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원 저

A Study on the Association between Tumor Necrosis Factor Alpha Gene Polymorphism and *Sasang* Constitution in Cerebral Infarction

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Objective : Tumor necrosis factor-α (TNF-α), a potent immuno-modulator and pro-inflammatory cytokine, has been implicated in many pathological processes. In this study, the author examined whether promoter region polymorphism in the TNF-α gene at position -308 affect the odds of cerebral infarction (CI) and whether genetic risk is enhanced by *sasang* constitutional classification.

Methods : 212 CI patients and 610 healthy controls were genotyped and determined according to *sasang* constitutional classification. The amplified genotypes were analyzed on 8% polyacrylamide gel. The alleles were visualized by ethidium bromide staining. Primers for TNF-α were designed to incorporate a polymorphic site at a position -308 bp of the TNF-α gene into an NcoI restriction site. Restriction digests generated products of 87 and 20 bp for G allele and 107 bp for A allele.

Results : A significant decrease was found for the TNF-α A allele in CI patients compared with controls (P=0.033, odds ratio, O.R.: 0.622). However, there was no significant association between TNF-α polymorphism and *sasang* constitution in CI patients.

Conclusion : My finding suggests that TNF-α promoter region polymorphism is responsible for susceptibility to CI in Koreans.

Key Words: tumor necrosis factor-α, TNF-α, *sasang* constitution, cerebral infarction, genotype, Korean

Introduction

The *Sasang* Constitutional Medicine classifies people's constitutions into four types, according to the strength and weakness in functions of the internal organs. *Sasang* Constitutional philosophy forms the basis of treatment by correcting the imbalance of the internal organs caused by the constitutional properties in each body type. Then it presents different treatments according to constitution[1)]. The different constitutions bring about different reactions to the same disease. Like this, the differences of disease severity to be shown in *Sasang* constitutional classification may be due to genetic factors.

Therefore much research for wishing to find the essential mechanism in gene level are tried vigorously to examine closely hereditary of *Sasang* Constitution recently. The examination of the association between

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Sasang Constitution and gene is taking a serious view as part of objectification research in order to suggest the solution for correct classification and distinction of *Sasang* Constitution. Up to now, much research for that association are gone based on Cho' s research about DNA-fingerprinting methods that is reported in 1996[2-3]. Specially, research for genetic factor of a stroke are expected to be still more useful in relation research with *Sasang* Constitution. Much valid research results were gained through much research about correlation between cerebrovascular disease and *Sasang* Constitutional medicine, since Song[4] made foundation of access about treatment and management of stroke in the view of *Sasang* Constitutional medicine.Lately, many theories that gene polymorphism increases the risk of cerebrovascular disease are proved thoroughly. Then Joo et al.[5] examined closely association between *Sasang* Constitution and ACE gene of cerebral infarction patients.

Cerebral infarction (CI) is a multifactorial disease caused by the interactions of several genetic and environmental factors, as with ischemic heart disease. Infiltration of leukocytes early in the ischemic region and development of brain edema characterizes the ischemia-induced inflammation[6]. Recently, it has become increasingly evident that this inflammatory response plays an important role in the pathogenesis of cerebral lesion following stroke. One of the gene polymorphism factor which contributes to the risk of cerebrovascular disease is justly TNF-α as a up-regulated cytokine in the brain after injury, which is worthy of notice.

TNF-α is known that it is concerned in the inflammatory response and the immune response[7], and it is a pleiotropic cytokine that promotes inflammation and signals leading to cell death. TNF contributes clearly to neuronal death in brain ischemia and HIV-1 infection. Contrary to the preponderance of evidence that TNF is toxic to neurons, it also exerts neuro-protective effects, particularly in conditions of excitotoxic death[8].

The TNF-α gene is located on the short arm of chromosome 6 between the class I and class II regions of the HLA complex. A striking feature of the entire HLA complex is a high degree of genetic variation. A number of polymorphisms have also been described for the TNF-α locus. A dimorphism with potential functional relevance is a guanine-to-adenosine transition at base pair -308 in the promoter region (termed the A allele)[9]. The A allele has been shown to be associated with increased TNF-α expression after in vitro stimulation[10-12]. Therefore this genetic variation might result in an altered TNF-α expression and thereby affect susceptibility and clinical severity of inflammatory diseases. Indeed, the A allele of TNF-α-308 is associated with a sevenfold increased risk for cerebral complications of malaria[13] and with a worse prognosis and longer disease duration in dermatitis herpetiformis[14-15].

In this study, the author investigated whether TNF promoter region polymorphism might be responsible for susceptibility to CI and whether genetic risk is enhanced by *Sasang* constitutional classification.

Materials and methods

1. Subjects and measurements

Patients with documented CI were identified from clinical records from December 1999 to July 2002 of one hospital in Iksan, Korea. Patients aged younger than 30 and older than 80 years were excluded. Final diagnosis of CI was confirmed on brain computed tomography or brain magnetic resonance imaging. I identified 212 patients with CI, excluding the subjects with cerebral hemorrhage. A total of 610 control subjects were recruited from individuals attending Wonkwang University Hospital (Iksan, South Korea)

undergoing general check-ups. None of the controls had a history of CI. The control group were randomly recruited and matched with study patients for age and gender.

2. Determination of genotypes

The genomic DNA was extracted by inorganic procedure[16]. A single base pair polymorphism at position 308 in the promoter region of the TNF-α gene was examined by the NcoI (Takara, Shiga, Japan) restriction fragment length polymorphism (RFLP) method described elsewhere[17]. PCR was performed in a reaction volume of 20μl using 1 units of Taq DNA (Takara, Japan) in the buffer furnished by the manufacturer supplemented with 1.5mM $MgCl_2$, 250μ M of each nucleotide, 200ng of DNA template and 0.2μ M of each

primer : 5' - AGGCAATAGGTTTTGAGGGCCAT-3(upstream)and 5' -TCCTCCCTGCTCCGATTCCG-3' (downstream).

Cycling conditions were as follows: 1 cycle of 94℃ for 3 min, 60℃ for 1 min, 72℃ for 1 min 35 cycles of 94℃ for 1 min, 60℃ for 1 min, 72℃ for 1 min; 1 cycle of 94℃ for 1 min, 60℃ for 1 min, 72℃ for 5 min. Products of 107 bp were generated. Primers for TNF-α were designed to incorporate a polymorphic site at a position 308 bp of the TNF-α gene into an NcoI restriction site. Restriction digests generated products of 87 and 20 bp for G allele and 107 bp for A allele (Fig.1).

3. Discrimination of *Sasang* Constitution of individuals

Individuals were discriminated into four types by QSCC II program; Taeumin, Taeyangin, Soyangin, and Soeumin. QSCC II is the program for "objective 4-constitutional body types" under PC, which is developed by the Department of *Sasang* Medical in Kyung Hee University Oriental Medicine Hospital, Seoul, Korea. It has been proved for providing its accuracy and universal logical ground with its standardized diagrams according to diagnostic clinical data.

4. Statistical analysis

The χ^2 test was used to compare genotype and allele frequencies between the CI and control populations. Odds ratios (O.R.) were calculated with 95% confidence intervals (C.I.). All statistical analyses were performed using SPSS v10.00 (SPSS Inc.) statistical analysis software. A p-value less than 0.05 considered statistically significant.

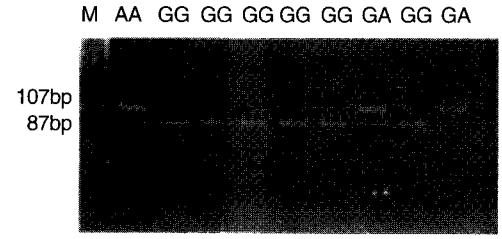

Fig. 1. Electrophoretic separation of TNF-α genotypes. The amplified genotypes were analyzed on 8% polyacrylamide gel. The alleles were visualized by ethidium bromide staining. Primers for TNF-α were designed to incorporate a polymorphic site at a position 308 bp of the TNF-α gene into an NcoI restriction site. Restriction digests generated products of 87 and 20 bp for G allele and 107 bp for A allele.

Results

1. Clinical characteristics of patients with CI

The characteristics of the patients with CI are summarized in Table 1. The patients comprised 101 females (47.6%) and 111 males (52.4%) with a mean age of 46.9±19.8 years of age.

2. Clinical characteristics of patients according to TNF-α polymorphism

The characteristics of the patients according to TNF-α genotypes and alleles are shown in Table 2 and Table 3. Mean values of total cholesterol and triglyceride were lower in GA/AA genotypes than in GG genotype (194.0 ±46.3 vs. 187.9±47.6) (Table 2). The tendency was also shown in patients with A allele (Table 3). However, the differences were not statistically significant ($p>0.05$).

Table 1. Clinical Characteristics of CI Patients (n=212)

Characteristics	
Age (year)	46.9±19.8
Male, %	52.4
Total cholesterol (mg/dl)	193.3±46.3
Triglyceride (mg/dl)	150.5±117.2
Hypertension, %	46.1
Diabetes, %	12.7

Values are means±S.D.

Table 2. Characteristics of CI Patients (n=212) according to TNF-α Genotype

	Genotype		Statistics[a]
Characteristics	GG	GA+AA	
Total cholesterol (mg/dl)	194.0±46.3	187.9±47.6	NS
Triglyceride (mg/dl)	153.9±123.1	125.4±54.8	NS
Hypertension, %	45.8	47.8	NS
Diabetes, %	12.7	13.0	NS

Values are means±S.D.

[a] Statistical tests by Student's *t*-test or χ^2-test (two-sided).

Table 3. Characteristics of CI Patients (n=212) according to TNF-α Allele

	Allele		Statistics[a]
Characteristics	G	A	
Total cholesterol (mg/dl)	193.4±46.2	192.7±48.2	NS
Triglyceride (mg/dl)	152.1±120.2	127.7±52.8	NS
Hypertension, %	45.7	52.0	NS
Diabetes, %	12.7	12.0	NS

Values are means±S.D.

[a] Statistical tests by Student's *t*-test or χ^2-test (two-sided).

Table 4. Characteristics of CI Patients (n=212) according to Sasang Constitution

	Sasang Constitution			Statistics[a]
Characteristics	Taeumin	Soyangin	Soeumin	
Total cholesterol (mg/dl)	192.7±46.0	196.3±46.8	171.0±39.8	NS
Triglyceride (mg/dl)	159.9±133.8	139.0±83.0	114.5±90.8	NS
Hypertension, %	54.3	47.2	23.5	p=0.027
Diabetes, %	16.2	13.5	0	NS

Values are means±S.D.
[a] Statistical tests by one-way ANOVA or Linear-by-Linear association test (two-sided).

Table 5. Distribution of TNF-α Genotype in CI Patients (n=212) and Control Subjects (n=610)

	Genotype			Statistics[a]
GG	GA	AA		
CI patients, n (%)	187(88.2)	24(11.3)	1(0.5)	p=0.104
Controls, n (%)	509(83.4)	86(14.1)	15(2.5)	

[a] By χ^2-test (two-sided).

Table 6. Distribution of TNF-α Allele in CI Patients and Control Subjects

	Allele		Statistics[a]
	G	A	
CI patients, n (%)	398(93.9)	26(6.1)	p=0.033, O.R. 0.622, C.I. 0.400-0.966
Controls, n (%)	1104(90.5)	116(9.5)	

[a] By χ^2-test (two-sided).

Table 7. Distribution of TNF-α Genotype according to Sasang Constitution in CI Patients

	Sasang constitution, n (%)			Statistics[a]
Genotype	Taeumin	Soyangin	Soeumin	
GG	90(85.7)	45(86.5)	16(94.1)	p=0.636
GA+AA	15(14.3)	7(13.5)	1(5.9)a	

[a] By χ^2-test (two-sided).
In CI patients, 174 cases of 212 cases were valid and the remaining 38 cases were omitted.

Table 8. Distribution of TNF-α Allele according to Sasang Constitution in CI Patients

	Sasang constitution, n (%)			Statistics[a]
Allele	Taeumin	Soyangin	Soeumin	
G	198(92.4)	97(93.3)	32(94.1)	p=0.91
A	16(7.6)	7(6.7)	2(5.9)	

[a] By χ^2-test (two-sided).
In CI patients, 174 cases of 212 cases were valid and the remaining 38 cases were omitted.

3. Clinical characteristics of patients according to *Sasang* Constitution

The characteristics of the patients according to *Sasang* Constitution are shown in Table 4. Mean values of total cholesterol and triglyceride were lower in Soeumin than in remaining constitutions, although the difference was not statistically significant. Of interest, the frequency of hypertension in Taeumin was significantly higher than those in remaining constitutions (p=0.027) (Table 4).

4. Frequencies of genotype

The distribution of -308G/A genotype and allele in the TNF promoter region is shown in Table 5 and Table 6. Both the patient and control populations were in Hardy-Weinberg equilibrium. The -308G/A genotype showed a marginally significant difference between CI patients and controls (χ^2=9.214, p=0.104). The distribution of -308G/A genotype in 212 patients with CI was as follows; G/G, 187 (88.2%); G/A, 24 (11.3%); and A/A, 1 (0.5%). It was different from the distribution in 610 control subjects: G/G, 509 (83.4%); G/A, 86 (14.1%); and A/A, 15 (2.5%) (Table 5). In addition, the difference was more obvious in the allele frequencies between CI patients and controls (χ^2=4.545; p=0.033; odds ratio, O.R.: 0.622; confidence interval, C.I.: 0.400-0.966). The allele frequencies of patients with CI were as follows; G, 398 (93.9%); and A, 26 (6.1%), which was significantly different from the distribution in control subjects: G, 1104 (90.5%); and A, 116 (9.5%).

5. Association between TNF-α polymorphism and *Sasang* Constitution

The distribution of *Sasang* Constitution in 212 patients with CI was as follows; Taeumin, 58.2%; Soyangin, 30.6%; and Soeumin, 11.2%. Of interest, the frequency of Soeumin with GA/AA genotypes was the lowest in CI patients (5.9%). (Table 7). The distribution of TNF-α alleles in Taeumin was as follows; G, 198(92.4%); A, 16(7.6%). That in Soyangin was as follows; G, 97(93.3%); A, 7(6.7%). It was not different from the distribution in Soeumin: G, 32 (94.1%); A, 2(5.9%). Therefore, the author did not find the association between TNF-α polymorphism and *Sasang* Constitution in CI patients (Table 8).

Discussion

In the《Longevity & Life Preservation In Oriental Medicine; The discourse upon four principles(東醫壽世保元; 四端論)》which is a basis text book of *Sasang* Constitutional medicine[1], spoke as "The nature and character are decided already and we need not mention these congenital formations (天稟之已定 固無可論)." And spoke as "There are four types of human beings based upon the congenital formations of the organs (人稟臟理 有四不同)." This is that *Sasang* Constitution is decided already by nature, and became theoretical basis of an immutable rule that *Sasang* Constitution does not change until die. Soon it brought a natural result that *Sasang* Constitution is hereditary. Up to now, although there is controvertible whether *Sasang* Constitution is hereditary or not, much research to examine closely the association between *Sasang* Constitution and gene are tried vigorously. Therefore DNA-fingerprinting methods is reported in 1996[2-3]. Korea Institute of Oriental Medicine and Kyung Hee University. reported to comparing each allele of *Sasang* Constitution group about STR locus as Genetic marker and examined each relationship of *Sasang* Constitution from 1996 to 1999[18]. And they announced association between the *Sasang* Constitution and ACE polymorphism[19]. In other way, there were more theoretical and clinical accesses about hereditary nature of *Sasang* Constitution[20-21]. Ha et al. instituted criticism that Microsatellite's polymorphism which is the non-functional part of

genome such as inter-gene and intron may be less relation with *Sasang* Constitution, so asserted that the functional gene polymorphism should be studied through comparative analysis about the past research for the association between *Sasang* Constitution and gene. And they presented that the studies about ACE and polymorphism of HLA types[22] and the method to use DNA chip might be useful[23].

But, at the same time Human Genome Project is discontinued, research about individual differences is accomplishing main axis recently. Specially, because single nucleotide polymorphisms (SNP) is rising as the marker that expresses each individual Special quality among this, The SNP Consortium (TSC) which were consisted of Enormous corporations like this Bayer, Glaxo, IBM, Motorola etc. announced that they constructed SNP database of 1.8 million at October, 2002[23].

In addition, Han et al. studied distribution of gene polymorphism about genes of β- Ⅱ AR (β-adrenaline receptor), β- Ⅲ AR (β-adrenaline receptor), UCP-1 (uncoupling protein-1), ALDH2 (aldehyde dehydrogenase) according to *Sasang* Constitution tendency, but did not show significant different[24].

On the other, many valid research results were gained for the treatment and management of stroke in the view of *Sasang* Constitutional Medicine since Song's report[4]. It was shown that the distribution of cerebral infarction patient by *Sasang* Constitution is agreed with Taeumin > Soyangin > Soeumin's order in several research as well as Choi 's report[25]. Then the research like Joo.'s[5] are gone to examine closely association between *Sasang* Constitution and various genetic factors that cause the cerebral infarction.

Recent attention to CI has focused on the inflammatory component of atherogenesis and acute ischemia[26-27]. Indeed, atherosclerosis is now described as an inflammatory disease[27] and flared plaque inflammation is considered a cause of intimate erosion and rupture and therefore of acute ischemia[26]. Most cerebrovascular disease is also related to atherosclerosis of the cerebral arteries, in which the inflammation system takes a serious view. The common and major pathological changes are atherosclerosis and thrombogenesis of that artery in ischemic heart disease (IHD) and CI. Specially, genetic traits contribute significantly to the global risk of IHD[26]. Therefore a number of studies have now addressed that the genetic variation theory of the inflammatory system that may increase the risk of disease[28].

However, the association between inflammatory cytokines and cerebrovascular disease has been less studied as compared with IHD. Here the author investigated the association between TNF-α-308 polymorphism, another powerful pro-inflammatory cytokine, and CI.

TNF is a kind of what is called biologic response material (BRM), and was found first by Memorial Sloan-kettering Cancer Center (MSKCC), States of America, in 1975. TNF is divided alpha(α) and beta(β), and TNF-α says cachectin in other name. TNF-α is created from Th1 cell, activated macrophage, monocyte, neutrophil and NK cell. It is concerned in partial cell-mediated immune responses and acts tumor necrosis, granuloma formation and increasing of toxicity of NK cell[29]. As well as autoimmune disease and infectious disease, this acts important role in obesity, insulin resistance, endothelial dysfunction, oxidative stress and arteriosclerosis. Also this acts fever, generating shock, macrophage activation, angiogenesis, bone resorption, cytotoxic to the many cells, stimulate polymorphonuclear leukocyte chemotoxin and etc in physiological conditions. TNF-α acts with IL-1 in cascade of immune response about infection and cancer as inflammatory cytokines, which can defend infectious or metastatic disease from pathogenic bacteria, Fungi, and protozoa[30-31].

When TNF-α is created in low concentration, it causes tissue remodeling and acts as fibroblast growth factor that causes deposition of angiogenesis factor and connective tissue[32-34]. But if TNF-α is created in high concentration according to non-regulated function, it can cause cachexia, auto immune disorder, meningococcal septicemia or septic shock that is mortal in life[35-36].

And it can cause rheumatoid arthritis in this state. United States of America UT Southwestern College of medicine research team developed new protein as anti TNF therapy that activation of TNF protein variants related with inflammation response in rheumatoid arthritis, and is studying pre-clinical testing. As well as Rheumatoid arthritis, it was reported that This anti TNF therapy is effective to autoimmune disease such as multiple sclerosis and systemic lupus erythematosis and to intercepting inflammation in neurodegenerative disorders such as Alzheimer' s disease[37].

Shin et al. found out that the risk of asthma is much higher when TNF-α-308th base is guanine(G) instead of adenine(A), through result of analysis of 700 asthmatic's gene for 3 years since 2001[38].

TNF-α aggravates progress of cerebral injury flowed in from outside after brain ischemia[39], but it is reported that if we reduce the function of TNF-α in cell interior through injection of anti TNF-antibody or soluble TNF-α receptor, TNF-α defends nerve cell from brain injury on brain ischemia[40-41].

TNF-α as a marker of monocyte/macrophage activation is elevated in vascular wall injury[42]. It stimulates the endothelial expression of adhesion molecules for mononuclear cells and induces endothelial apoptosis[43]. It also down-regulates endothelial nitric oxide synthase[44] and increases oxidative stress in endothelial cells[45]. Since atherosclerosis is a main cause of CI, it can be expected that frequency of A allele in CI group should be increased. However, the author observed a significant increase for the TNF-α A allele in controls compared with CI patients as a result. The mechanism by which the TNF-α gene polymorphisms might have an essential role for CI is probably related to a different TNF-α synthesis, secretion and activity. Wilson et al. reported that the AA genotype significantly increased the transcriptional activity of the TNF-α-308 gene with respect to the GG genotype, and that a slight increase of the protein levels was also observed in the plasma[10]. Therefore, G allele carriers are regarded to be low producers of TNF-α, whereas A allele carriers are high producers of TNF-α. Therefore my results may be explained by assuming that the A allele and resulting increased TNF-α concentration in circulation may induce the neuro-protective effects, particularly in conditions of excitotoxic death, and thereby affect susceptibility of CI. These explanations could be supported by several reports on the beneficial effects of TNF-α. Nawashiro et al. have demonstrated that pre-treatment with TNF-α is neuro-protective[46]. In parallel, Gary et al. have shown that p55, a high-affinity receptor RI of TNF-α, knock-out mice are more susceptible than wild type to cerebral ischemia[47], and other studies suggest that TNF-α may have a potential neuro-protective role[8, 48].

However, concerning the use of TNF-α as a neuro-protective agent, the results are ambiguous. Nawashiro et al. have reported that administration of TNF-α binding protein (which inhibits TNF-α) is also neuro-protective[40]. For instance, although TNF-α is the archetypal pro-inflammatory cytokine, it can be both neurotoxic and neuro-protective in models of cerebral ischemia and brain injury[39, 49-54]. It has been suggested that over-expression of TNF-α is deleterious in the early stages of injury, while at later time points it may aid recovery of injured tissue[51, 54]. Consequently, the role of TNF-α in the pathogenesis of cerebral ischemia should be further investigated.

In addition, the author investigated the relationship

between TNF-α polymorphism and *Sasang* Constitution in CI patients. As a result, any difference in distributions of the genotype was not observed in *Sasang* constitution. However, the author found a possibility that *Sasang* Constitution could enhance the risk for CI associated with TNF-α genotype. For instance, the author observed a significant increase for the TNF-α A allele in controls compared with CI patients. In addition, the mean values of total cholesterol and triglyceride were lower in GA/AA genotypes than in GG genotype, and tended to be lower in Soeumin than remaining constitutions, but not significantly so. Of interest, the frequency of Soeumin with GA/AA genotype was the lowest in CI patients (5.9%). These results indicate that the A allele and Soeumin constitution may induce the neuro-protective effects, and thereby decrease susceptibility of CI. These explanations could be supported by the viewpoint of *Sasang* constitutional medicine on Taeumin. Taeumin, who resembled the typical abdominal type of obesity in Western populations, is thought to have a higher rate of stroke, hypertension and hyperlipidemia than the other types because he or she has a large liver and small lung. Here my data showed a consistent result with the viewpoint of *Sasang* Constitutional Medicine.

In summary, I suggest the apparent relationship between gene polymorphism and CI, as well as the novel possibility of molecular genetic understanding of *Sasang* constitutional medicine.

References

1. Lee JM, Choi SH(Eds). Dongyi Suse Bowon (Longevity & Life Preservation In Oriental Medicine). Seoul. Korea:Kyung Hee University Press. 1996:10-15.
2. Cho DW, Cho WS et al. Genetic analysis study of *Sasang* Constitution classification. Korean Journal of Oriental Medicine. 1996;2:402-406.
3. Cho DW et al. Genetic Analysis study of *Sasang* Constitution Classification by DNA-fingerprinting methods. J. of *Sasang* Constitutional Medicine. 1996;8(1):151-163.
4. Song IB. Clinical Study on the Management of Stroke Patients in the View of *Sasang* Constitutional Medicine. J. of *Sasang* Constitutional Medicine. 1996;8(2):117-130.
5. Joo JC. et al. Angiotensin Converting Enzyme (ACE) Gene Polymorphism and *Sasang* Constitution in Patients with Cerebral Infarction. J. of *Sasang* Constitutional Medicine. 2002; 14(1):132-139.
6. Stoll G, Jander S, Schroeter M. Inflammation and glial responses in ischemic brain lesions. Prog. Neurobiol. 1998;56:149-171.
7. Estrada C, Gomez C, Martin C. Effects of TNF-α on the production of vasoactive substances by cerebral endothelial and smooth muscle cells in culture. J. Cereb Blood Flow Metab. 1995;15: 920-928.
8. Cheng B, Christakos S, Mattson MP. Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. Neuron. 1994;14:139-153.
9. Wilson AG, DiGiovine FS, Duff GW. Genetics of tumor necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. J. Inflamm. 1995;45:1-12.
10. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation, Proc. Natl. Acad. Sci. USA. 1997;94: 3195-3199.
11. Braun N, Michel U, Ernst BP, Metzner R, Bitsch A, Weber F, Rieckmann P. Gene polymorphism at position -308 of the tumor-necrosis-factor-alpha (TNF-alpha) in multiple sclerosis and it' s influence on the regulation of TNF-alpha production, Neurosci. Lett. 1996;215:75-78.
12. Wu WS, McClain K. DNA polymorphisms and mutation of the tumor necrosis-alpha (TNF-

alpha) promoter in Langerhans cell histiocytosis (LCH). J. Interferon Cytokine Res. 1997;17:631-635.
13. McGuire W, Hill AV, Allsopp CEM, Greenwood BM, Kwiatkowski D. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. Nature. 1994;371:508-510.
14. Wilson AG, Clay FE, Crane AM, Cork MJ, Duff GW. Genetics of tumor necrosis factor alpha in dermatitis herpetiformis. J. Invest. Dermatol. 1995;104:856-858.
15. Collier PM, Wojnarowska F, McGuire B, Welsh KI. Polymorphism of tumor necrosis factor a promoter region of the MHC is strongly associated with linear IgA disease and affects prognosis. Br. J. Dermatol. 1994;131:22.
16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
17. Cabrera M, Shaw MA, Sharples C, Williams H, Castes M, Convit J, Blackwell JM. Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. J. Exp. Med. 1995;182:1259-1264.
18. Cho DW. et al. Genetic Analysis of *Sasang* Constitution by Amp-FLP Method, J. of *Sasang* Constitutional Medicine. 1997;9(2):163-174.
19. Choi SH. et al. Relationship Between the *Sasang* Constitution and ACE Polymorphism. J. of *Sasang* Constitutional Medicine. 1998;10(2): 283-290.
20. Kim MH. Study on *Sasang* Constitution by Genetic Analysis Using Four Short Tandem Repeat Loci. J. of *Sasang* Constitutional Medicine. 1999;11(1):169-183.
21. Cho WS. *Sasang* Constitution and genetics. J. of *Sasang* Constitutional Medicine. 1998;10(1):13-24.
22. Han SK. et al. A Study on the analysis of constitutional genes by HLA typing. J. of *Sasang* Constitutional Medicine. 2001;13(1):97-103.
23. Ha MS, Song IB. A Study on the Methodologies for the Classification of *Sasang* Constitution by Analysis of Genetic Polymorphism. J. of *Sasang* Constitutional Medicine. 1999;11(2):185-194.
24. Han SK. et al. A Study on the analysis of constitutional genes. J. of *Sasang* Constitutional Medicine. 2003;15(1):109-117.
25. Choi JY, Park SS. A Clinical Study for 157 Cases of CVA by *Sasang* Constitutional Medicine. J. of *Sasang* Constitutional Medicine. 1998;10(2):431-454.
26. Woods A, Brull DJ, Humphries SE, Montgomery HE. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. Eur. Heart J. 2000;21:1574-1583.
27. Ross R. Atherosclerosis an inflammatory disease. N. Engl. J. Med. 1999;340:115-126.
28. Andreotti F, Porto I, Crea F, Maseri A. Inflammatory gene polymorphisms and ischemic heart disease: review of population association studies. Heart. 2002;87:107-112.
29. Kindler V, Apappino S, Grau GE, Pignet PF, and Vassalli P. The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. Cell. 1989;56:731-739.
30. Duncombe AS, Meager A, Prentice HG., Grundy JE, Heslop HE, Hoffbrand AV, Brenner MK. Gamma-interferon and tumor necrosis factor production after bone marrow transplantation is augmented by exposure to marrow fibroblasts infected with cytomegalovirus. Blood. 1990;76(5) :1046-1053.
31. Goeddel DV, Aggarwal BB, Gray PW, Leung DW, Nedwin GE, Palladino MA, Patton JS, Pennica D, Shepard HM, Sugarman BJ. et al. Tumor necrosis factors: gene structure and biological activities. Cold Spring Harb. Symp. Quant. Biol. 1986;51(1):587-609.
32. Kiemer AK, Vollmar AM. The atrial natriuretic peptide regulates the production of inflammatory mediators in macrophages. Ann. Rheum. Dis.. 60 Suppl. 2001;3:68-70.
33. Mann DL. Recent insights into the role of tumor necrosis factor in the failing heart. Heart Fail.

Rev. 2001;6(2):71-80.
34. Tilg H. Cytokines and liver diseases. Can J Gastroenterol. 2001;15(10):661-668.
35. Oliff A. The role of tumor necrosis factor (cachectin) in cachexia. Cell. 1988;54(2): 141-142.
36. Tracey KJ, Fong Y, Hess DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, and Cerami A. Anti cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature. 1987;330(6149):662-664.
37. Paul M, Steed et al. Inactivation of TNF Signaling by Rationally Designed Dominant-Negative TNF Variants. Science. 2003;301 (5641):1895-1898.
38. Sin HD, Park CS. Association of Tumor Necrosis Factor (TNF) Polymorphisms With Asthma and Serum Total IgE.. Human Molecular Genetics Advance Access published online ahead of print. 2003:17.
39. Barone FC, Arvin B, White RF, Miller A, Webb CL, Willette RN, Lysko PG, Feuerstein GZ. Tumor necrosis factor-alpha. A mediator of focal ischemic brain injury. Stroke. 1997;28:1233-1244.
40. Nawashiro H, Martin D, Hallenbeck JM. Neuroprotective effects of TNF binding protein in focal cerebral ischemia. Brain Res. 1997;778:265-271.
41. Lavine SD, Hofman FM, Zlokovic BV. Circulating antibody against tumor necrosis factor-alpha protects rat brain from reperfusion injury. J. Cereb. Blood Flow Metab. 1998;18:52-58.
42. Elneihoum AM, Falke P, Hedblad B, Lindgarde F, Ohlsson K. Leukocyte activation in atherosclerosis : correlation with risk factors. Atherosclerosis. 1997;131:79-84.
43. Paleolog EM, Delasalle SA, Buurman WA, Feldmann M. Functional activities of receptors for tumor necrosis factor-alpha on human vascular endothelial cells. Blood. 1994;84:2578-2590.
44. Yoshizumi M, Perrella MA, Jr. Burnett, JC, Lee ME. Tumor necrosis factor down regulates an endothelial nitric oxide synthase mRNA by shortening its half-life. Circ. Res. 1993;73:205-209.
45. Toborek M, Blanc EM, Kaiser S, Mattson MP, Hennig B. Linoleic acid potentiates TNF-mediated oxidative stress, disruption of calcium homeostasis, and apoptosis of cultured vascular endothelial cells. J. Lipid. Res. 1997;38:2155-2167.
46. Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM. TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. J. Cereb. Blood Flow Metab. 1997;17: 483-490.
47. Gary DS, Bruce-Keller AJ, Kindy MS, Mattson MP. Ischemic and excitotoxic brain injury is enhanced in mice lacking the p55 tumor necrosis factor receptor. J. Cereb. Blood Flow Metab. 1998;18:1283-1287.
48. Clark WM. Cytokines and reperfusion injury. Neurology. 1997;49:510-514.
49. Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ, Carpenter MK, Holtsberg FW, Mattson MP. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nat. Med. 1996;2:788-794.
50. Dawson DA, Martin D, Hallenbeck JM. Inhibition of tumor necrosis factor-alpha reduces focal cerebral ischemic injury in the spontaneously hypertensive rat. Neurosci. Lett. 1996;218:41-44.
51. Shohami E, Ginis I, Hallenbeck JM. Dual role of tumor necrosis factor alpha in brain injury. Cytokine & Growth Factor Rev. 1999;10:119-130.
52. Nawashiro H, Martin D, Hallenbeck JM. Inhibition of tumor necrosis factor and amelioration of brain infarction in mice. J. Cereb. Blood Flow Metab. 1997;17:229-232.
53. Shohami, Bass R, Wallach D, Yamin A, Gallily R. Inhibition of tumor necrosis factor alpha (TNFalpha) activity in rat brain is associated with cerebroprotection after closed head injury. J.

Cereb. Blood Flow Metab. 1996;16:378-384.
54. Scherbel U, Raghupathi R, Nakamura M, Saatman KE, Trojanowski JQ, Neugebauer E, MW. N, McIntosh TK. Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. Proc. Natl. Acad. Sci. USA. 1999;96(15):8721-8726.