclusion were the lack of hs-CRP data in 18 subjects and insufficient samples for DNA analysis in nine subjects. No difference in genotype distribution was noted between these 18 individuals and the 507 subjects recruited into the study.

Study group II consisted of 245 patients, 219 of whom were included (17 lacked hs-CRP data on admission, insufficient samples for DNA analysis in five patients, while four patients refused to participate). The mean ± SD age was 66.6 ± 13.3 years and there were 143 males. We identified 116 patients with lacunar stroke, 50 with large-artery atherosclerotic stroke, 42 with TIA, six with cardioembolic stroke, four with stroke of undetermined etiology and one patient with stroke of other determined etiology. No difference in genotype distribution was noted between the 17 individuals excluded from the study due to lack of hs-CRP data and the 219 patients who were recruited. Ninety-six of the 219 stroke/TIA patients were examined 6 months following the acute event. The remaining patients were not examined because of the following reasons: 14 patients died, 48 had another thrombotic event or a major inflammatory condition during the 6 months and 61 were lost to follow-up or refused follow-up assessments. The characteristics of group I and II are shown in Table 1.

Serum CRP concentrations were significantly higher within 24 h of admission for acute stroke/TIA than 6 months later in group II (1.73 ± 1.6 vs. 1.43 ± 1.61 mg/l, P < 0.001).

**Identification of polymorphism**

Genotype of CRP gene −717A/G frequencies is shown in Table 2. The genotype and allele frequencies were in Hardy–Weinberg equilibrium in both stroke/TIA patients and controls, and did not differ significantly between the patients and the controls.

Genotype distribution of the CRP −717A/G locus for stroke/TIA patients were: AA: 60.7%, AG: 34.7%, GG: 4.6%. The genotype distribution of the CRP −717A/G locus for the controls were: AA: 69%, AG: 27.2%, GG: 3.7%. The genotype frequencies did not differ significantly from Hardy–Weinberg proportions. A trend was suggested in the allelic and genotype frequencies between the stroke/TIA patients and the controls, but no significant difference was found (χ² = 4.75, d.f. = 2, P = 0.093). The −717G allele was associated with hyperlipidemia (P = 0.017) in patients, while in the controls, it was associated with diabetes mellitus (P = 0.028) (Table 2).

**Effect of CRP genotype on basal hs-CRP concentrations**

The CRP gene polymorphism −717A/G was not associated with basal hs-CRP concentrations of the controls (group I) (Table 3). When the acute stroke/TIA patients (group II) were analyzed 6 months following the event, no association was found between the CRP −717A/G polymorphism and their basal hs-CRP concentrations (Table 3).

**Effect of CRP genotype on triggered hs-CRP concentrations**

The polymorphism was significantly associated with hs-CRP concentrations of the stroke/TIA patients within 24 h of hospital admission (Table 3); the hs-CRP
concentrations being $1.73 \pm 1.69$ mg/l for individuals with the AA genotype and $2.02 \pm 1.59$ mg/l for those with the AG/GG genotype ($P = 0.027$). The difference in CRP level between admission and 6 months was significantly higher in patients carrying the CRP $-717G$ allele vs. CRP $-717$ AA genotype (Table 3).

We defined a new parameter named 'CRP increment' (CRP increment = admission CRP divided by 'baseline'...
Table 3 CRP concentrations by genotype of −717 A/G genotype

<table>
<thead>
<tr>
<th></th>
<th>CRP −717AA</th>
<th>CRP −717AG/GG</th>
<th>P</th>
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<tbody>
<tr>
<td>Stroke/TIA patients</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>n</td>
<td>133</td>
<td>86</td>
<td>0.027</td>
</tr>
<tr>
<td>CRP at admission, mg/l</td>
<td>1.73 ± 1.69</td>
<td>2.02 ± 1.59</td>
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<tr>
<td>Stroke/TIA patients</td>
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<tr>
<td>n</td>
<td>53</td>
<td>43</td>
<td>0.975</td>
</tr>
<tr>
<td>CRP 6 months following the event, mg/l</td>
<td>1.43 ± 1.57</td>
<td>1.44 ± 1.67</td>
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<tr>
<td>Controls (group I)</td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>350</td>
<td>157</td>
<td>0.899</td>
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<tr>
<td>CRP, mg/l</td>
<td>1.41 ± 1.57</td>
<td>1.42 ± 1.62</td>
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TIA, transient ischemic attack; CRP, C-reactive protein.

aData were logarithmically transformed before analysis, presented as geometric mean (±SD).

bMann–Whitney test.

or 6-month CRP) or ‘multiples of baseline CRP’. The effect of the CRP −717A/G genotype on the CRP increment between the basal state of the stroke/TIA patients and the acute phase was 3.22-fold higher in patients carrying the CRP −717G allele vs. CRP −717AA genotype (5.59 ± 12.54 vs. 1.74 ± 1.82, P = 0.05) (Fig. 1).

We further performed a subgroup analysis of the CRP increment by stroke etiology: small and large arteries. In the small arteries group, the CRP increment was 3.7-fold higher in patients carrying the CRP −717G allele vs. CRP −717AA genotype (4.74 ± 8.92 vs. 1.28 ± 0.98, P = 0.034).

In the large arteries group, the CRP increment was 3.34-fold higher in patients carrying the CRP −717G allele vs. CRP −717 AA genotype (9.21 ± 22.06 vs. 2.76 ± 2.74, P = 0.408).

Furthermore, the basal hs-CRP concentrations, 6 months following the acute event, were the same as in the control group, regardless of the genotype, the respective values being 1.43 ± 1.61 mg/l and 1.41 ± 1.58 mg/l (P = 0.740). When both groups were analyzed by genotype, the AA stroke/TIA patients after 6 months had an average hs-CRP of 1.43 ± 1.57 mg/l vs. 1.41 ± 1.57 mg/l for the AA controls (P = 0.796), whereas the AG/GG stroke/TIA patients had an average hs-CRP of 1.44 ± 1.67 mg/l vs. 1.42 ± 1.62 mg/l for the AG/GG controls, (P = 0.861).

Multivariate stepwise linear regression models including the CRP −717A/G genotype, age, body mass index (BMI), diabetes mellitus, hyperlipidemia, hypertension, past smoking, present smoking, β-blockers, angiotensin-converting enzyme (ACE) inhibitors, aspirin, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) and clopidogrel as independent variables were tested to assess their relationship with serum hs-CRP concentrations in groups I and II. In the controls, the linear combination of BMI (P < 0.0001, r = 0.368), present smoking (P < 0.001, r = 0.155) and gender (P < 0.007, r = 0.122) was significantly correlated with serum hs-CRP concentrations, whereas the linear combination of acetylsalicylic acid (P < 0.002, r = −0.216), hypertension (P < 0.003, r = 0.209), BMI (P < 0.01, r = 0.182) and CRP −717A/G genotype (P < 0.027, r = 0.157) was significantly correlated with serum hs-CRP concentrations in the acute stroke/TIA patients.

**Discussion**

The present study identifies a genetic variation in the promoter region of the CRP gene which influences the triggered serum hs-CRP concentrations following acute ischemic stroke/TIA. In both groups, the common CRP −717A/G polymorphism was not associated with basal CRP concentrations. However, following an acute ischemic stroke/TIA, a 3.22-fold difference was noted in individuals carrying the CRP −717A allele.

Seven polymorphisms have been hitherto identified in the CRP gene: two located in the promoter region, −717A/G and −286C/T/A; one in the second exon, +1059G/C; two within the intron, GT repeat and T/A substitution and the last two in the 3′-UTR, +1444C/T and +2147A/G.

Several studies have tested the association of the polymorphisms with basal CRP levels [7,11–20], while others investigated its triggered levels [6,8]. However, the results from the different studies are controversial.

The −717A/G polymorphism has been described previously [6,8,11], and the frequency of the G allele varies from 0.138 to 0.73 in the different reports, which
analyzed different populations. No association was found between the polymorphism and basal CRP concentrations in either study.

Brull et al. analyzed the triggered CRP concentrations following strenuous exercise and following coronary artery bypass grafting (CABG) in coronary artery disease (CAD) patients and found no association between the −717A/G polymorphism and triggered CRP concentrations in healthy and in atherothrombotic patients [8].

We found that the −717 single-nucleotide polymorphism (SNP) is involved in determining the CRP response to acute ischemic event. Our study showed that the effect of the −717 SNP on serum concentrations of CRP was limited only to the acute phase of the ischemic stroke/TIA. In fact, serum CRP concentrations increased up to 3.22-fold from the patient’s basal concentrations in the acute phase of ischemic stroke/TIA.

A possible reason for the diverse effect on serum CRP concentrations in patients and controls in our cohort is the medication effect. Apparently, the vast majority of patients (91.8%) were not treated with HMG-CoA reductase inhibitors (statins), whereas 30.6% of the controls were. Statins have been shown to lower serum CRP levels [21], but as patient’s serum CRP levels remain greater than its effect on the controls, we assume that statins are unlikely to influence the results. ACE inhibitor treatment was associated with lower CRP levels in acute ischemic stroke [22]. The prevalence of acute stroke/TIA patients treated with ACE inhibitors was higher than the controls, but their CRP concentrations remained elevated compared with controls. The use of other medications with potential anti-inflammatory effects did not significantly differ between patients and controls in our study and between carriers of the −717G allele vs. individuals with the AA genotype (data not shown).

Of special interest is that the basal concentrations of CRP, 6 months following the acute stroke/TIA, reached the same concentrations of CRP of the controls in the entire group and by genotypes. This observation supports our hypothesis of a stimulated CRP response regulated by −717 SNP. This adenine to guanine transition at position −717 creates a potential binding site for the transcription factor GR, a glucocorticoid receptor, which may result in conformational changes as opposed to levels of gene product [11].

The study has a few limitations: a preponderance of the patients were males (65.3% vs. 47.5% in the controls), hypertensive (62.8% vs. 46.4% in the controls) and there were more smokers and diabetics among patients vs. controls. Another weakness of the study is the relatively small proportion of cases analyzed after 6 months. This was largely due to reasonable conditions, as mentioned above, and any effort was made to contact and analyze the remaining patients. All subjects who signed informed consent at study entrance were asked to give contact details including a first degree family member. In addition, they were all being informed of the next visits. Six months from study entrance we have contacted each subject by phone and mail, inviting each one of them for follow-up visits.

In conclusion, we report a polymorphism in the promoter region of the CRP gene associated with stimulated CRP concentrations following acute ischemic stroke/TIA, which does not influence the basal (unstimulated) concentrations of CRP. These findings might shed more light on mechanisms of CRP increment during an acute ischemia/infarction.

References

13. Szalai AJ, McCrory MA, Cooper GS, Wu J, Kimberly RP. Association between baseline levels of C-reactive protein (CRP) and a dinucleotide repeat polymorphism in the intron of the CRP gene. *Genes Immunity* 2002; **3**: 14–19.