

SHORT REPORT

The -148 C/T fibrinogen gene polymorphism and fibrinogen levels in ischaemic stroke: a case-control study

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Background: To determine whether -148 C/T fibrinogen gene promoter polymorphism increases stroke risk by modifying the fibrinogen level.

Design: A case-control study of patients with first ever ischaemic stroke, confirmed by computed tomography.

Methods: Venous blood samples were collected for fibrinogen and routine coagulation tests one week after the stroke, and after three months in about half the patients. Population controls were age and sex matched. -148 C/T fibrinogen polymorphism was determined by polymerase chain reaction followed by digestion with restriction enzymes HindIII/AluI.

Results: There were 124 patients and 125 controls, mean age 56 years (range 18 to 75); 34 patients (27%) and 41 controls (33%) were heterozygous for -148 C/T fibrinogen polymorphism; six patients (5%) and five controls (4%) had the T/T genotype. The odds ratio of ischaemic stroke associated with CC homozygotes v T carriers was 0.8 (95% confidence interval, 0.5 to 1.4). Relative risk for ischaemic stroke associated with fibrinogen levels in the highest quartile was 3.9 (1.9 to 8.4) at one week, decreasing to 1.4 (0.6 to 3.3) at three months.

Conclusions: -148 C/T fibrinogen gene polymorphism was not a strong risk factor for ischaemic stroke. High fibrinogen levels early after acute stroke probably represent an acute phase response.

Fibrinogen is a well known risk factor for myocardial infarction and stroke.¹⁻⁴ Whether the relation between fibrinogen concentrations and stroke is a marker of the inflammatory status of the vessel wall or contributes causally to the occurrence of ischaemic stroke is much debated.⁵⁻⁸

Fibrinogen promoter polymorphisms are associated with fibrinogen levels in the general population.⁹⁻¹¹ These genetic features could therefore increase stroke risk. An alternative view is that fibrinogen is merely a marker of acute phase reactions and hence an innocent bystander rather than a causative agent in stroke. However, a combination of both hypotheses is also possible—that is, the presence of the T allele, which causes higher levels, could also mediate the fibrinogen increase as part of the acute phase response after stroke.

Fibrinogen is a glycoprotein consisting of two sets of three polypeptide chains—known as α , β , and γ chains—which are encoded by three different genes clustered on chromosome 4.¹² Several promoter polymorphisms of the β chain are described which are in almost complete linkage disequilibrium.¹³

Only a few case-control studies have focused on the possible association between fibrinogen promoter gene polymorphisms and stroke, and their results are contradictory.¹⁴⁻¹⁷ The extent to which the increased risk of stroke

resulting from raised fibrinogen levels is genetically determined or represents an acute phase response remains unknown.

We designed a case-control study to investigate further the association between -148 C/T fibrinogen gene polymorphism, fibrinogen level, and ischaemic stroke.

METHODS

We carried out a case-control study with prospective inclusion of the participants. Cases were consecutively recruited patients with first ever acute ischaemic stroke, admitted to the neurology department of a university hospital between January 1999 and December 2001. We used population controls—partners, friends, or neighbours of the patients—who were age and sex matched, of the same race, without a history of stroke, and not related to the patient. Patients, controls, and their parents were all white and born in northern Europe. Exclusion criteria were age over 75 years, a definite non-atherosclerotic cause of stroke, and the use of oral anticoagulants.

In both patients and controls, we collected detailed information about cardiovascular risk factors. Ischaemic stroke was defined as the acute onset of focal cerebral dysfunction caused by cerebral ischaemia, with symptoms lasting more than 24 hours. In all patients, computed tomography of the brain was done within three days of the onset of symptoms to confirm the diagnosis of ischaemic stroke and to rule out haemorrhagic stroke. The aetiology of the stroke was classified using the TOAST criteria.¹⁸

At one week and three months after the stroke, venous blood samples were taken under strictly standardised conditions (the patients were fasted and the sampling took place after 15 minutes of rest). Fibrinogen levels, cholesterol, glycosylated haemoglobin, and C reactive protein levels were determined in the blood samples. C reactive protein was used as a control variable for the acute phase response. Fibrinogen was measured as described by von Clauss¹⁹ on an automated coagulation analyser (Sysmex CA 1500, Dade Behring, Leusden, Netherlands) (coefficient of variation, 3.5%) during both acute and convalescent phases.

Genomic DNA was isolated from the white cell fraction of citrated blood, using the high salt concentration standard procedure.²⁰ The -148 C/T fibrinogen polymorphism was detected by polymerase chain reaction (PCR) followed by digestion with restriction enzymes HindIII/AluI.¹³

For the -148 C/T mutation with an expected prevalence of the T allele of about 20% in the control group,²¹ the minimum detectable odds ratio would be 2.2 ($\beta = 20\%$, $\alpha = 5\%$). The relation between -148 C/T fibrinogen polymorphism and ischaemic stroke was expressed as an odds ratio with 95% confidence interval (CI). The fibrinogen levels were categorised into quartiles and the odds ratio associated with ischaemic stroke for the highest quartile fibrinogen versus the lower three quartiles was estimated. Multiple logistic regression analysis was used to adjust for possible confounding

variables, such as smoking and hypertension, as these vascular risk factors are known to affect fibrinogen levels.

RESULTS

The study population consisted of 123 patients and 123 controls. Their mean age was 56 years (range 18 to 75). Forty seven per cent of the participants were female. Patients were more often smokers and more often had hypertension or diabetes, or to have known cardiovascular disease, than the controls.

Thirty four patients (27%) and 41 controls (33%) were heterozygous for -148 C/T fibrinogen gene polymorphism, and six patients (5%) and five controls (4%) were homozygous for the T allele. The genotype frequencies in the controls were in Hardy-Weinberg equilibrium. The odds ratio of ischaemic stroke associated with the -148 C/T genotype was 0.8 (95% CI, 0.4 to 1.3), and with the -148 T/T genotype, 1.1 (0.5 to 1.4).

In the stroke patients, the highest fibrinogen levels were found in combination with the TT genotype and the lowest in combination with the CC genotype, but this difference was not statistically significant (table 1). In controls, there was no association between fibrinogen level and genotype.

The mean fibrinogen concentration was higher in patients than in controls (3.7 v 3.4 g/l, $p < 0.02$, Student's *t* test). The blood sample could be repeated at least three months after the ischaemic stroke in 64 patients. At that time, the mean fibrinogen concentration had decreased to 3.3 g/l, which did not differ significantly from the level in controls.

The odds ratio associated with ischaemic stroke and fibrinogen in the highest quartile fibrinogen versus the lower three quartiles was 3.9 (95% CI, 1.9 to 8.4) at one week after the stroke. After adjustment for smoking and hypertension the odds ratio was 3.5 (1.7 to 7.2). The odds ratio based on fibrinogen levels in the second blood samples, taken three months after the index event, fell to 1.4 (0.6 to 3.3).

The mean C reactive protein concentration was 16 mg/l (median 5 mg/l, range 1 to 215) in patients, and 4 mg/l (median 3 mg/l, range 1 to 21) in controls one week after the stroke; after three months the values were equal in both groups and lower than during the first blood examination. The C reactive protein level was strongly related to fibrinogen early after the stroke ($r = 0.56$, $p = 0.001$).

DISCUSSION

We see it as a strong point of this study that cases were included prospectively and consecutively by a neurologist. Furthermore, all patients underwent neuroimaging to rule out haemorrhage. We were able to include population controls, thus avoiding the biases induced by "hospital controls". We collected detailed information about cardiovascular risk factors, medical history, and family history from patients as well as controls. All subjects participating were white north Europeans, and allele frequencies of the polymorphism in the control group were in Hardy-Weinberg

equilibrium, indicating that we were studying a representative group.

It could be argued that our study was not large enough to exclude an association between ischaemic stroke and -148 C/T fibrinogen gene polymorphism. The odds ratio in our study was 0.8, with a narrow 95% confidence interval of 0.5 to 1.4. The 95% confidence interval indicates that even if the polymorphism is a risk factor, it cannot be a strong one. One other (nested case-control) study confirms this finding.¹⁴

We found a statistically significant difference between fibrinogen levels in patients and controls one week after the stroke. This finding confirms the already accepted association between fibrinogen level and cardiovascular events.¹⁻⁴ The odds ratio of 1.4 (95% CI, 0.6 to 3.3) for the highest quartile three months after the stroke is compatible with the results of several nested case-control studies.^{1 5 22}

The fibrinogen concentration in the controls was not related to genotype in our study, in agreement with two other cross sectional cohort studies.^{9 23}

There are several explanations for the differences in fibrinogen levels between stroke patients and controls being highly significant shortly after the event but mostly inapparent after three months. Fibrinogen concentrations increase as a result of an acute phase reaction, which might explain the higher fibrinogen levels in patients one week after the stroke. This is supported by concomitant changes in C reactive protein. Secondly, other cardiovascular risk factors are associated with higher fibrinogen levels and advanced atherosclerosis.²⁴ The lower fibrinogen at three months after the stroke could in theory have resulted from rigorous treatment of these risk factors, particularly smoking. However, only five patients (4%) stopped smoking. This therefore only partly explains our observations.

Apart from the cardiovascular risk factors, interleukin-6 (IL-6) increases both fibrinogen and C reactive protein.^{1 22 25} It may be that a polymorphism of the IL-6 gene is associated with the occurrence of ischaemic stroke. As the -148 C/T polymorphism lies close to the putative IL-6 responsive element in the fibrinogen gene, one could hypothesise that this polymorphism is also within the IL-6 responsive region, and therefore its intrinsic effect on fibrinogen levels—determined by the presence of a C or T allele—is also modulated by IL-6. This might explain why the fibrinogen levels were associated with the -148 C/T fibrinogen promoter polymorphic alleles only in patients and not in the controls.

Conclusions

The -148 C/T fibrinogen gene polymorphism is not associated with an increased risk of ischaemic stroke in north European white patients. The high fibrinogen levels shortly after the stroke most probably represent a non-specific acute phase response, although a causative role cannot be excluded. Future studies should focus on other polymorphisms, and their phenotypic expression, which may be related to increased acute phase proteins.

Table 1 Fibrinogen concentrations (g/l) according to genotype in controls and patients one week after the stroke (first blood sample) and three months after the stroke (second blood sample)

Genotype	Controls		Patients, 1st sample		Patients, 2nd sample	
	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n
CC	3.45 (0.6)	70	3.62 (1.1)	81	3.25 (0.8)	42
CT	3.34 (0.5)	38	3.82 (0.9)	34	3.43 (0.6)	19
TT	3.38 (0.4)	4	3.75 (0.6)	6	4.07 (0.7)	3

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