J Biomed Sci (2008) 15:545–552
DOI 10.1007/s11373-008-9242-2

ORIGINAL PAPER

Promoter sequence variants of LIGHT are associated with female vascular dementia

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Received: 20 December 2007 / Accepted: 19 February 2008 / Published online: 5 March 2008

Abstract LIGHT (*homologous to **L**ymphotoxins, exhibits **I**nducible expression, and competes with herpes simplex virus **G**lycoprotein D for **H**erpes virus entry mediator, a receptor expressed by **T** lymphocytes*) is implicated in the inflammation by disrupted T cell homeostasis, primarily at a transcriptional level. We investigated the association of LIGHT promoter with ischemic stroke and vascular dementia induced by such inflammation. We determined transcription factor binding sites altered by promoter SNPs using transcription factor prediction programs. Six common haplotypes composed of the selected SNPs (C-770T, G-607T, G-543A, and A-399G) were used for the assay of reporter activity. The most frequent haplotype construct, CGGA, induced the highest luciferase activity. The haplotype TTGA showed the lowest expression with 0.39-fold activity ($P < 0.001$) of CGGA. The substitution from C to T at the locus of C-770T (TGGA) decreased the reporter activity by 47% ($P < 0.001$). The SNPs and haplotypes were further investigated to see their association with ischemic stroke and vascular dementia in 455 controls and 478 patients. Significant association with vascular dementia was shown in the allele T of C-770T (odds ratio [OR] = 1.54; $P < 0.05$) and the haplotype TTGA (OR = 10.59; $P < 0.05$) in females. We concluded that the allele T of C-770T and the haplotype TTGA of the promoter SNPs in LIGHT gene might decrease the expression of LIGHT and subsequently increase the susceptibility to vascular dementia in females.

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Keywords Estrogen · Genetic risk factor · Inflammation · Vascular dementia

Introduction

Ischemic stroke and vascular dementia might be induced by inflammation independently or interactively with conventional risk factors such as hypertension, smoking, and cardiac diseases [1]. Inflammatory responses are generally orchestrated by T cells, and proinflammatory cytokines are secreted also by the T lymphocytes [2]. Although identifying susceptibility genes that promote T cell activation has raised a great concern for early recognition and therapeutic targets of ischemic stroke and vascular dementia, the knowledge on the gene profile is still quite limited. Especially, among the genes that could directly activate T cells, only C-reactive protein (CRP; Leu184Leu in exon 2) [3] and toll-like receptor 4 (TLR4; A119C in intron 1) [4] have been found to be associated with the ischemic stroke so far.

One of the candidate immune-related molecules was the tumor necrosis factor superfamily (TNFSF) that functions as co stimulator in T cell activation by delivering second signal for T cell proliferation/differentiation and cytokine production [5]. Especially, LIGHT (*homologous to **L**ymphotoxins, exhibits **I**nducible expression, and competes with herpes simplex virus **G**lycoprotein D for **H**erpes virus entry mediator, a receptor expressed by **T** lymphocytes*), the 14th member of TNFSF (TNFSF14), plays a crucial role in T cell homeostasis by stimulating T cell activation, triggering T cell proliferation, and secreting T helper cell (Th) 1 cytokines. A variety of T cell immune responses were stimulated and modulated interactively by the LIGHT and herpes virus entry mediator (HVEM) [6]. Lack of LIGHT led to a considerable impairment of proliferative

responses in $CD3^+$ and $CD8^+$ T cells [7]. Dysregulation of the LIGHT activity ultimately resulted in the breakdown of peripheral tolerance [8]. Furthermore, the activity could induce not only autoimmune diseases such as graft versus host disease [9] and imunoglobulin A nephropathy [10], but also inflammatory diseases such as rheumatoid arthritis [11, 12] and Crohn's disease [13].

The objective of this study was to investigate the association of the human LIGHT gene with ischemic stroke and vascular dementia. We focused on the association with single nucleotide polymorphisms (SNPs) in its promoter region because primary regulation of LIGHT on T cell occurred at the transcriptional level [14]. We performed a promoter assay and a case-control association study.

Materials and methods

Patients

Patients with ischemic stroke were recruited from Hallym University Hospital in Korea with the diagnosis by performing computed tomography or magnetic resonance imaging scan from acute stroke patients within 7 days of onset (2002–2005). A total of 271 patients with ischemic stroke were partitioned into its subtypes, large artery atherosclerosis (LAA), small vessel occlusion (SVO), cardioembolism (CE), and the other strokes with rare or undetermined etiology (Table 1), using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification [15]. Additionally, we included another group of 207 patients with vascular dementia diagnosed according to the DSM-IV [16] and NINDS-AIREN [17]. The vascular dementia is defined as a progressive deterioration in memory, behavior, thinking, motor, and cognitive function, accompanied with a cerebrovascular disease. Patients presenting both vascular and Alzheimer features were excluded. A control group of 455 Korean subjects without any history of cerebral ischemic events were randomly recruited from routine health checkups at the same hospital. Clinical characteristics of the patients and controls were presented in our previous study [18]. Written informed consent was obtained from all subjects, and the study protocol was approved by the Ethical Committee.

Identification of LIGHT promoter SNPs

We discovered SNPs in promoter of the LIGHT gene from 120 unrelated individuals by direct sequencing. Genomic DNA was isolated from peripheral blood cells using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany).

Table 1 The number of case and control subjects by gender

Subject	Total	Male	Female
Control	455	238	217
IS	271	154	117
LAA	95	54	41
SVO	110	65	45
CE	20	10	10
VD	207	114	93

IS—Ischemic stroke; LAA—Large artery atherosclerosis; SVO—Small vessel occlusion; CE—Cardioembolism; VD—Vascular dementia

The sequence polymorphisms were genotyped using the TaqMan polymerase chain reaction (PCR) assay (Applied Biosystems, Foster City, CA, USA). Reactions were carried out following the manufacturer's protocol, and the products were analyzed using ABI PRISM 7900HT (Applied Biosystems, Foster City, CA, USA).

We selected SNPs according to transcription factor-binding sites. We determined the potential transcription factor-binding sites altered by one base substitution using their prediction programs such as MatInspector (Genomatix, http://www.genomatix.de/), TFBIND (IFTI-MIRAGE, http://www.ifti.org/), and TRANSFAC (BIOBASE, http://www.gene-regulation.de/).

Plasmid constructs

We proceeded with a promoter-reporter assay to characterize their functional significance in vitro. Common haplotypes was selected based on their frequencies estimated from the study of SNP discovery. The 1,220 bp fragment containing the selected SNPs was amplified using PCR. The amplified product (25 μl) was loaded on 1% agarose gel and purified using QIAquick gel extraction kit (Qiagen, Charsworth, CA, USA). The products were inserted into pGEM T Easy vector (Promega, Madison, WI, USA) to facilitate construction of experimental plasmids and then subcloned for the luciferase reporter plasmid pGL3-Basic (Promega, Madison, WI, USA). The final plasmid fragments were transformed into *E. coli* DH5α competent cells (Intron, Sungnam, South Korea). The sequence of each pGL3-Basic-haplotype from screened colonies was confirmed by direct sequencing. The other common haplotypes were produced by using the QuickChange Site-Directed Mutagenesis Kit (Stratagene, LA Jolla, CA, USA) with the primers listed in Supplemental Table (available online at http://grad.hallym.ac.kr/~clee/LIGHT) and also confirmed by direct sequencing. Midipreps (Intron, Sungnam, South Korea) were prepared to generate sufficient DNA for transfection.

Transient transfection and reporter assays

We determined reporter activity of each construct using the human embryonic kidney (HEK) 293 cells that could endogenously express LIGHT. The pCMV β-gal as an internal control was co-transfected with the other reporter vectors. Luciferase activity was assessed by the Luciferase Assay System kit (Promega, Madison, WI, USA), and β-galactosidase activity by *o*-nitrophenyl-β-D-galactopyranoside cleavage rate. Three parallels were applied to every transfection, and all experiments were performed in triplicate. The details on all experiments are described in Supplemental Appendix, available online at http://www.hallym.ac.kr/~clee/LIGHT.

Association study

An association study was performed to examine the genetic effects of the selected promoter SNPs and their haplotypes on the susceptibility of ischemic stroke and vascular dementia. Genomic DNA extraction and sequencing were carried out as presented above. Genotyping was performed by laboratory personnel blind to case-control status of the samples.

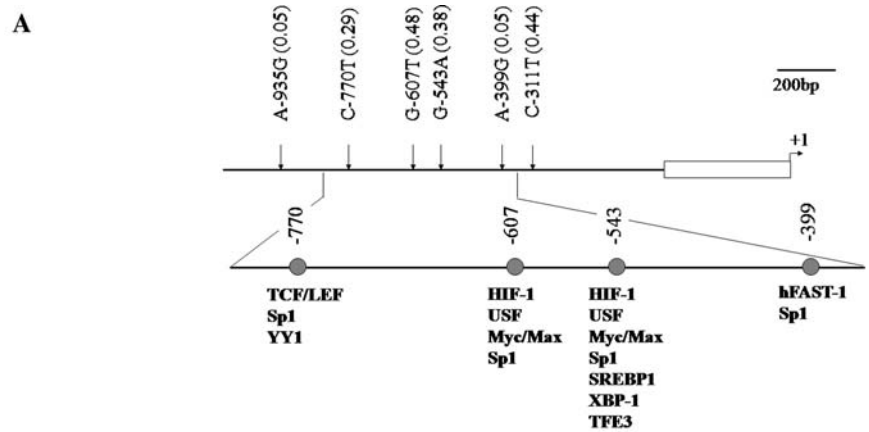
Statistical analysis

Pairwise linkage disequilibrium (LD) among the promoter SNPs was estimated by D′ and r^2 using SNPAnalyzer (ISTECH Inc., Seoul, Korea). Haplotype frequencies were estimated by an expectation-maximization (EM) algorithm using Arlequin program (version 2.0, http://lgb.unigene.ch/arlequin). Significances for comparing reporter activities of the haplotypes were tested after Bonferroni correction. Odds ratios (ORs) and the confidence intervals (CIs) for alleles, genotypes, and haplotypes were obtained using SAS Release 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

Identification of promoter SNPs in LIGHT gene

We discovered six SNPs in promoter region of the LIGHT gene by direct sequencing of 1,500 bp (Fig. 1a). In silico analysis with these SNPs showed that four out of the six SNPs have altered transcription factor binding sites, and the transcription factors altered by each SNP are presented in Fig. 1a. Of these, Sp1 affected by all the four SNPs was the only transcription factor previously reported for regulating promoter activity of LIGHT [19]. Its promoter activity might be also regulated considerably by the other transcription factors discovered in the current study. For example, T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) is known as a transcriptional activator essential for lymphoid cell development [20]. Yin Yang 1 (YY1) is a ubiquitously expressed transcription factor that can regulate the central nervous system during embryogenesis [21], and its target genes are known for regulating cell growth and differentiation [22]. TFEB is to regulate normal vascularization of the placenta [23], and X-box binding protein-1 (XBP-1) activates transcription of major



B

	SNPs	-770	-607	-543	-399
		|D′|			
r^2	-770	-	1.000	0.950	1.000
	-607	0.448	-	0.977	1.000
	-543	0.620	0.622	-	1.000
	-399	0.024	0.053	0.034	-

C

Haplotype	-770	-607	-543	-399	Frequency
ht1	C	G	G	A	0.46
ht2	T	T	A	A	0.28
ht3	C	T	G	A	0.10
ht4	C	T	A	A	0.09
ht5	C	G	G	G	0.05
ht6	T	T	G	A	0.01

Fig. 1 Promoter SNPs for human LIGHT gene on chromosome 19p13.3; Location, linkage disequilibrium, and allelic and haplotypic frequencies. (**a**) Map for human LIGHT promoter region. The promoter SNPs are presented with their minor allele frequencies in parentheses. The transcription factors altered by these SNPs are presented in bold. (**b**) Linkage disequilibrium coefficient among the SNPs. The upper triangular elements indicate |D′|, and the lower triangular elements indicate r^2. (**c**) Haplotypes composed of the SNPs. Common haplotypes with frequency >0.01 are presented

histocompatibility complex class II genes [24]. The human forkhead activin signal transducer-1 (hFAST-1) can mediate transcriptional responses to transforming growth factor β (TGFβ) in a ligand-, receptor-, and Smad-dependent manner [25].

The identified four SNPs were all selected for the association study because they were not in complete linkage equilibrium (Fig. 1b). The distributions of their genotypes were all in Hardy-Weinberg equilibrium ($P > 0.05$). Six natural haplotypes composed of the four SNPs were selected based on their frequencies greater than 0.01 and used for promoter activity analysis (Fig. 1c).

Activity of promoter SNPs in LIGHT gene

The promoter-reporter assay of the constructs corresponding to the six haplotypes showed remarkable differences in transcriptional activity at 24 h (Fig. 2a). The transcriptional activity somewhat increased at 48 h for the constructs, but their proportional changes were corresponding to one another (data not shown). The highest luciferase activity was induced by CGGA, the most common haplotype construct. The TTGA was found to be the lowest expressing haplotype with 0.39-fold activity ($P < 0.001$). The largest difference by one base substitution was observed between CTGA and TTGA (41%), indicating a potential role of C-770T. A stronger effect of the C-770T on gene expression could be detected by further investigating promoter activity for TGGA, the haplotype construct with the first base substitution from C to T within the most common haplotype (Fig. 2b). The difference in promoter activity between CGGA and TGGA were 47% ($P < 0.001$), which was corresponding to that between CTGA and TTGA.

Another dramatic reduction in the promoter activity was observed by two base substitutions from CGGA to CTAA (48%, $P < 0.001$). This might be explained by marginal reductions by two one-base substitutions from CGGA to CTGA (20%, $P < 0.001$) and CTGA to CTAA (28%, $P < 0.001$). This indicated that substituting the second and the third SNPs might influence on gene expression.

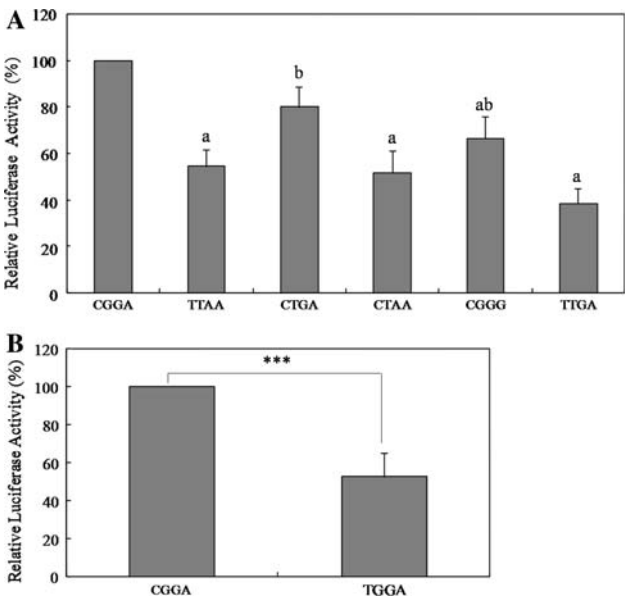


Fig. 2 Effect of haplotypes on the LIGHT promoter activity in HEK293 cells at 24 h. (**a**) In vitro luciferase expression levels for wild-type (CGGA) and five variant (TTAA, CTGA, CTAA, CGGG, and TTGA) haplotypes. Luciferase activity was normalized to the β-galactosidase activity. Data are expressed as percentages over the common haplotype activity and shown as mean ± SD. Three transfections were performed per experiment, and all experiments were triplicated. Reporter activities without the same letter mean statistical difference ($P < 0.001$) by multiple testing after Bonferroni correction. (**b**) An effect of −770C/T on the transcriptional activity of the LIGHT promoter. Statistical difference was detected between the transcriptional activities of the two constructs possessing C or T allele in this cell line. ***$P < 0.001$

Association of promoter SNPs in LIGHT gene with ischemic stroke

A preliminary logistic regression analysis showed that ORs for SNPs and their haplotypes using age, body mass index, hyperlipidemia, smoking, and hypertension did not differ from those unadjusted for these factors ($P > 0.05$; data not shown). On the other hand, a difference between ORs with and without gender was significantly observed ($P < 0.05$), and further analysis of the data partitioned by gender revealed an association of C-770T with vascular dementia (Table 2). Its T allele was associated with an increased risk of vascular dementia in females ($P < 0.05$). The significance, however, did not reach in males ($P > 0.05$). There was also a haplotypic association with vascular dementia in females demonstrating even more increased OR estimates of 10.59 (Table 3, TTGA, $P < 0.05$). Furthermore, the haplotypic risk increased in female CE patients (OR = 49.24, $P < 0.001$).

Discussion

The human LIGHT gene was examined in the current study for its association with ischemic stroke and vascular dementia using four promoter SNPs; C-770T, G-607T, G-543A, and A-399G. The case-control association study showed significant associations of C-770T and the haplotype, TTGA, with vascular dementia that was accompanied with progressive deterioration in memory, behavior, thinking, motor, and cognition. The associations were supported by the influential promoter activity of the haplotype constructs. The largest change in the promoter

Table 2 Allele frequency of selected LIGHT promoter SNPs in case and control subjects by gender

SNP	Subject	Male				Female			
		Frequency		OR (95% CI)	*P*-value	Frequency		OR (95% CI)	*P*-value
C-770T		C	T	T		C	T	T	
	Control	0.65	0.35	Reference		0.73	0.27	Reference	
	IS	0.70	0.30	0.82(0.58–1.17)	0.272	0.71	0.29	1.14(0.78–1.68)	0.497
	LAA	0.73	0.27	0.69(0.42–1.13)	0.143	0.70	0.30	1.20(0.70–2.06)	0.510
	SVO	0.68	0.32	0.87(0.56–1.36)	0.534	0.67	0.33	1.37(0.82–2.28)	0.232
	CE	0.85	0.15	0.33(0.10–1.16)	0.085	0.60	0.40	1.82(0.72–4.63)	0.207
	VD	0.71	0.29	0.78(0.54–1.15)	0.208	**0.64**	**0.36**	**1.54(1.03–2.30)**	**0.034**
G-607T		G	T	T		G	T	T	
	Control	0.50	0.50	Reference		0.57	0.43	Reference	
	IS	0.55	0.45	0.83(0.60–1.16)	0.275	0.54	0.46	1.12(0.79–1.59)	0.521
	LAA	0.57	0.43	0.75(0.48–1.18)	0.215	0.59	0.41	0.94(0.57–1.56)	0.822
	SVO	0.53	0.47	0.90(0.59–1.36)	0.610	0.51	0.49	1.28(0.79–2.05)	0.317
	CE	0.60	0.40	0.68(0.27–1.71)	0.408	0.50	0.50	1.33(0.54–3.31)	0.535
	VD	0.53	0.47	0.91(0.64–1.30)	0.613	0.53	0.47	1.17(0.81–1.70)	0.405
G-543A		G	A	A		G	A	A	
	Control	0.59	0.41	Reference		0.67	0.33	Reference	
	IS	0.62	0.38	0.90(0.64–1.25)	0.516	0.61	0.39	1.30(0.91–1.87)	0.155
	LAA	0.63	0.37	0.85(0.54–1.35)	0.490	0.60	0.40	1.38(0.83–2.29)	0.217
	SVO	0.61	0.39	0.93(0.61–1.43)	0.753	0.59	0.41	1.43(0.88–1.14)	0.154
	CE	0.70	0.30	0.62(0.23–1.66)	0.342	0.55	0.45	1.67(0.67–4.18)	0.271
	VD	0.63	0.37	0.86(0.60–1.23)	0.412	0.66	0.34	1.07(0.72–1.59)	0.728
A-399G		A	G	G		A	G	G	
	Control	0.95	0.05	Reference		0.94	0.06	Reference	
	IS	0.95	0.05	0.89(0.43–1.86)	0.756	0.96	0.04	0.62(0.27–1.42)	0.256
	LAA	0.95	0.05	0.84(0.30–2.37)	0.739	0.98	0.02	0.39(0.09–1.71)	0.210
	SVO	0.93	0.07	1.28(0.55–3.02)	0.566	0.97	0.03	0.53(0.15–1.86)	0.325
	CE	1.00	0.00	–		0.90	0.10	1.72(0.37–8.03)	0.491
	VD	0.97	0.03	0.55(0.22–1.37)	0.196	0.94	0.06	0.97(0.45–2.13)	0.944

IS—Ischemic stroke; LAA—large artery atherosclerosis; SVO—Small vessel occlusion; CE—Cardioembolism; VD—Vascular dementia. Boldface indicates $P < 0.05$

activity by one base substitution was observed at C-770T from C to T, showing 47% reduction from CGGA to TGGA and 41% from CTGA to TTGA. Also, the haplotype associated with vascular dementia showed the smallest promoter activity, only 39% activity of the most common haplotype.

The transcription factors altered specifically by C-770T were TCF/LEF and YY1. The TCF/LEF might be influential in the development of vascular dementia by destroying vasculature in human brain. Wnt signaling pathway plays an important regulatory role in the vasculature as factor of angiogenesis through its downstream transcription factors, TCF/LEF and β-catenin [26]. The YY1 might also contribute developing vascular dementia by repressing the promoters of pro-atherogenic genes [27] and regulating the expression of several T cell cytokines [28]. Moreover, YY1 inhibits the growth of vascular smooth muscle cells without influencing endothelial cell proliferation [29]. Functional interaction of YY1 with c-Myc might further explain the haplotypic effect caused by additional allelic substitutions of G-607T and G-543A from the most common haplotype [30].

The association specifically observed with vascular dementia might be produced by additional inflammatory roles of LIGHT on the pathogenesis related to cognitive impairment or memory function after ischemic cerebrovascular events. The pathogenic etiology of not only ischemic stroke but also further development of vascular dementia could depend on neuroinflammatory mechanisms. For example, a proinflammatory cytokine, interleukin 1β (IL1β) increased in patients with ischemic stroke and vascular dementia [31]. On the other hand,

Table 3 Haplotype frequency of selected LIGHT promoter SNPs in case and control subjects by gender

Haplotype	Subject	Male			Female		
		Frequency	OR (95% CI)	*P*	Frequency	OR (95% CI)	*P*
CGGA	Control	0.45	Reference		0.51	Reference	
(ht1)	IS	0.49	1.20(0.86–1.66)	0.283	0.49	0.92(0.65–1.31)	0.652
	LAA	0.53	1.37(0.88–2.14)	0.165	0.52	1.07(0.65–1.75)	0.784
	SVO	0.46	1.05(0.69–1.60)	0.812	0.47	0.85(0.53–1.37)	0.504
	CE	0.60	1.84(0.73–4.65)	0.196	0.40	0.65(0.26–1.63)	0.358
	VD	0.50	1.20(0.84–1.72)	0.308	0.47	0.86(0.58–1.27)	0.444
TTAA	Control	0.34	Reference		0.26	Reference	
(ht2)	IS	0.30	0.84(0.59–1.19)	0.321	0.27	1.07(0.72–1.58)	0.744
	LAA	0.26	0.69(0.42–1.14)	0.148	0.27	1.04(0.60–1.81)	0.891
	SVO	0.32	0.91(0.44–2.08)	0.684	0.32	1.35(0.80–2.26)	0.257
	CE	0.15	0.35(0.10–1.22)	0.100	0.25	0.95(0.33–2.69)	0.916
	VD	0.30	0.84(0.57–1.24)	0.389	0.35	1.51(0.99–2.29)	0.053
CTGA	Control	0.08	Reference		0.10	Reference	
(ht3)	IS	0.07	0.84(0.45–1.57)	0.593	0.08	0.80(0.43–1.46)	0.462
	LAA	0.06	1.48(0.58–3.77)	0.406	0.05	0.46(0.16–1.36)	0.160
	SVO	0.08	0.95(0.44–2.08)	0.907	0.09	0.88(0.39–2.00)	0.757
	CE	0.10	1.27(0.28–5.85)	0.757	–	–	
	VD	0.09	1.18(0.63–2.23)	0.607	0.10	1.04(0.55–1.97)	0.902
CTAA	Control	0.07	Reference		0.06	Reference	
(ht4)	IS	0.08	1.18(0.64–2.16)	0.595	0.10	1.59(0.83–3.02)	0.159
	LAA	0.11	1.59(0.75–3.37)	0.229	0.10	1.57(0.66–3.76)	0.308
	SVO	0.08	1.06(0.48–2.33)	0.888	0.08	1.23(0.50–3.04)	0.658
	CE	0.15	2.24(0.61–8.30)	0.227	0.10	1.62(0.35–7.52)	0.540
	VD	0.10	1.38(0.73–2.62)	0.322	0.03	0.46(0.17–1.26)	0.129
CGGG	Control	0.05	Reference		0.06	Reference	
(ht5)	IS	0.05	1.04(0.49–2.21)	0.909	0.04	0.62(0.27–1.42)	0.256
	LAA	0.04	0.71(0.23–2.22)	0.561	0.02	0.39(0.09–1.71)	0.210
	SVO	0.07	1.38(0.58–3.28)	0.464	0.03	0.53(0.15–1.86)	0.325
	CE	–	–		–	–	
	VD	–	–		–	–	
TTGA	Control	0.01	Reference		0.00	Reference	
(ht6)	IS	–	–		0.00	1.20(0.07–19.25)	0.899
	LAA	–	–		–	–	
	SVO	–	–		–	–	
	CE	–	–		**0.15**	**49.24(4.86–498.77)**	**0.001**
	VD	0.01	1.93(0.32–11.68)	0.472	**0.04**	**10.59(1.26–88.80)**	**0.030**
TGAA	Control	–	–		0.00	Reference	
(ht7)	IS	–	–		0.02	4.85(0.54–43.71)	0.159
	LAA	–	–		**0.04**	**10.59(1.09–103.26)**	**0.042**
	SVO	–	–		0.01	3.13(0.19–50.63)	0.421
	CE	–	–		–	–	
	VD	–	–		–	–	

IS—Ischemic stroke; LAA—Large artery atherosclerosis; SVO—Small vessel occlusion; CE—Cardioembolism; VD—Vascular dementia. Boldface indicates $P < 0.05$. The haplotype TGAA (ht7) was included in the association analysis because its frequency was larger than 0.01 in ischemic stroke patients

another proinflammatory cytokine, tumor necrosis factor α (TNFα), increased in patients only with vascular dementia [31]. The increased TNFα could induce an immunosuppression by apoptotic loss of T cell, resulting in indispensable influence on T cell homeostasis of LIGHT [32]. Furthermore, chronic inflammatory processes have been known to produce oxidative stress that was recognized as one of contributing factors in the pathogenesis of vascular dementia [33]. Additionally, vascular dementia might be partially induced by causes associated with autoimmune inflammatory diseases such as systemic lupus erythematosus [34] and temporal arteries [35].

The current study showed a female-specific association of LIGHT sequence variants with vascular dementia. Such gender-specific effects of autosomal genes might be explained by their epistasis with sex-linked genes [36]. The epistasis is often resulted from hormonal effects on gene expression and regulation. The female-specific effects found in this study might be accountable for potential interaction between LIGHT and female hormones such as estrogen and progesterone. Effects of the female hormones on cytokine production can control the balance between Th1 and Th2 responses [37]. Especially, the estrogen and LIGHT could produce proinflammatory Th1 cytokine and thus stimulate immune responses. The female-specific effect of LIGHT might be also supported by the association studies where LIGHT increased as a mediator of bone resorption in patients with rheumatoid arthritis [11] and in mice with collagen-induced arthritis [38]. The association might reflect modulation of estrogen on collagen-induced arthritis [37]. This supports that female hormones might act as a detonator for the C-770T polymorphism and further TTGA haplotype to demonstrate genetic effects, and consequently the polymorphisms might produce female-specific effects. The female-specific effects could be additionally supported by female-specific functions of the transcriptional factors altered by the polymorphisms. Wnt4, one of the Wnt family, operates during the development of the female reproductive system, and it is implicated in forming sex-specific vasculature, inhibiting steroidogenesis, and inducing female-specific cell migration events [39]. Also, YY1 is involved in signaling by TNFα that regulates osteoclast differentiation and function [40].

We could speculate a scenario about the effect of LIGHT on female vascular dementia. Reduction of LIGHT at the post-stroke stage would result in a decrease of T cell proliferation and thus an increase of inflammation. Such inflammatory response would generate oxidative stress, which becomes more serious accompanied with a deficit of neuroprotective effects of estrogens. Finally, cognitive impairment in postmenopausal females might be developed as a result cumulated from oxidative stress by decreased estrogen. Our results suggest that the allele T of C-770T and the haplotype TTGA of the promoter SNPs in LIGHT gene might decrease the expression of LIGHT and subsequently increase the susceptibility of vascular dementia in females. Our findings should be replicated with a larger sample for practical application with a precise estimate of the risk. A large-scale study would enable us to confirm authenticity of the genetic effects of LIGHT on ischemic stroke and its subtypes.

Acknowledgements We thank healthy participants, patients with ischemic stroke and vascular dementia, and their family members whose contribution made this work possible. We also thank all neurologists who recruited their patients for this study and our laboratory personnel who collected clinical data and participated in SNP discovery. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (A020007).

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