Original Article

Transforming growth factor-β1 (C509T, G800A, and T869C) gene polymorphisms and risk of ischemic stroke in North Indian population: A hospital-based case-control study

Pradeep Kumar, Shubham Misra, Amit Kumar, Mohammad Faruq[1], Sunil Shakya, Gyan Vardhan, Subiah Vivekanandhan[2], Achal Kumar Srivastava, Kameshwar Prasad

Departments of Neurology and [2]Neurobiochemistry, All India Institute of Medical Sciences, [1]Department of Functional Genomics, Institutes of Genomics and Integrative Biology, New Delhi, India

Abstract

Background: Transforming growth factor-beta 1 (TGF-β1) is a multifunctional pleiotropic cytokine involved in inflammation and pathogenesis of cerebrovascular diseases. There is limited information on the association between variations within the TGF-β1 gene polymorphisms and risk of ischemic stroke (IS). The aim of this study was to investigate the association of the TGF-β1 gene (C509T, G800A, and T869C) polymorphisms, and their haplotypes with the risk of IS in North Indian population. **Methods:** A total of 250 IS patients and 250 age- and sex-matched controls were studied. IS was classified using the Trial of Org 10172 in Acute Stroke Treatment classification. Conditional logistic regression analysis was used to calculate the strength of association between TGF-β1 gene polymorphisms and risk of IS. Genotyping was performed using SNaPshot method. **Results:** Hypertension, diabetes, dyslipidemia, alcohol, smoking, family history of stroke, sedentary lifestyle, and low socioeconomic status were found to be associated with the risk of IS. The distribution of C509T, G800A and T869C genotypes was consistent with Hardy-Weinberg Equilibrium in the IS and control groups. Adjusted conditional logistic regression analysis showed a significant association of TGF-β1 C509T (odds ratio [OR], 2.1; 95% CI; 1.2–3.8; $P = 0.006$), G800A (OR, 4.4; 95% CI; 2.1–9.3; $P < 0.001$) and T869C (OR, 2.6; 95% CI; 1.5–4.5; $P = 0.001$) with the risk of IS under dominant model. Haplotype analysis showed that C509-A800-T869 and T509-G800-C869 haplotypes were significantly associated with the increased risk of IS. C509T and T869C were in strong linkage disequilibrium (D' =0.51, $r^2 = 0.23$). **Conclusion:** Our results suggest that TGF-β1 polymorphisms and their haplotypes are significantly associated with the risk of IS in North Indian population.

Key Words

Cytokine, inflammatory gene, ischemic stroke, single nucleotide polymorphisms, transforming growth factor beta

For correspondence:
Dr. Kameshwar Prasad, Department of Neurology, Neurosciences Centre, All India Institute of Medical Sciences, Room No. 702, 7th Floor, Ansari Nagar, New Delhi, India.
E-mail: kp0704@gmail.com

Ann Indian Acad Neurol 2017;20:5-12

Introduction

Stroke is the major leading cause of long-term disability and mortality worldwide. It has accounted for nearly 5.7 million deaths worldwide, and 87% of these deaths occur in low and middle-income countries.[1] The incidence of stroke in South Asian countries has increased by >100%, whereas this is decreased by 42% in developed European countries in the past four decades.[2] Ischemic stroke (IS) accounts for 85% of overall stroke, and its pathophysiology is regulated by a combination of lifestyle, environmental, and unclear genetic risk factors.[3]

Access this article online

Quick Response Code:

Website: www.annalsofian.org

DOI: 10.4103/0972-2327.199910

How to cite this article: Kumar P, Misra S, Kumar A, Faruq M, Shakya S, Vardhan G, *et al*. Transforming growth factor-β1 (C509T, G800A, and T869C) gene polymorphisms and risk of ischemic stroke in North Indian population: A hospital-based case-control study. Ann Indian Acad Neurol 2017;20:5-12.

Received: 07-07-16, **Revised:** 07-10-16, **Accepted:** 30-10-16

The pathogenesis of stroke is based on atherosclerosis, which can be reviewed as an inflammatory process. Inflammation and genetics are both prominent mechanisms in the pathogenesis of IS.[4] Numerous recent studies have focused on the inflammatory reactions after stroke and identifying the roles of important inflammatory signaling molecules, mainly cytokines.[5-8] Cytokines are up-regulated in the brain after stroke and are expressed not only in immunological cells but also in glial cells and neurons.[9] However, the mechanisms leading to increased release of inflammatory cytokines in patients with stroke remain unclear. Recent advances in genetic epidemiology have revealed that genetic variants increase the risk of IS including polymorphisms in inflammatory cytokine genes.

Transforming growth factor-beta (TGF-β) is a prototype of a large family of cytokines and in mammals; TGF-β has been shown to have three isoforms (TGFβ-1, 2, and 3) with very similar biological properties. TGF-β1 has been observed to inhibit the proliferation and migration of vascular smooth muscle cells in culture.[10,11] TGF-β1 is a multifunctional pleiotropic cytokine produced in particular by astrocytes and microglia in response to brain injury.[12,13] It plays an important role in inflammatory inhibition, immune regulation, cell apoptosis, lipid metabolism, hypertension, and atherosclerotic plaque formation and its levels are mainly determined by genetic factors under physiological condition.[14-16] Most specifically after a stroke, it reduces glial activation, decreases the expression and efficacy of other cytokines, suppresses the release of harmful oxygen and nitrogen derived products, promotes angiogenesis in the penumbra area, causes less brain edema with less neutrophil adherence to endothelial cells, and stimulates the release of interleukin-1 receptor antagonist.[17,18]

The human TGF-β1 gene is mapped on chromosome 19q13.2, full length of 23.56 kB, includes seven exons and six introns with multiple polymorphism loci, more than 147 single nucleotide polymorphism (SNP) sites that have been known.[19] Shah *et al*. analyzed the regulating region of TGF-β1 gene and found 10 SNPs initially except the common mutation of G800A (rs1800468), C509T (rs1800469), and T869C (rs1800470; Leu10/Pro10; T29->C), which presents that the regulating region was large span and rich in polymorphisms that could affect on the progression, development, and prognosis of TGF-β1 related disease by means of influencing the transcription and expression of TGF-β1.[20] The G800A and C509T polymorphisms are located in the promoter region; their precise effect is still unknown, but due to their location, they are considered possible modulators of the expression of TGF-β. T869C gene polymorphism has been indicated to be associated with elevated TGF-β gene and levels.[16,21] T869C polymorphism is located in the signal peptide sequence; this sequence is involved in the export of synthesized protein across the membranes of the endoplasmic reticulum and is located at potentially important positions that influence the activation of TGF-β protein.[22]

Till date, a few studies have been published on the association between TGF-β1 polymorphisms[23-29] and risk of IS with one SNP and only two studies have examined the TGF-β1 haplotype.[23,27] The study by Peng *et al*.[23] suggested that C-509T and T869C gene polymorphisms in TGF-β1 might be a critical risk factor for genetic susceptibility to confidence interval (CI) and the study by Tao *et al*.[27] suggested that haplotypes in the TGF-β1 gene might be genetic markers for cerebral infarction in the Chinese population. Although a previously published meta-analysis of six studies by Peng *et al*.[30] found no significant association between T869C gene polymorphism of TGF-β1 and risk of IS. Due to conflicting results from the previously published literature, well-designed prospective were warranted to reach a definitive conclusion. To the best of our knowledge, most of the studies have been conducted in an Asian population, whereas no study has been conducted in Indian population so far. Due to large variations in the ethnic and genetic background between the Asian and Indian population, the results from the Asian studies cannot be used to ascertain the risk of these polymorphisms in Indian settings. Therefore, we conducted this case–control study to investigate the association of the TGF-β1 gene (C509T, G800A, and T869C) polymorphisms, and their haplotypes with the risk of IS in North Indian population.

Methods

Subjects

The study was conducted in the Department of Neurology, All India Institute of Medical Sciences, New Delhi in collaboration with Institute of Genomics and Integrative Biology, New Delhi. The study was a hospital-based case–control study and was completed in 1½ years (October 2013 to April 2015). Patients with a history of transient ischemic attack, fever, rheumatologic disease, autoimmune disease, any acute or chronic infection, computed tomography (CT) or magnetic resonance imaging (MRI) proven hemorrhagic stroke, and a history of regular immunosuppressive or analgesic therapies were excluded from the study. Patients were recruited after radiologic confirmation of IS by CT or MRI scans of the brain. All patients had clinical signs consistent with the World Health Organization definition of stroke. Control subjects with age- and sex-matched individuals was recruited from volunteers and healthy persons accompanying the patients in the general outpatient department (OPD) and was assessed by questionnaire for verifying stroke-free status (QVSFS). Written informed consent was obtained from all subjects before the collection of information and blood samples. The study was approved by the Local Institutional Ethics Committee. Inclusion and exclusion criteria for cases and controls are listed below:

Inclusion criteria for cases

a. Age 18–85 years (both sexes)
b. Diagnosis of stroke as defined by the World Health Organization
c. Noncontrast computed tomography head/MRI consistent with ischemic or intracerebral hemorrhage
d. Stroke onset within 3 years before the recruitment
e. Should be Resident of North India (residing for the last 1 year or longer).

Exclusion criteria for cases

a. Unwillingness to provide written informed consent (by self or legally authorized representative [LAR])
b. Stroke associated with pregnancy
c. Stroke associated with surgery.

Inclusion criteria for controls

a. Age 18–85 years (both sexes)

b. Age (±5 years) and sex matched
c. Controls have not had prior stroke assessed by QVSFS
d. Spouse or friends but not a relative (by blood)
e. Should be Resident of North India (residing for the last 1 year or longer)
f. No evidence of any serious brain disorders.

Exclusion criteria for controls

a. Unwillingness to provide written informed consent (by self or LAR)
b. Pregnancy
c. Subjects with any serious brain disorder.

Sample size

The sample size was calculated using software StatsDirect (StatsDirect Ltd.). Sample size calculation was based on our primary hypothesis that there is an association between TGF-β1 (C509T, G800A, and T869C) gene polymorphisms and risk of IS. We took parameters of published article by Peng *et al*.[23] in which probability of exposure in controls was 0.35 and in cases was 0.5. Taking into an account, controls per case subject = 1, alpha = 0.05/3 = 0.017, power = 0.8. The estimated minimum sample size (cases required) =239. The final sample size of 250 cases and 250 controls were taken considering any sample loss.

Clinical examination

A detailed history of hypertension, diabetes, stroke, dyslipidemia, alcohol intake, smoking, myocardial infarction, atrial fibrillation, migraine, asthma, clinical investigation, including lipid profile, blood chemistry, and severity of stroke using National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS), and Barthel index (BI) were carried out. IS was categorized using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.[31] TOAST defines small-vessel disease only if the subcortical lesions are smaller than 15 mm and large artery atherosclerosis only if there is stenosis (>50%) in the corresponding artery. Therefore, subcortical infarction of >15 mm in size and infarctions with a vulnerable plaque with stenosis <50% should be classified as stroke of undetermined etiology.[31] At 6 months, disability and functional independence were assessed telephonically by mRS and BI scales.

Definition of variables

Definitions of variables were adapted from our published study protocol[32] and are as follows: Hypertension: Subjects will be considered to have hypertension if they either have the diagnosis of hypertension or treated for hypertension before the stroke or reference date. Diabetes: If a subject will have the diagnosis documented by a physician in the medical record or if fasting blood sugar level will be >126 mg/dl. Dyslipidemia: If they either have the diagnosis of dyslipidemia or treated for dyslipidemia. Smoker: person will be defined as regular smokers if a person smoking ≥1 cigarettes daily, Bidis and cigar for proceeding >3 months.

Genotype determination

Four milliliters of venous blood samples was taken from IS patients and controls in a tube containing ethylene diamine tetraacetic acid. Genomic deoxyribose nucleic acid (DNA) was isolated from whole blood through standard phenol-chloroform method. The primers were designed for the selected SNPs using the Primer3 online tool, (http://www.bioinfo.ut.ee/primer3-0.4.0/). Primers were obtained from Imperial Life Sciences (P) Limited; Haryana, India. TGF-β1 regions were amplified using the primer sequences and conditions for polymerase chain reaction (PCR) listed in Table 1 and were carried out in T-100 thermal cycler (Bio-Rad, Gurgaon, and Haryana, India). The PCR amplification was performed in a total volume of 10 μl mixture containing: 1 μl (50 ng) genomic DNA, 1 μl ×10 buffer solution, 0.1 μl 2.5 U Taq DNA polymerase ((Bangalore Genei, Bangalore, India), 0.2 μl (20 pmol) of each primer, and 0.2 μl (200 μmol/l) of each deoxynucleotide triphosphate. Genotyping was performed through the SNaPshot method on 3130xl automated DNA sequencer (Applied Biosystems, USA).

Statistical analysis

Conditional logistic regression analysis was used to estimate the strength of association between TGF-β1 gene polymorphisms with risk of IS using odds ratio (OR) and 95% confidence interval (CI). Multivariate logistic regression analysis was used to manage the confounding effects of demographic and risk factor variables associated with IS. $P < 0.05$ was considered statistically significant. Data were analyzed using the STATA, version 13.0 (StataCorp LP, StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX, USA). Haplotypes were reconstructed by PHASE 2.0 and patterns of linkage disequilibrium (LD) were analyzed using HaploView 4.2 software.[33] The threshold value of the frequencies of the haplotypes included in the analysis was set to 2%. All the haplotypes below the threshold value were excluded from the analysis.

Table 1: List of primer sequences and polymerase chain reaction conditions used for transforming growth factor-β1 gene polymorphisms

SNPs	rsID	Primers	Annealing (°C)	Amplicon size (bp)
C509T	rs1800469	F.P: 5-CAGACTCTAGAGACTGTCAG-3	60	418
		R.P: 5-GTCACCAGAGAAAGAGGAC-3		
		S.P: 5- GCCTCCTGACCCTTCCATCC-3		
G800A	rs1800468	F.P: 5-GGCAGTTGGCCGAGAACAGT-3	60	600
		R.P: 5-ACCCAGAACGGAAGGAGAGT-3		
		S.P: 5- TGAGGGACTCTGCCTCCAAC-3		
T869C	rs1800470	F.P: 5-ACCACACCAGCCCTGTTCGC-3	66	123
		R.P: 5-AGTAGCCACAGCAGCGGTAGCAGCTGC-3		
		S.P: 5-CTCCGGGCTGCGGCTGCTGC-3		

SNPs = Single nucleotide polymorphisms, F.P = Forward primer, R.P = Reverse primer, S.P = SNaPshot primer, bp = Base pair

Results

After screening 389 stroke cases, 250 IS cases were included in the study. For the control group, 321 people were screened, and 250 age- and sex-matched controls were recruited for the study. The mean age of IS patients was 52.8 ± 12.5 years and control group was 50.9 ± 12.7 years, and both groups included 203 males and 47 females. The clinical, demographic, and risk factor characteristics of IS patients and controls are presented in Tables 2 and 3. The risk factors examined such as history of hypertension (cases 58.4% vs. controls 16.8%), diabetes (cases 31.6% vs. controls 10.4%), smoking (cases 38.8% vs. controls 26.8%), alcohol intake (cases 32.4% vs. controls 22.4%), and dyslipidemia (cases 22.8% vs. controls 5.6%) were found significantly more often in cases than in controls ($P < 0.05$).

Of 250 cases, 157 (62.8%) cases were recruited from the OPD, and 93 (37.2%) cases were recruited from the inpatient department. 240 cases (96.0%) completed full 6 months telephonic follow-up, seven patients (2.8%) died, two patients (0.4%) had a recurrence of IS, and 10 (4.0%) were lost to follow-up. The mean and standard deviation (SD) was 12.55 ± 13.42 for the NIHSS at admission, 3.06 ± 1.05 for mRS and 63.56 ± 16.69 for BI at discharge. After a telephonic follow-up at 6 months, the mean and SD was 1.30 ± 1.16 for mRS and 85.31 ± 15.26 for BI.

Table 2: Characteristics of ischemic stroke patients and controls

Characteristics	IS						Controls (*n*=250)
	Number of observed	OPD (*n*=157)	Number of observed	IPD (*n*=93)	Number of observed	Total (*n*=250)	
Age in years (mean±SD)	157	53.4±12.8	93	51.7±12.2	250	52.83±12.59	50.97±12.70
Male/female, *n*	157	125/32	93	78/15	250	203/47	203/47
SBP, mmHg (mean±SD)	157	135.9±24.5	93	137.6±22.1	250	136.58±23.67	132.06±19.43
DBP, mmHg (mean±SD)	157	81.8±12.9	93	81.8±11.5	250	81.68±12.43	81.26±10.74
Random blood sugar, mg/dl (mean±SD)	106	119.2±42.4	64	119.8±45.9	173	119.44±43.68	-
Total cholesterol, mg/dl (mean±SD)	102	169.1±45.8	68	167.9±59.1	170	168.67±51.42	-
Triglycerides (mg/dl) (mean±SD)	101	128.6±65.1	65	129±59	166	126.58±64.33	-
History of stroke, *n* (%)	157	29 (18.4)	93	10 (10.7)	250	39 (15.6)	-
Stroke in young (<50 years), *n* (%)	157	42 (26.7)	93	32 (34.4)	250	74 (29.6)	-
LVD	157	65 (41.4)	93	42 (45.1)	250	107 (43)	-
SVD	157	52 (33.1)	93	31 (33.3)	250	83 (33)	-
CE	157	14 (8.9)	93	12 (12.9)	250	26 (10)	-
Others	157	26 (16.5)	93	8 (8.6)	250	34 (14)	-
NIHSS at admission (mean±SD)	157	8.2±4.7	93	14.3±6.8	250	12.55±13.42	-
BI score at admission (mean±SD)	157	69.7±13.3	93	52.4±16.7	250	63.56±16.69	-
BI score at 6 months (mean±SD)	151	90.6±9.8	89	74.7±22.6	243	85.51±15.26	-
mRS score at discharge (mean±SD)	157	2.7±0.9	93	3.7±0.8	250	3.06±1.05	-
mRS score at 6 months (median [IQR])	151	1 (0-1)	89	2 (1-3)	243	1 (0-2)	-

SBP = Systolic blood pressure, DBP = Diastolic blood pressure, NIHSS = National Institute of Health Stroke Scale, mRS = Modified Rank in Scale, BI = Barthel index, LVD = Large vessel disease, SVD = Small vessel disease, CE = Cardioembolic; others includes - stroke due to undetermined etiology+other determined etiology and negative evaluation, IQR = Inter quartile range, IS = Ischemic stroke, OPD = Outpatient department, IPD = Inpatient department, SD = Standard deviation

Table 3: Demographic and risk factors details in ischemic stroke cases and control subjects

Characteristics	Controls (*n*=250), *n* (%)	IS (*n*=250), *n* (%)	Crude OR (95% CI), *P*	Adjusted OR* (95% CI), *P*
Hypertension, *n* (%)	42 (16.8)	146 (58.4)	8.4 (4.8-14.6), <0.0001	6.2 (3.2-12), <0.0001
Diabetes, *n* (%)	26 (10.4)	79 (31.6)	3.5 (2.1-5.7), <0.0001	2.1 (1.1-4.2), 0.02
Dyslipidemia, *n* (%)	14 (5.6)	57 (22.8)	5.2 (2.6-10.4), <0.0001	2.4 (1.0-5.7), 0.04
Smoking, *n* (%)	67 (26.8)	97 (38.8)	1.7 (1.1-2.5), 0.005	1.1 (0.6-1.9), 0.69
Alcohol, *n* (%)	56 (22.4)	81 (32.4)	1.8 (1.1-2.8), 0.008	1.8 (0.9-3.5), 0.05
Myocardial infarction, *n* (%)	4 (1.6)	17 (6.8)	5.3 (1.5-18.3), 0.008	1.8 (0.4-7.1), 0.36
Migraine, *n* (%)	12 (4.8)	15 (6)	1.2 (0.5-2.7), 0.55	1.6 (0.4-5.8), 0.40
Low socioeconomic status, *n* (%)	14 (5.6)	66 (26.4)	5.0 (2.7-9.0), <0.0001	7.5 (3.0-18.2), <0.0001
High BMI, *n* (%)	89 (35.6)	77 (30.8)	0.7 (0.4-1.0), 0.11	0.8 (0.4-1.4), 0.50
Sedentary occupation, *n* (%)	106 (42.4)	127 (50.8)	1.4 (1.0-2.1), 0.04	1.1 (0.6-1.9), 0.6
Family history of stroke, *n* (%)	9 (3.6)	32 (12.8)	3.5 (1.6-7.4), 0.001	6.8 (2.2-20.9), 0.001
Family history of diabetes, *n* (%)	28 (11.2)	48 (19.2)	1.8 (1.1-3.1), 0.015	3.1 (1.4-6.9), 0.004
Family history of hypertension, *n* (%)	34 (13.6)	61 (24.4)	2.0 (1.2-3.1), 0.003	1.8 (0.9-3.4), 0.06
Family history of heart attack, *n* (%)	14 (5.6)	19 (7.6)	1.4 (0.6-3.1), 0.33	1.7 (0.5-5.9), 0.38

*Adjusted variable includes hypertension, diabetes, dyslipidemia, smoking, family stroke record, alcohol, sedentary occupation, and low socioeconomic status. BMI = Body mass index, OR = Odds ratio, CI = Confidence interval, SD = Standard deviation, IS = Ischemic stroke

All genotypes and allelic frequencies were in accordance to Hardy-Weinberg Equilibrium (HWE) in both IS cases and control subjects. Genetic analysis of TGF-β1 (C509T, G800A, and T869C) gene polymorphisms was conducted for all 250 IS cases and 250 controls and are summarized in Table 4. Adjusted conditional logistic regression analysis showed a significant association of C509T (OR, 2.1; 95% CI; 1.2–3.8), G800A (OR, 4.4; 95% CI; 2.1–9.3), and T869C (OR, 2.6; 95%

Table 4: Allelic and genotypic frequencies of Transforming growth factor-β1 (C509T, G800A, and T869C) gene polymorphisms in ischemic stroke cases and controls

Polymorphisms	LVD (*n*=107)	SVD (*n*=83)	CE (*n*=26)	Others (*n*=34)	IS (*n*=250)	Controls (*n*=250)
C509T						
Genotype, *n* (%)						
CC	66 (61.6)	55 (66.2)	15 (57.6)	21 (61.7)	157 (62.8)	193 (77.2)
CT	39 (36.4)	25 (30.1)	9 (34.6)	13 (38.2)	86 (34.4)	55 (22)
TT	2 (1.8)	3 (3.6)	2 (7.6)	0	7 (2.8)	2 (0.8)
Allele, *n* (%)						
C	171 (79.9)	135 (81.3)	39 (75)	55 (80.8)	400 (80)	441 (88.2)
T	43 (20.1)	31 (18.7)	13 (25)	13 (19.1)	100 (20)	59 (11.8)
Dominant (CC + CT vs. TT)						
Adjusted OR* (95% CI), *P*	2.22 (1.21-4.08), 0.01	1.98 (1.03-3.82), 0.04	3.39 (1.18-9.79), 0.02	2.11 (0.81-5.45), 0.12	2.19 (1.25-3.83), 0.006	
Unadjusted OR (95% CI), *P*	2.10 (1.28-3.43), 0.003	1.72 (1.00-2.96), 0.004	2.48 (1.08-5.70), 0.03	2.09 (0.98-4.44), 0.05	1.87 (1.28-2.74), 0.001	
Recessive (CC vs. TT + CT)						
Adjusted OR* (95% CI), *P*	4.09 (0.35-46.86), 0.25	4.69 (0.57-38.56), 0.15	NE	NE	3.71 (0.60-22.85), 0.15	
Unadjusted OR (95% CI), *P*	2.36 (0.32-16.90), 0.39	4.65 (0.76-28.32), 0.09	NE	NE	3.5 (0.72-16.84), 0.11	
Allelic (C vs. T)						
OR (95% CI), *P*	1.87 1.22-2.89), <0.001	1.71 (1.06-2.76), 0.02	2.49 (1.25-4.93), 0.007	1.76 (0.91-3.42), 0.08	1.86 (1.31-2.64), <0.001	
G800A						
Genotype, *n* (%)						
GG	73 (68.2)	65 (78.3)	18 (69.2)	25 (69.4)	181 (72.4)	226 (90.4)
GA	30 (28)	18 (21.6)	8 (30.7)	7 (20.5)	63 (25.20	22 (8.8)
AA	4 (3.7)	0	0	2 (5.8)	6 (2.4)	2 (0.8)
Allele (%)						
G	176 (82.2)	148 (89.1)	44 (84.6)	55 (80.8)	425 (85)	474 (94.8)
A	38 (17.7)	18 (10.8)	8 (15.4)	13 (19.2)	75 (15)	26 (5.2)
Dominant (GG + GA vs. AA)						
Adjusted OR* (95% CI), *P*	4.80 (2.35-9.78), <0.001	2.81 (1.25-6.32), 0.01	3.28 (1.0210.55), 0.04	4.40 (1.49-13.0), 0.007	4.43 (2.10-9.32), <0.001	
Unadjusted OR (95% CI), *P*	4.38 (2.44-7.87), <0.001	2.60 (1.33-5.09), 0.005	4.18 (1.64-10.64), 0.003	3.39 (1.41-8.09), 0.006	3.14 (1.92-5.13), <0.001	
Recessive (GG vs. AA + GA)						
Adjusted OR* (95% CI), *P*	3.86 (0.52-28.56), 0.18	NE	NE	3.76 (0.29-47.79), 0.30	2.16 (0.33-13.85), 0.41	
Unadjusted OR (95% CI), *P*	4.81 (0.86-26.70), 0.07	NE	NE	7.75 (1.05-56.93), 0.04	3.00 (0.60-14.86), 0.17	
Allelic (G vs. A)						
OR (95% CI), *P*	3.39 (2.32-6.67), <0.001	2.21 (1.18-4.15), 0.01	3.31 (1.41-7.79), 0.009	3.64 (1.70-7.78), <0.001	3.21 (2.02-5.12), <0.001	
T 869C						
Genotype, *n* (%)						
TT	66 (56)	55 (66.2)	19 (73)	24 (70.5)	164 (65.6)	215 (86)
TC	37 (34.5)	26 (31.3)	6 (23)	10 (29.5)	79 (31.6)	33 (13.2)
CC	4 (3.7)	2 (2.4)	1 (3.8)	0	7 (2.8)	2 (0.8)
Allele (%)						
T	169 (78.9)	136 (81.9)	44 (84.6)	55 (80.8)	407 (81.4)	463 (92.6)
C	45 (21.1)	30 (18.1)	8 (15.4)	13 (19.2)	93 (18.6)	37 (7.4)
Dominant (TT + TC vs. CC)						

Contd...

Table 4: Contd...

Polymorphisms	LVD (*n*=107)	SVD (*n*=83)	CE (*n*=26)	Others (*n*=34)	IS (*n*=250)	Controls (*n*=250)
Adjusted OR* (95% CI), *P*	3.65 (1.91-6.97), <0.001	2.84 (1.43-5.66), 0.003	1.15 (0.34-3.83), 0.81	2.57 (0.96-6.88), 0.06	2.63 (1.524.54), <0.001	
Unadjusted OR (95% CI), *P*	3.81 (2.24-6.47), <0.001	3.12 (1.75-5.57) <0.001	2.26 (0.88-5.77), 0.08	2.55 (1.12-5.80), 0.02	2.45 (1.65-3.63) <0.001	
Recessive (TT vs. CC + TC)						
Adjusted OR* (95% CI), *P*	NE	6.56 (0.71-59.94), 0.09	NE	NE	6.96 (1.22-39.70), 0.02	
Unadjusted OR (95% CI), P	4.81 (0.86-26.70), 0.07	3.06 (0.42-22.08), 0.26	NE	NE	3.5 (0.72-16.84), 0.11	
Allelic (T vs. C)						
OR (95% CI), P	3.33 (2.08-5.32), <0.001	2.76 (1.64-4.63), <0.001	2.27 (0.99-5.18), 0.06	2.27 (1.07-4.82), 0.02	2.85 (1.90-4.28), <0.001	

*Adjusted analysis was done by adjusting hypertension, diabetes, dyslipidemia, smoking, family history of stroke, alcohol, sedentary occupation, low socioeconomic status variables. LVD = Large vessel disease, SVD = Small vessel disease, CE = Cardioembolic stroke, others includes - stroke due to undetermined etiology + other determined etiology, IS = Ischemic stroke, NE = Not estimable, OR = Odds ratio, CI = Confidence interval

CI; 1.5–4.5) polymorphisms of TGF-β1 gene with the risk of IS under dominant model.

Stratification analysis based on TOAST classification, we observed a significant association between TGF-β1 C509T gene polymorphism and risk of IS under dominant and allelic models for large vessel disease (LVD), small vessel disease (SVD), and cardioembolic (CE) except for others (stroke due to determined and undetermined etiology) subtype of IS. For TGF-β1 G800A gene polymorphism, a significant association with the risk of IS was observed for all subtypes of IS under dominant and allelic models. For TGF-β1 T869C gene polymorphism, a significant association with the risk of IS was observed for LVD, SVD, others (Stroke due to determined and undetermined etiology) except for CE subtype of IS under dominant and allelic models.

The haplotype frequency distribution of the TGF-β1 and its association with IS are represented in Table 5. Among all combinations of haplotypes, C509-A800-T869 and T509-G800-C869 haplotypes were found to be significantly associated with the increased risk of IS. Strong LD (D′ = 0.51, r^2 = 0.23) was identified between two SNPs (C509T and T869C) of the TGF-β1 gene [Figure 1].

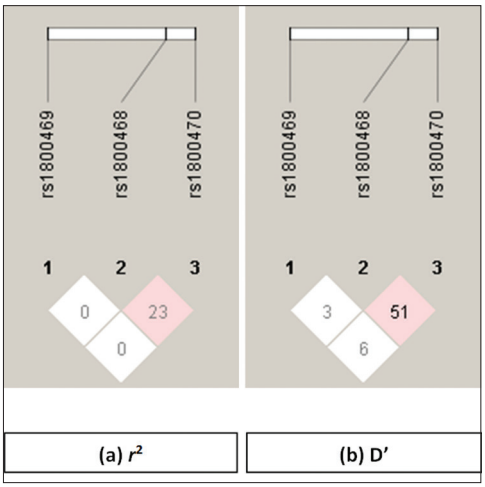

Figure 1: Linkage disequilibrium plots of the three single nucleotide polymorphisms (C509T, G800A, and T869C) of transforming growth factor-beta 1 gene in North Indian population. The figures in the squares are the pair-wise calculations of (a) r^2 or (b) D'. The squares with the "0" indicate r^2 = 0 (i.e., No linkage disequilibrium between a pair of single nucleotide polymorphisms). The square with "51" indicates D' = 0.51

Discussion

This study was the first study from North India, which revealed that the C509T, G800A, and T869C polymorphisms of TGF-β1 gene and their haplotypes were significantly associated with increased risk of IS. Our findings confirm a significant association between TGF-β1 gene polymorphisms and risk of IS. A high degree of LD was observed between pairs of G800A and T869C SNPs in North Indian population. In this regard, the further haplotype analysis of the three polymorphisms was conducted, which is more useful for the identification of predisposing genes of complicated diseases.[34] Our findings demonstrated that the haplotypes C509-A800-T869 (OR = 10.7; 95% CI 4.5–25.3) and T509-G800-C869 (OR = 2.09; 95% CI 1.47–2.99) were associated with a higher risk of developing IS. Our findings imply that these haplotypes may be a risk factor in independently modifying individual susceptibility to the development of IS in North Indian population.

Only a few studies are available which investigated the relationship between human TGF-β1 SNPs and stroke.[11,18-20] The case–control study published by Xie *et al.* did not show any significant association between TGF-β1 G800A gene polymorphism and risk of Cerebral Infarction in Chinese Population.[24] A meta-analysis of six studies conducted by Peng *et al.* involving 1701 cases did not show any significant association between TGF-β1 T869C polymorphism and risk of IS under all genetic models.[30] However, this meta-analysis was conducted only in a Chinese population with a significant amount of heterogeneity present between the studies including different study designs. The observed differences in the results may be due to different dietary and lifestyle choices of Chinese and Indian populations.[35,36] Therefore, the results of this meta-analysis cannot be used to draw any profound evidences in the North Indian population. The results in the present study provide more convincing evidence of the association between TGF-β1 gene polymorphisms and risk of IS after adjusting the

Table 5: Frequencies and association of transforming growth factor-β1 (C509T, G800A, and T869C) haplotypes in ischemic stroke cases and controls

Haplotypes	IS cases, *n* (%)	Controls, *n* (%)	χ^2	OR (95% CI)	*P*
C509-G800-C869	314 (62.8)	389 (77.8)	Reference		
C509-G800-T869	14 (2.8)	27 (5.4)	1.74	0.64 (0.33-1.24)	0.19
C509-A800-T869	52 (10.4)	6 (1.2)	43.44	10.73 (4.55-25.32)	<0.0001
C509-A800-C869	20 (4)	19 (3.8)	0.65	1.30 (0.68-2.48)	0.42
T509-G800-C869	100 (20)	59 (11.8)	17.26	2.09 (1.47-2.99)	<0.0001
Total	500		500		

IS = Ischemic stroke, OR = Odds ratio, CI = Confidence interval

confounding variables, including hypertension, alcohol, diabetes, dyslipidemia, smoking, family history of stroke, sedentary lifestyle, and low socioeconomic status. Genotype distribution in all the controls did not deviate from HWE, reassuring the representation of control samples. In addition, we conducted an adjusted analysis for multiple comparisons under all genetic models.

IS a clinically heterogeneous disorder encompassing stroke's attributable to various etiologies and thus, we investigated the heterogeneous genetic effect of TGF-β1 on IS subtypes based on TOAST classification. The effect of these polymorphisms was mainly confined to LVD and SVD among IS subtypes. Although the pathogenesis of SVD and LVD is not clearly understood, thrombosis is a major contributor to SVD. TGF-β1 is an anti-inflammatory cytokine that is believed to play a key role in thrombosis and therefore, it might be related to thrombotic occlusion of small vessels by regulating inflammatory process. Several studies have confirmed that the involvement of TGF-β1 in atherosclerosis plays a key role by inhibiting inflammation and increasing atherosclerotic plaque stabilization, and hence appears to be anti-atherogenic. The potential explanation for these inconsistent findings is that dissimilarities in the prevalence of conventional risk factors for IS, including coronary artery disease, arterial hypertension, hypercholesterolemia, and cigarette smoking may have also contributed to the divergent results between the study populations and other reasons include the small sample size (164 cases and 167 controls) in Peng *et al*. study.

However, recent studies demonstrated that TGF-β1 is associated with vascular stenosis and thrombogenesis by promoting fibrosis and inhibiting endothelial regeneration and hence is a pro-atherogenic factor.

However, there were a few limitations in our study. First, the study was conducted in a single hospital, and the participants might not have been the representatives from other areas. Therefore, further large sample size and multicentric studies are needed to confirm our findings. Second, no levels of TGF-β were determined; therefore, we were unable to elucidate the effect of the polymorphism on TGF-β levels. Third, more than 147 SNP sites have been known; but in our study, we focused on three SNPs of TGF-β1 gene which might infer the effect of other SNPs on phenotypes which further needs to be studied. Despite these limitations, our study provides strong evidence for the association between TGF-β1 gene polymorphisms and risk of IS.

Conclusion

Our results suggest that TGF-β1 polymorphisms and their haplotypes are significantly associated with the risk of IS in North Indian population.

Financial support and sponsorship

Institute of Genomics and Integrative Biology, New Delhi, India.

Conflicts of interest

There are no conflicts of interest.

References

1. Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, *et al.* Global and regional burden of stroke during 1990-2010: Findings from the Global Burden of Disease Study 2010. Lancet 2014;383:245-54.
2. Feigin VL. Stroke in developing countries: Can the epidemic be stopped and outcomes improved? Lancet Neurol 2007;6:94-7.
3. Della-Morte D, Guadagni F, Palmirotta R, Testa G, Caso V, Paciaroni M, *et al.* Genetics of ischemic stroke, stroke-related risk factors, stroke precursors and treatments. Pharmacogenomics 2012;13:595-613.
4. Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999;138(5 Pt 2):S419-20.
5. Ormstad H, Aass HC, Lund-Sørensen N, Amthor KF, Sandvik L. Serum levels of cytokines and C-reactive protein in acute ischemic stroke patients, and their relationship to stroke lateralization, type, and infarct volume. J Neurol 2011;258:677-85.
6. Ormstad H, Aass HC, Amthor KF, Lund-Sørensen N, Sandvik L. Serum cytokine and glucose levels as predictors of poststroke fatigue in acute ischemic stroke patients. J Neurol 2011;258:670-6.
7. Perini F, Morra M, Alecci M, Galloni E, Marchi M, Toso V. Temporal profile of serum anti-inflammatory and pro-inflammatory interleukins in acute ischemic stroke patients. Neurol Sci 2001;22:289-96.
8. Worthmann H, Tryc AB, Goldbecker A, Ma YT, Tountopoulou A, Hahn A, *et al.* The temporal profile of inflammatory markers and mediators in blood after acute ischemic stroke differs depending on stroke outcome. Cerebrovasc Dis 2010;30:85-92.
9. Vila N, Castillo J, Dávalos A, Chamorro A. Proinflammatory cytokines and early neurological worsening in ischemic stroke. Stroke 2000;31:2325-9.
10. Grainger DJ, Kirschenlohr HL, Metcalfe JC, Weissberg PL, Wade DP, Lawn RM. Proliferation of human smooth muscle cells promoted by lipoprotein(a). Science 1993;260:1655-8.
11. Kojima S, Harpel PC, Rifkin DB. Lipoprotein (a) inhibits the generation of transforming growth factor beta: An endogenous inhibitor of smooth muscle cell migration. J Cell Biol 1991;113:1439-45.

12. Boche D, Cunningham C, Gauldie J, Perry VH. Transforming growth factor-beta 1-mediated neuroprotection against excitotoxic injury *in vivo*. J Cereb Blood Flow Metab 2003;23:1174-82.
13. Feng Z, Ko CP. Schwann cells promote synaptogenesis at the neuromuscular junction via transforming growth factor-beta1. J Neurosci 2008;28:9599-609.
14. August P, Suthanthiran M. Transforming growth factor beta signaling, vascular remodeling, and hypertension. N Engl J Med 2006;354:2721-3.
15. Ramji DP, Singh NN, Foka P, Irvine SA, Arnaoutakis K. Transforming growth factor-beta-regulated expression of genes in macrophages implicated in the control of cholesterol homoeostasis. Biochem Soc Trans 2006;34(Pt 6):1141-4.
16. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, *et al.* Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999;8:93-7.
17. Fèvre-Montange M, Dumontel C, Chevallier P, Isnard AK, Guigard MP, Trouillas J. Localization of transforming growth factors, TGFbeta1 and TGFbeta3, in hypothalamic magnocellular neurones and the neurohypophysis. J Neuroendocrinol 2004;16:571-6.
18. Helmy A, Carpenter KL, Menon DK, Pickard JD, Hutchinson PJ. The cytokine response to human traumatic brain injury: Temporal profiles and evidence for cerebral parenchymal production. J Cereb Blood Flow Metab 2011;31:658-70.
19. Toyoda T, Nakamura K, Yamada K, Thanseem I, Anitha A, Suda S, *et al.* SNP analyses of growth factor genes EGF, TGFbeta-1, and HGF reveal haplotypic association of EGF with autism. Biochem Biophys Res Commun 2007;360:715-20.
20. Shah R, Rahaman B, Hurley CK, Posch PE. Allelic diversity in the TGFB1 regulatory region: Characterization of novel functional single nucleotide polymorphisms. Hum Genet 2006;119:61-74.
21. Cambien F, Ricard S, Troesch A, Mallet C, Générénaz L, Evans A, *et al.* Polymorphisms of the transforming growth factor-beta 1 gene in relation to myocardial infarction and blood pressure. The Etude Cas-Témoin de l'Infarctus du Myocarde (ECTIM) Study. Hypertension 1996;28:881-7.
22. Syrris P, Carter ND, Metcalfe JC, Kemp PR, Grainger DJ, Kaski JC, *et al.* Transforming growth factor-beta1 gene polymorphisms and coronary artery disease. Clin Sci (Lond) 1998;95:659-67.
23. Peng Z, Zhan L, Chen S, Xu E. Association of transforming growth factor-ß1 gene C-509T and T869C polymorphisms with atherosclerotic cerebral infarction in the Chinese: A case-control study. Lipids Health Dis 2011;10:100.
24. Xie Y, Zeng K, Wang Y, Xu H. TGF-beta1-800G>A polymorphism and cerebral infarction in Chinese Han population. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2009;34:1166-70.
25. Kim Y, Lee C. The gene encoding transforming growth factor beta 1 confers risk of ischemic stroke and vascular dementia. Stroke 2006;37:2843-5.
26. Katakami N, Kaneto H, Osonoi T, Kawai K, Ishibashi F, Imamura K, *et al.* Transforming growth factor ß1 T868C gene polymorphism is associated with cerebral infarction in Japanese patients with type 2 diabetes. Diabetes Res Clin Pract 2011;94:e57-60.
27. Tao HM, Chen GZ, Cheng GP, Shan XY. The haplotype of the TGFß1 gene associated with cerebral infarction in Chinese. Can J Neurol Sci 2012;39:626-31.
28. Tao HM, Chen GZ, Lu XD, Chen GP, Shao B. TGF-ß1 869T/C polymorphism and ischemic stroke: Sex difference in Chinese. Can J Neurol Sci 2010;37:803-7.
29. Sie MP, Uitterlinden AG, Bos MJ, Arp PP, Breteler MM, Koudstaal PJ, *et al.* TGF-beta 1 polymorphisms and risk of myocardial infarction and stroke: The Rotterdam Study. Stroke 2006;37:2667-71.
30. Peng L, Li P, Chen J, Yan K, Huo F, Han L, *et al.* Association between transforming growth factor-beta 1 T869C polymorphism and ischemic stroke: A meta-analysis. PLoS One 2013;8:e67738.
31. Adams HP Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, *et al.* Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35-41.
32. Kumar A, Sagar R, Kumar P, Sahu JK, Grover A, Srivastava AK, *et al.* Identification of genetic contribution to ischemic stroke by screening of single nucleotide polymorphisms in stroke patients by using a case control study design. BMC Neurol 2013;13:136.
33. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
34. Weiss KM, Terwilliger JD. How many diseases does it take to map a gene with SNPs? Nat Genet 2000;26:151-7.
35. Gezmu T, Schneider D, Demissie K, Lin Y, Gizzi MS. Risk factors for acute stroke among South Asians compared to other racial/ethnic groups. PLoS One 2014;9:e108901.
36. Bourke J, Sylvester R, Sharma P. Ethnic variations in the management of patients with acute stroke. Postgrad Med J 2006;82:13-5.