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**Original Paper**

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# Association between Beta-Fibrinogen C148T Gene Polymorphism and Risk of Ischemic Stroke in a North Indian Population: A Case-Control Study

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## Key Words

Ischemic stroke · Polymorphisms · Endothelial nitric oxide synthase · North India · Cerebrovascular disease

## Abstract

**Background and Purpose:** Stroke is a multifactorial disease influenced by both genetic and environmental factors. The aim of this case-control study was to determine the association between  $\beta$ -fibrinogen C148T (rs1800787) gene polymorphism and susceptibility to ischemic stroke (IS) in a North Indian population. **Methods:** In the present case-control study, genotyping was performed using the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method on 250 IS patients and 250 age- and sex-matched controls. Frequency distributions of genotypes and alleles were compared between the cases and controls by conditional logistic regression. **Results:** Hypertension, diabetes, dyslipidemia, low socioeconomic status, and family history of stroke were found to be independent risk factors for IS. The mean age of the cases and controls was  $52.83 \pm 12.59$  and  $50.97 \pm 12.70$  years, respectively. Multivariate logistic regression analysis showed an independent association between  $\beta$ -fibrinogen C148T (rs1800787) polymorphism and risk of IS in dominant (OR = 2.19; 95% CI 1.23–3.90;  $p = 0.007$ ) and allelic (OR = 1.66; 95% CI 1.19–2.33;  $p = 0.002$ ) models. Based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification, an independent association of small vessel disease with risk of IS was observed in the dominant (OR = 2.09; 95% CI 1.10–3.96;  $p = 0.02$ ) and allelic (OR = 1.75; 95% CI 1.12–2.75;  $p = 0.01$ ) models, and a significant association of cardioembolic stroke with risk of IS was seen in the allelic model (OR = 2.11; 95% CI 1.07–4.17;  $p = 0.02$ ). All the genotype frequencies observed were in accordance with Hardy-Weinberg equilibrium in both cases and controls. **Conclusion:** The findings of the

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present study suggest that polymorphism in the C148T position of the  $\beta$ -fibrinogen gene might be a risk factor for IS mainly for the small vessel disease stroke subtype in a North Indian population. Further, large prospective studies are required to confirm these findings.

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## Introduction

Stroke is known to be a major, devastating neurological disorder having a high mortality rate. By the year 2030, it has been estimated that the death toll due to stroke will rise to 12 million, with only 70 million stroke survivors and more than 200 million disability-adjusted life years lost worldwide [1, 2]. Ischemic stroke (IS), which accounts for 85% of the stroke subtypes, is only the second most common cause of mortality across the globe after ischemic heart disease [3]. Stroke has been known to result from a combination of various vascular, environmental, and genetic factors [4]. Heritability estimates of IS vary from 17 to 38%.

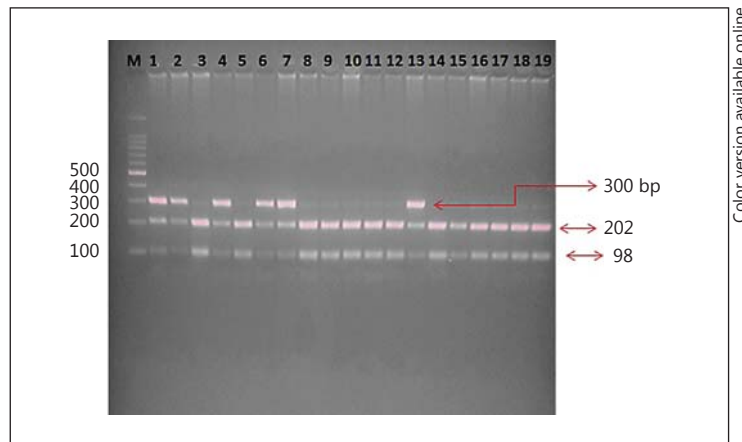
Plasma fibrinogen is known to be a risk factor for stroke and myocardial infarction [5]. It is a pivotal constituent of the coagulation cascade and a crucial determinant of blood viscosity and flow. Fibrinogen is located on the long arm of chromosome number 4, which encodes  $\alpha$ -,  $\beta$ -, and  $\gamma$ -chains. It is encoded by 3 separate genes in a cluster of 50 kb [6]. Synthesis of the  $\beta$ -polypeptide chain is the rate-limiting step in fibrinogen formation which is regulated by a  $\beta$ -fibrinogen promoter [7]. Several studies in the past have described an association between plasma fibrinogen levels and stroke, coronary heart disease, and carotid atherosclerosis. Previous studies have shown that the C148T polymorphism of  $\beta$ -fibrinogen is associated with an increased plasma fibrinogen level in both men and women [8–10]. A meta-analysis of 20 studies in a Chinese population including 2,561 cases and 2,402 controls has shown a significant association between C148T gene polymorphism and risk of IS with a pooled OR of 1.56 (95% CI 1.39–1.75) [11]. However, these studies were confined to a single population; therefore, studies with large sample sizes from different populations are required. The present study was undertaken to establish a firm relationship between C148T gene polymorphism of  $\beta$ -fibrinogen and risk of IS in a North Indian population.

## Subjects and Methods

### *Study Participants*

The present study was completed at the Department of Neurology, Neurosciences Centre, All India Institute of Medical Sciences, New Delhi, India. The study design was a hospital-based case-control study, and it was completed in a period of 2 years (November 2012 to October 2014). A total of 450 patients were screened for eligibility criteria. Patients with a history of autoimmune disease, transient ischemic attack, rheumatologic disease, any acute or chronic infection, hemorrhagic stroke proven by computed tomography or magnetic resonance imaging, and stroke due to surgery or pregnancy were excluded. The World Health Organization definition of stroke was used. Age- and sex-matched healthy individuals were used as controls in the study. Stroke-free status of the controls was assessed by the Questionnaire for Verifying Stroke-Free Status (QVSFS) [12]. Informed consent was obtained from all subjects before data collection. The study was approved by the local institutional ethics committee.

Detailed history-taking and a clinical evaluation were carried out. IS was further classified using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [13]. The National Institutes of Health Stroke Scale (NIHSS) was used for determination of clinical severity on the day of admission. Definitions of variables were modified from the study protocol published by Kumar et al. [14].



**Fig. 1.** RFLP gel image of  $\beta$ -fibrinogen C148T polymorphism. Loading order: M = 100 bp Ladder. Lanes 1, 2, 4, 6, 7, and 13: C/T genotype (heterozygous genotype). Lanes 3, 5, 8–12, and 14–19: T/T genotype (wild type).

Color version available online

#### Laboratory Investigations

Single-time 1-teaspoon (4-ml) blood samples were taken in an ethylenediaminetetraacetic acid vial from the patients and controls. Total genomic DNA was isolated from whole blood with the standard phenol-chloroform method.

#### Genotype Determination

The genotypes of the  $\beta$ -fibrinogen C148T gene were detected with the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) technique. The primers were designed for the single nucleotide polymorphism using the Primer3 online tool (<http://bioinfo.ut.ee/primer3-0.4.0/>). The  $\beta$ -fibrinogen C148T region was amplified using the primers forward 5'-CCTAACTTCCCATCATTTTGTC-3' and reverse 5'-ATGGTTTTAAGTTTGTGGAAGC-3' in a T100 thermal cycler (Bio-Rad). The primers were synthesized by Imperial Life Sciences (Gurgaon, India) under standard conditions. The conditions for amplification were as follows: an initial melting step of 4 min at 94°C, followed by 35 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 56.7°C, 45 s of extension at 72°C, and a final elongation step of 7 min at 72°C. Genotyping was performed by digesting the PCR products using the *Hind*III restriction enzyme and incubation at 37°C overnight. After amplification and digestion, the products were confirmed by agarose gel electrophoresis. Bands of 202 and 300 bp were observed after digestion, as seen through the UV transilluminator (fig. 1).

#### Statistical Analysis

The  $\chi^2$  test was used to determine whether the allelic frequencies were in accordance with Hardy-Weinberg equilibrium or not. Conditional logistic regression analysis was used to estimate ORs and 95% CIs for the strength of association between  $\beta$ -fibrinogen C148T polymorphism and risk of IS. Multivariate logistic regression was used to control for the confounding effects of demographic and risk factor variables. Tests were considered significant at  $p < 0.05$ . The data were analyzed using Stata version 13.0 (StataCorp, College Station, Tex., USA).

## Results

After screening 450 stroke cases, 250 IS cases were included in the study. For the control group, 393 people were screened and 250 age- and sex-matched controls were recruited to the study. The mean age of the IS patients was  $52.8 \pm 12.5$  years, and that of the control group was  $50.9 \pm 12.7$  years; both groups consisted of 203 males and 47 females. There was no statistical difference in age between the cases and controls ( $p > 0.05$ ). The demographic and risk factor variables of the IS patients and controls are presented in table 1. The control group was matched for age and sex, and, as expected, hypertension (cases vs. controls: 58.4 vs.

**Table 1.** Demographic and risk factor variables for IS patients and control subjects

Characteristics	Controls (n = 250)	IS patients (n = 250)	Crude OR [95% CI]	p value	Adjusted <sup>a</sup> OR [95% CI]	p value
Mean age ± SD, years	52.83 ± 12.59	50.97 ± 12.70				
Male/female, n	203/47	203/47			Matched	
Hypertension	42 (16.8)	146 (58.4)	<b>8.4 [4.8–14.6]</b>	<b>&lt;0.0001</b>	<b>6.2 [3.2–12]</b>	<b>&lt;0.0001</b>
Diabetes	26 (10.4)	79 (31.6)	<b>3.5 [2.1–5.7]</b>	<b>&lt;0.0001</b>	<b>2.1 [1.1–4.2]</b>	<b>0.02</b>
Dyslipidemia	14 (5.6)	57 (22.8)	<b>5.2 [2.6–10.4]</b>	<b>&lt;0.0001</b>	<b>2.4 [1.0–5.7]</b>	<b>0.04</b>
Smoking	67 (26.8)	97 (38.8)	<b>1.7 [1.1–2.5]</b>	<b>0.005</b>	1.1 [0.6–1.9]	0.69
Alcohol	56 (22.4)	81 (32.4)	<b>1.8 [1.1–2.8]</b>	<b>0.008</b>	1.8 [0.9–3.5]	0.05
Myocardial infarction	4 (1.6)	17 (6.8)	<b>5.3 [1.5–18.3]</b>	<b>0.008</b>	1.8 [0.4–7.1]	0.36
Migraine with aura	8 (3.2)	10 (4)	1.2 [0.4–3.1]	0.63	1.6 [0.4–5.8]	0.40
Migraine without aura	4 (1.6)	5 (2)	1.2 [0.3–4.6]	0.33	1.3 [0.1–10.9]	0.78
Low socioeconomic status	14 (5.6)	66 (26.4)	<b>5.0 [2.7–9.0]</b>	<b>&lt;0.0001</b>	<b>7.5 [3.0–18.2]</b>	<b>&lt;0.0001</b>
High BMI	89 (35.6)	77 (30.8)	0.7 [0.4–1.0]	0.11	0.8 [0.4–1.4]	0.50
Sedentary life style	106 (42.4)	127 (50.8)	<b>1.4 [1.0–2.1]</b>	<b>0.04</b>	1.1 [0.6–1.9]	0.6
Physical activity	136 (54.4)	107 (42.8)	<b>0.6 [0.4–0.8]</b>	<b>0.009</b>	0.7 [0.4–1.3]	0.36
Family history of stroke	9 (3.6)	32 (12.8)	<b>3.5 [1.6–7.4]</b>	<b>0.001</b>	<b>6.8 [2.2–20.9]</b>	<b>0.001</b>
Family history of diabetes	28 (11.2)	48 (19.2)	<b>1.8 [1.1–3.1]</b>	<b>0.015</b>	<b>3.1 [1.4–6.9]</b>	<b>0.004</b>
Family history of hypertension	34 (13.6)	61 (24.4)	<b>2.0 [1.2–3.1]</b>	<b>0.003</b>	1.8 [0.9–3.4]	0.06
Family history of heart attack	14 (5.6)	19 (7.6)	1.4 [0.6–3.1]	0.33	1.7 [0.5–5.9]	0.38

Values are presented as n (%) unless specified otherwise. Conditional logistic regression analysis. Boldface denotes significance ( $p < 0.05$ ). IS = Ischemic stroke; BMI = body mass index; OR = odds ratio; CI = confidence interval; SD = standard deviation. <sup>a</sup> Adjusted variables include hypertension, diabetes, dyslipidemia, smoking, family history of stroke, alcohol, sedentary lifestyle, and low socioeconomic status.

16.8%), diabetes (cases vs. controls: 31.6 vs. 10.8%), and dyslipidemia (cases vs. controls: 22.8 vs. 5.6%) were found significantly more often in cases than in controls ( $p < 0.05$ ).

All genotype and allelic frequencies were in Hardy-Weinberg equilibrium in both IS patients and controls. The genetic analysis of  $\beta$ -fibrinogen gene polymorphism was conducted on all 250 IS cases and 250 age- and sex-matched controls; the results are summarized in tables 2 and 3. The adjusted conditional logistic regression analysis showed a significant association of  $\beta$ -fibrinogen gene polymorphism with risk of IS in the dominant (OR = 2.19; 95% CI 1.23–3.90;  $p = 0.007$ ) and allelic (OR = 1.66; 95% CI 1.19–2.33;  $p = 0.002$ ) models.

In the subgroup analysis as per the TOAST classification, an independent association with small vessel disease was observed in the dominant (OR = 2.09; 95% CI 1.10–3.96;  $p = 0.02$ ) and allelic (OR = 1.75; 95% CI 1.12–2.75;  $p = 0.01$ ) models. A significant association was also observed with the cardioembolic subtype of IS in the allelic model (OR = 2.11; 95% CI 1.07–4.17;  $p = 0.02$ ).

## Discussion

The present case-control study suggests that polymorphism in the C148T position of the  $\beta$ -fibrinogen gene is associated with risk of IS in a North Indian population. An elevated level of  $\beta$ -fibrinogen has been found to be associated with an independent risk of stroke, venous thrombosis, ischemic heart disease, and peripheral artery disease [15, 16]. Fibrinogen is synthesized in the liver and is involved in blood coagulation. On activation, it is converted into fibrin and forms thrombi with subsequent hemostasis. It is located on chromosome 4, consisting of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -chains located in a 50-kb region. Although mRNA is independently synthesized for each of the chains, transcriptional regulation of these genes is known to be closely related.

G-A substitution in the promoter region of  $\beta$ -fibrinogen has been found to be associated with an increase in plasma fibrinogen levels [17, 18]. One study demonstrated that the  $\beta$ -fibrinogen 148 TT genotype was associated with genetic risk factors for carotid atherosclerosis [19]. A meta-analysis which included 12 case-control studies involving 1,536 cases and 1,329 control subjects demonstrated that 148 CT polymorphism of the  $\beta$ -fibrinogen gene is a

**Table 2.** Genotype and allelic frequencies of  $\beta$ -fibrinogen C148T gene polymorphism in IS patients and controls

Group	Subjects, n	Genotypes, n (%)			HWE p value	Allele, n (%)		Allele C vs. T		
		CC	CT	TT		C	T	OR	95% CI	p value
Controls	250	188 (75.2)	56 (22.4)	6 (2.4)	0.45	432 (86.4)	68 (13.6)			
IS	250	153 (61.2)	90 (36)	7 (2.8)	0.14	396 (79.2)	104 (20.8)	<b>1.66</b>	<b>1.19–2.33</b>	<b>0.002</b>
LVD	107	67 (62.6)	39 (36.4)	1 (0.94)	0.06	173 (80.8)	41 (19.1)	1.50	0.98–2.30	0.058
SVD	83	50 (60.2)	30 (36.1)	3 (3.7)	0.55	130 (78.3)	36 (21.7)	<b>1.75</b>	<b>1.12–2.75</b>	<b>0.01</b>
CE	26	15 (57.7)	9 (34.6)	2 (7.7)	0.69	39 (75)	13 (25)	<b>2.11</b>	<b>1.07–4.17</b>	<b>0.02</b>
Others	34	21 (61.8)	12 (35.3)	1 (2.9)	0.64	54 (79.4)	14 (20.6)	1.64	0.86–3.12	0.12

CI = Confidence interval; OR = odds ratio; C = cytosine; CC = cytosine cytosine; T = thymine; CT = cytosine thymine; TT = thymine thymine; IS = Ischemic stroke; LVD = large vessel disease; SVD = small vessel disease; CE = cardioembolic; HWE = Hardy-Weinberg equilibrium. Boldface denotes significance ( $p < 0.05$ ).

**Table 3.** Association between  $\beta$ -fibrinogen C148T gene polymorphism and risk of IS

Group	Subjects, n	Variants, n (%)	Dominant model CT + TT vs. CC				Variants, n (%)	Recessive model TT vs. CT + CC			
			Crude OR [95% CI]	p value	Adjusted <sup>a</sup> OR [95% CI]	p value		Crude OR [95% CI]	p value	Adjusted <sup>a</sup> OR [95% CI]	p value
Controls	250	62 (24.8)					6 (2.4)				
IS	250	97 (38.8)	<b>2.06</b> [1.35–3.12]	<b>0.001</b>	<b>2.19</b> [1.23–3.90]	<b>0.007</b>	7 (2.8)	1.16 [0.39–3.47]	0.78	0.88 [0.20–3.89]	0.87
LVD	107	40 (37.3)	1.81 [1.11–2.94]	0.017	1.69 [0.92–3.08]	0.08	1 (0.93)	0.38 [0.04–3.22]	0.15	0.32 [0.01–7.64]	0.48
SVD	83	33 (39.7)	<b>2.001</b> [1.18–3.38]	<b>0.01</b>	<b>2.09</b> [1.10–3.96]	<b>0.02</b>	3 (3.6)	1.52 [0.37–6.23]	0.55	1.96 [0.32–12.01]	0.46
CE	26	11 (42.3)	2.22 [0.97–5.09]	0.059	1.81 [0.67–4.86]	0.23	2 (7.7)	3.38 [0.64–17.72]	0.14	4.07 [0.48–34.57]	0.19
Others	34	13 (38.2)	1.87 [0.88–3.96]	0.09	1.22 [0.48–3.05]	0.66	1 (2.9)	1.23 [0.14–10.55]	0.84	0.53 [0.03–8.53]	0.66

CI = Confidence interval; OR = odds ratio; C = cytosine; CC = cytosine cytosine; T = thymine; CT = cytosine thymine; TT = thymine thymine; IS = ischemic stroke; LVD = large vessel disease; SVD = small vessel disease; CE = cardioembolic. Boldface denotes significance ( $p < 0.05$ ). <sup>a</sup> Adjusted analysis was done by adjusting for hypertension, diabetes, dyslipidemia, smoking, family history of stroke, alcohol, sedentary lifestyle, and low economic status.

susceptibility marker for risk of IS [20]. Another meta-analysis which involved 32 independent studies with 4,311 cases and 4,124 controls demonstrated that 148 CT polymorphism of  $\beta$ -fibrinogen contributes to susceptibility to IS [11].

The findings of the present study also confirm a significant association between the T allele of the  $\beta$ -fibrinogen gene and susceptibility to IS mainly in the small vessel subtype of stroke. Multivariate logistic regression analysis also observed an independent association between 148 CT polymorphism and risk of IS in the dominant model of inheritance. The analysis based on the TOAST classification demonstrated that this association was mainly with the small vessel subtype of stroke. These findings are consistent with those of a study which observed that polymorphism in the  $\beta$ -fibrinogen gene is associated with risk of the small vessel subtype of stroke [21].

### Conclusion

The findings of the present study suggest that polymorphism in the C148T position of the  $\beta$ -fibrinogen gene might be a risk factor for IS mainly for the small vessel disease stroke subtype in a North Indian population. Further, large prospective studies are required to confirm these findings.

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## Disclosure Statement

The authors declare that no competing interests exist.

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