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원 저

A Study on the Relationship between Polymorphism of Interleukin 4 Receptor and Korean Patients with Cerebral Infarction

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국문초록

Interleukin 4 Receptor 유전자 다형성과 한국인 뇌경색 환자와의 상관성에 대한 연구

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목적 : 본 연구는 뇌경색에서 일반적으로 많이 사용하는 한방치료가 뇌경색 환자의 단일유전자 염기 다형성에 미치는 영향에 대하여 분석하였다.

2003년 3월부터 2003년 12월까지 경희대학교 한의과대학 부속한방병원 침구과에 입원한 뇌경색 환자 146명과 경희의료원 종합검진센터에 건강검진을 위해 내원한 건강인 192명을 대상으로 하였다.

방법 : 한국인 뇌경색 환자와 건강인에서 혈액을 채취하여 개인마다 DNA를 분리 정제하고 *Taq* polymerase로 증폭한 후 Pyrosequencing을 통하여 IL4R(interleukin 4 receptor)의 유전형을 관찰하였다.

결과 : 본 연구 결과 IL4R 유전자의 경우 한국인 뇌경색 환자군과 대조군 사이에 유의성 있는 차이가 나타나지 않았다.

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결론 : 이상의 결과를 통하여 IL4R 유전자 다형성은 한국인에서 뇌경색의 발병에 관련이 적은 것으로 사려되며 더 많은 환자를 대상으로 다른 환경요인 또는 유전자와의 연관성에 대한 심도 깊은 연구가 필요할 것으로 사려된다.

핵심 단어 : Interleukin 4 Receptor(IL4R), gene, polymorphism, cerebral infarction, Pyrosequencing

Ⅰ. Introduction

Cerebral infarction is a leading cause of morbidity and mortality in Korea. Physical and psychological impairment from cerebral infarction may negatively affect the quality of life. Cerebral infarction is a multifactorial disease and various factors such as atherosclerosis, hypertension, diabetes mellitus, hyperlipidemia and smoking interact to increase the risk of developing cerebral infarction. Lou et al revealed platelet hyperaggregability was seen in young patients with completed stroke[1].

Cytokines in cerebral infarction may play an important role in the response to injury. Especially anti-inflammatory cytokines such as IL4 may act in a feedback loop to inhibit continued proinflammatory cytokine production[2]. Whether cytokines is a result or cause of the brain injury process is not certain.

The mechanisms of cytokines involved in stroke progression is not revealed completely yet. But ischemic brain injury such as cerebral infarction is characterized by acute local inflammatory response mediated by cytokines.[2] Recently it was reported that the mean Interleukin 4(IL4) serum levels were significantly higher in the patients with cerebral infarction than in the normal groups[2-3].

IL4 is an anti-inflammatory cytokine that reduces the production of proinflammatory cytokines and destructive enzymes[4-5]. For many cytokines and their receptors, genetic variants have been described[6-10].

The Interleukin 4 Receptor(IL4R)'s chain genetic polymorphism was associated with immunoglobulin E(IgE) secretion and atopy in familial studies[11]. Gene expression can be regulated by a number of genetic elements located in the 5-upstream region of the gene. Variances in this upstream sequence can result in a different level of gene expression. So far, many genetic polymorphisms in the 5'-flanking region of the IL4R gene have been reported, but in cerebral infarction IL4R gene polymorphism has rarely been described.

Whether cytokines is a result or cause of the brain injury process is not certain. We hypothesized that the IL4R gene would be an important candidate in the development of cerebral infarction. Through this study, we investigated the relationship between single-nucleotide polymorphism (SNP) of IL4R and Koeran patients with cerebral infarction.

Ⅱ. Patients and methods

1. Study population

The control group consisted of 192 apparently healthy Koreans. Controls were selected from healthy subjects who has visited for the health examinations at Kyung Hee medical center from March, 2003 to December, 2003. The group of 146 are cerebral infarction patients. At first, there were 153 cerebral infarction subjects, who were admitted to the stroke service center of the department of acupuncture & moxibustion, college of Oriental Medicine at Kyung Hee University. Among these patients, 7 subjects were excluded from this study (4 were transported to other

hospitals, and 3 declined to give consent). Ultimately, 146 Koreans were enrolled in the current analysis.

2. Definition of cerebral infarction

We included cerebral infarction patients with neurological symptoms lasting more than 24 hours accompanied by corresponding focal density changes detected by brain computerized tomography (CT) or magnetic resonance imaging(MRI). This study excluded patients suffering from epidural (or subdural) hematoma, brain tumors, and accidental or iatrogenic cerebral infarction. Final diagnosis of cerebral infarction was confirmed by serial CT or MRI findings. In case of CT, cerebral infarction was identified by gradual or sometimes rapid development of focal neurological symptoms and signs, such as hemiparesis, sensory impairment, and a low-density area in the CT image.

3. Blood sample collection

Blood samples were obtained from the antecubital vein without regarding to the time of the last meal. This study was approved by the ethics review committee of Kyung Hee medical center. Informed consent was obtained from all subjects. If patients were incommunicative, it was obtained from close relatives.

4. DNA preparation and genotyping

Blood samples from all subjects were obtained for DNA extraction and collected in EDTA tube. Genomic DNA was extracted using DNA isolation kit for Mammalian Blood (Boehringer Mannheim, IN, USA). According to the method of Lesch et al, with minor modifications, the extracted DNA was amplified by polymerase chain reaction(PCR)[12]. The IL4R gene (110-bp) was amplified using 25 ng of DNA, 5 pmol of each primer. IL4R forward was 5′-GAAACCTGGGAGCAGATCCT-3′ and IL4R reverse was 5′-TCCACCGCATGTACAAACTC-3′.

The PCR amplification was performed by using 0.5 unit Taq polymerase (HT Biotechnology Ltd., Cambridge, United Kingdom). The 30 ul of PCR reaction mixtures were 10 mM Tris-HCl, pH 9.0, 1.5 mM magnesium chloride, 50 mM potassium chloride, 0.1% Triton-X 100, 0.01 % [v/v] stabilizer, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 0.1 M of each oligonucleotide primer. The PCR steps were denaturation of 5 minutes at 95℃, 30 cycles of 30 seconds at 95℃, 30 seconds at 60℃, and 30 seconds at 72℃ with a Thermocycler (Astech, Fukuoka, Japan). The reverse primer was biotinylated to allow the preparation of single-stranded DNA. The quality of PCR products was controlled by 1.5% of agarose gel electrophoresis.

The performance of DNA Preparation for pyrosequencing was based on manufacturer's standard protocol (Pyrosequencing AB, Uppsala, Sweden)[13]. The streptavidin sepharose beads (Streptavidin Sepharose HP, Amersham Pharmacia Biotech, Uppsala, Sweden) were immobilized to PCR products. The sequencing primer of IL4R was 5′-CCCACCAGTGGCTAT-3′ and it was designed so that the terminal residue hybridized to the base immediate adjacent to the A/G mutation at position +1902 of the IL4R gene (refSNP ID: rs1801275) from Pyrosequencing AB (http://www.pyrosequencing.com)[13]. By incubation at room temperature for 10 minutes, 20 ul of biotinylated PCR products were immobilized onto streptavidin-coated sepharose beads, the immobilized PCR products were transferred to a Millipore 96-well filter plate (Millipore, Bedford, MA, USA). Vacuum was used to eliminate the different solutions and reagents to obtain pure, single-stranded DNA while the beads remained in the wells. In 55 ul of 4 M acetic acid containing 0.35 uM of IL4R sequencing primer the beads with the immobilized template were resuspended. Then, the 45 ul of suspension was transferred to a PSQ 96 plate (Pyrosequencing AB, Uppsala,Sweden)[14]. By using PSQ 96 Sample Prep Thermoplate (Pyrosequencing AB, Uppsala, Sweden) the PSQ 96 plate

containing the samples were heated at 90℃ for 5 minutes for sequencing primer annealing, and moved to room temperature for 10 minutes. Next the PSQ 96 Plate was placed into the process chamber of the PSQ 96 instrument (Pyrosequencing AB, Uppsala, Sweden)[15]. The enzymes, substrates, and nucleotides were dispensed from a reagent cassette into the wells by using the PSQ 96 SNP Reagent kit (Pyrosequencing AB, Uppsala, Sweden), The light was generated when a nucleotide is incorporated into a growing DNA strand[14]. Through this process the polymorphism of the IL4R was genotyped automatically.

5. Statistical analysis

Students' t-test was used in order to compare the age of cerebral infarction patients and controls. χ^2 tests or Fisher's exact test was used to compare the sex, the distribution of the genotypes and the frequencies of alleles between cerebral infarction patients and controls. The genotype data were tested for Hardy-Weinberg equilibrium using the goodness-of-fit test. *P*-values of less than 0.05 were considered significant.

Ⅲ. Results

1. Clinical characteristics of cerebral infarction patients and controls

The characteristics of the patients and controls are shown in Table 1. There was no significant difference between the patients and controls in age and sex.

2. Genotype and allele frequencies of polymorphism in IL4R gene in cerebral infarction patients and controls

The homozygote and heterozygote genotypes of IL4R gene are seen in Figure 1,2 and 3. The distribution of genotypes and allelic frequencies are shown in Table 2 and 3. The observed genotype frequencies of cerebral infarction patients (*P*=0.147) and the control group (*P*=0.207) did not show significant difference predicted by the Hardy-Weinberg equation. As for IL4R genotypes distribution of A/A homozygotes, A/G heterozygotes, and G/G homozygotes, there was no significant difference between control and cerebral infarction group (P=0.919) . About allele frequencies distribution, there was no significant difference between control and cerebral infarction group (*P*=0.908).

3. Genotype and allele frequencies of polymorphism in IL4R gene in hypertension or diabetes mellitus patients and controls

We analysed the genotypes distribution between controls and hypertension or diabetes mellitus patients. The observed genotype frequencies of hypertension patients (*P*=0.274) and diabetes mellitus patients (*P*=0.523) did not show significant difference predicted by the Hardy-Weinberg equation. There was no significant difference (*P*=0.954, *P*=0.919 respectively). As for the comparison of allele frequencies distribution between controls and hypertension or diabetes mellitus patients there was no significant difference (*P*=0.756, *P*=0.692 respectively).

Table 1. Clinical characteristics of cerebral infarction patients and controls

	Controls (n=192)	Cerebral infarction patients (n=146)	P value
Male / Female	100 / 92	79 / 67	0.714
Age, mean±SD, y	62.3 ± 5.7	64.1 ± 11.4	0.060
Hypertension (%)		81 (55.4)	
Diabetes mellitus (%)		59 (40.4)	

x^2 test was used to compare the values of cerebral infarction patients and controls for sex. Age was compared by Students' t test

Table 2. Genotype and allele frequencies of polymorphism in IL4R gene in cerebral infarction patients and controls

	Genotype (%)				Allele Frequency (%)		
	AA	AG	GG	P value	A	G	P value
Controls (n=192)	145 (75.5)	41 (21.4)	6 (3.1)		331 (86.2)	53 (13.8)	
Cerebral infarction patients (n=146)	113 (77.4)	29 (19.8)	4 (2.8)	0.919	225 (85.9)	37 (14.1)	0.908

x^2 tests or Fisher's exact test was used to compare genotypes and allele frequencies between controls and cerebral infarction patients

Table 3. Genotype and allele frequencies of polymorphism in IL4R gene in hypertension or diabetes mellitus patients. and controls

	Genotype (%)				AlleleFrequency (%)			
	AA	AG	GG	P value	A	G	G	P value
Controls (n=192)	145 (75.5)	41 (21.4)	6 (3.1)		331 (86.2)		53 (13.8)	
Hypertension (n= 81)	60 (74.1)	18 (22.2)	3 (3.7)	0.954	138 (85.2)		24 (14.8)	0.756
Diabetes Mellitus (n= 59)	43 (72.9)	14 (23.7)	2 (3.4)	0.919	100 (84.8)		18 (15.2)	0.692

x^2 tests or Fisher's exact test was used to compare genotypes and allele frequencies between controls and hypertension or diabetes mellitus patients

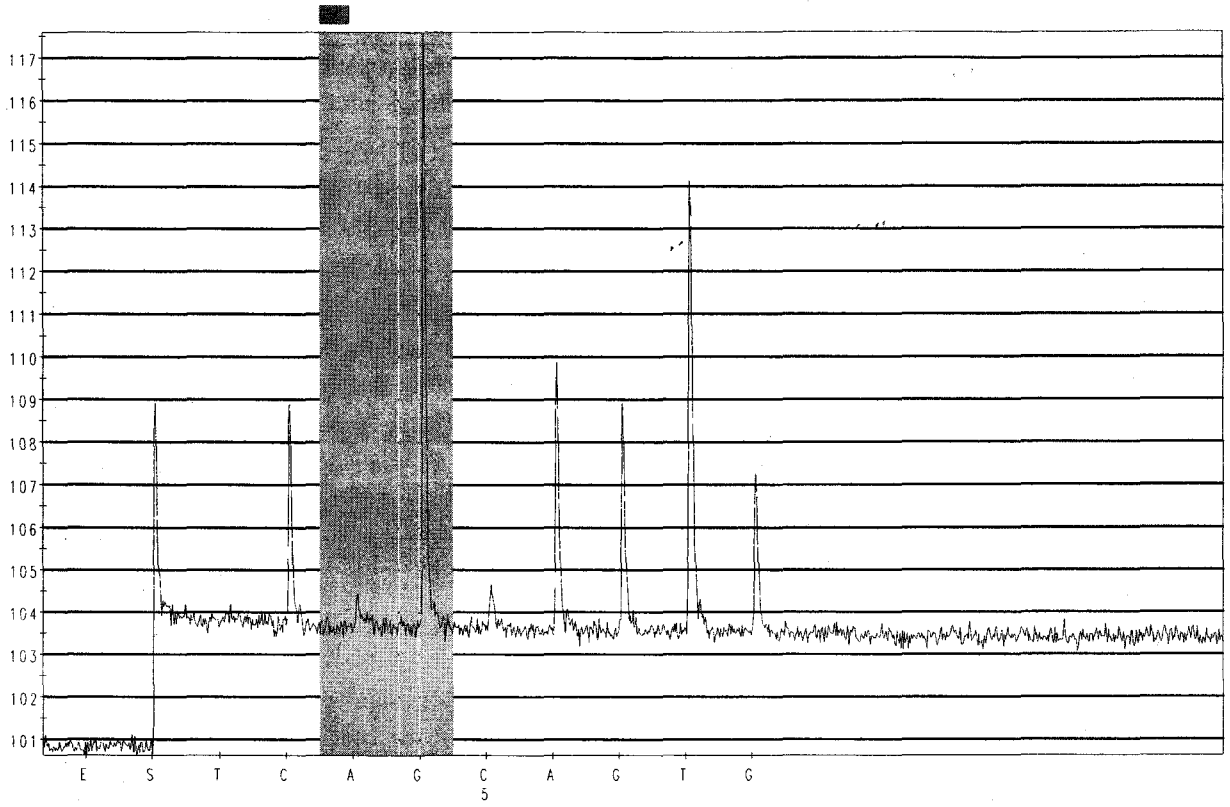

Fig. 1. Pyrosequencing result of G/G homozygote genotype of the IL4R gene

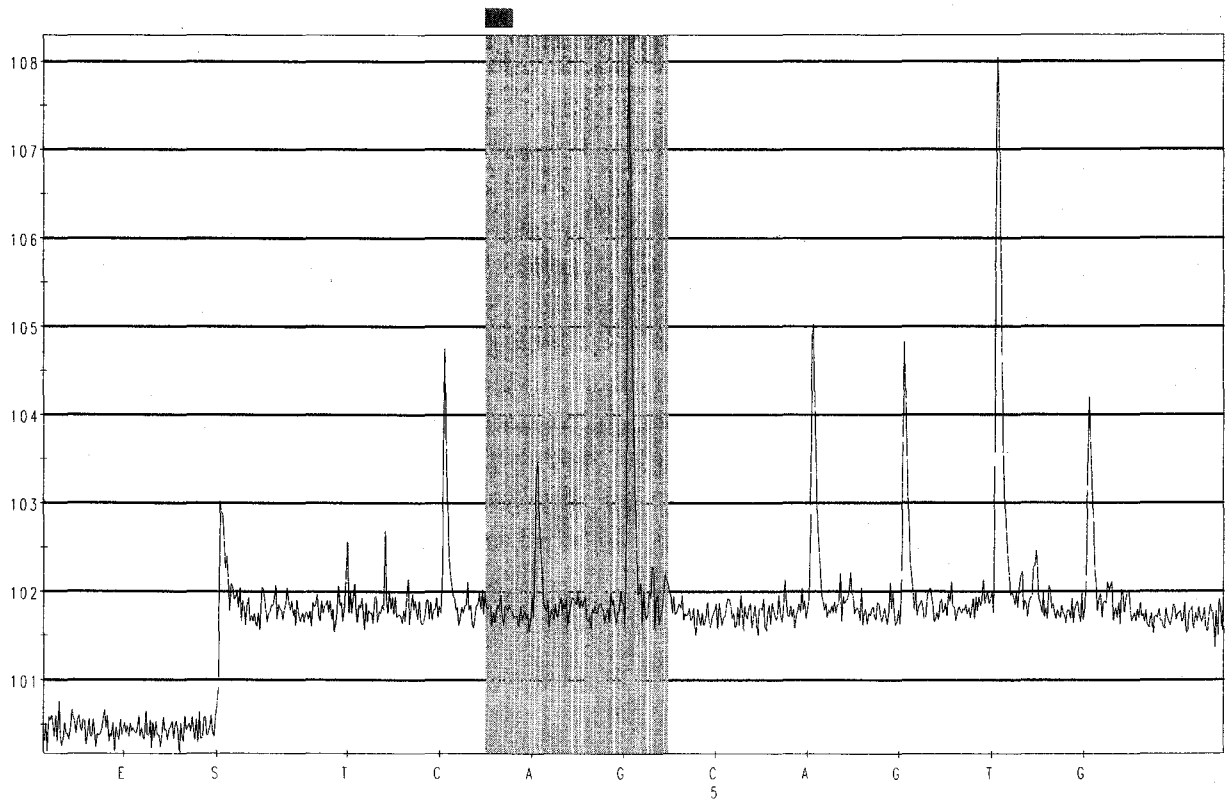

Fig. 2. Pyrosequencing result of A/G heterozygote genotype of the IL4R gene

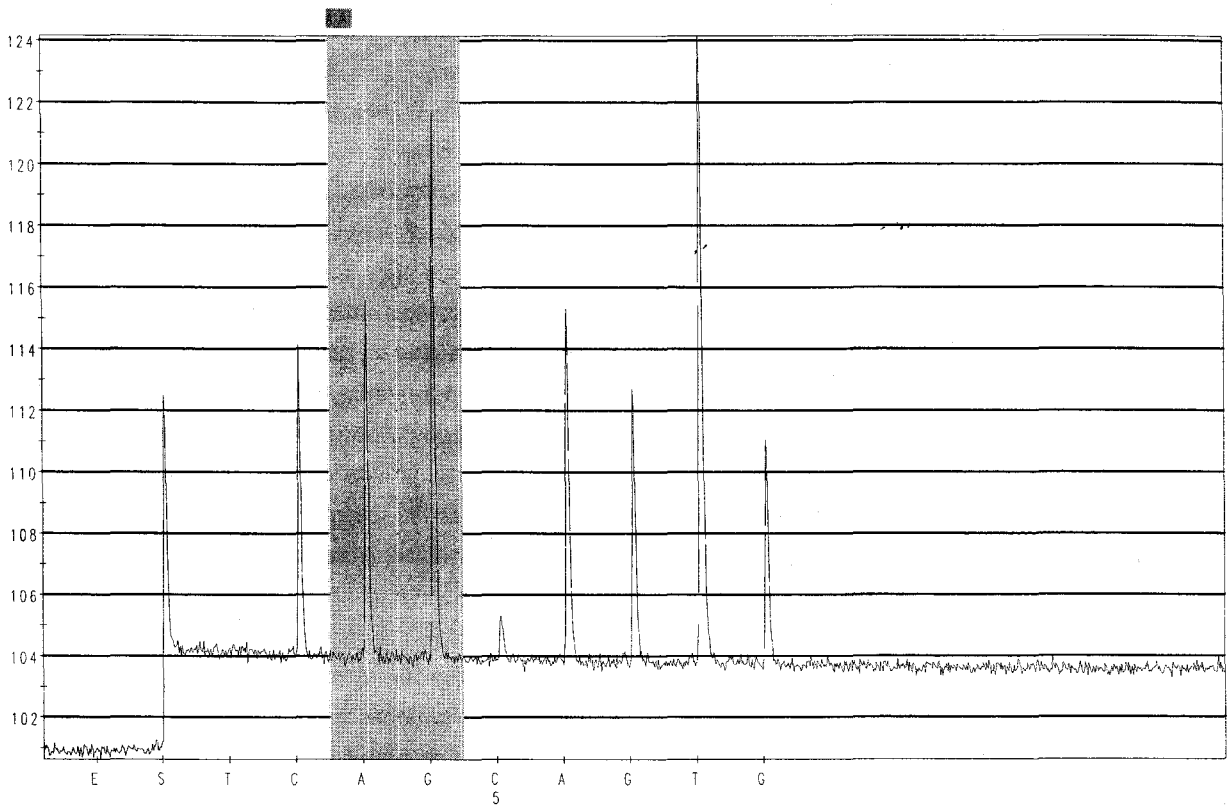

Fig. 3. Pyrosequencing result of A/A homozygote genotype of the IL4R gene

IV. Discussion

Stroke is a clinical concept of neurological disorder characterized by an acute faint, unconsciousness, excessive phlegm, hemiparalysis, dysphasia, facial palsy and motor disorder, etc., also, is called cerebro-vascular accident (CVA) in Western Medicine[15]. Stroke develops several complications, among that sequela of stroke like motor disorder affects the family as well as the patient with great psychological and financial stress. Stroke is the one of the most fatal disease following cancer in Korea. It is the most frequent disease as the cause of admission of most Oriental Medical hospitals.

In Oriental Medicine, cerebral infarction has been called wind-stroke syndrome (Chungpung). It is syndrome due to external affection by the pathogenic wind. To treat wind-stroke syndrome, it has used acupuncture, moxibustion and herbal medicines.

Genetic factors appear to contribute to virtually every human disease, conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental influences. Trying to get the information about genetic variation is important for understanding how genes function or malfunction, and genetic and functional variation are related.

IL4 plays a major role in IgE production. Its signal is conferred to effector cells through binding to the alpha chain of the IL4R. IL4 is a cytokine produced by T cells that regulates proliferation and differentiation of a variety of cells. It modulates the activity of these cells following binding to cell surface receptors. By cross-species hybridization using a cDNA

(complementary DNA) encoding the murine IL4R, Idzerda et al[16] isolated cDNAs encoding the human IL4R. The amino acid sequence of its extracellular domain defined it as a member of the hematopoietin receptor superfamily.

About IL4R gene function Zurawski et al[17]. presented data demonstrating that IL4R is a complex of at least 2 components. One is a novel affinity converting subunit that is critical for cellular signal transduction. Ihle and Kerr[18] reviewed the activation cascade involving cytokines, IL4R and other cytokine receptors, the Janus kinases, and the signal transducers and activators of transcription.

Kotanides and Reich[19] identified a specific STAT6(Signal transducer and activator of transcription 6) DNA-binding target site in the promoter of IL4R and showed that STAT6 activates IL4 gene expression via this site. Because IL4, IL13 and their specific signaling pathways are considered attractive targets for the treatment of allergy and asthma, Kelly-Welch et al.[22] reviewed the signaling connections of these cytokines. IL4 interacts with IL4R in high affinity, leading to dimerization with either the common gamma chain, a component of receptors for a number of cytokines, to create a type I receptor, or with IL13RA1(Interleukin 13 receptor alpha 1) to form a type II receptor. Kelly-Welch et al[20]. proposed that subtle differences in IL4 and IL13 signaling due to polymorphisms near docking sites in IL4R may have profound implications for allergy and asthma. By examining genes regulated by NFATC2 (Nuclear factor of activated T-cells, cytoplasmic 2) in muscle, Horsley et al[21]. identified the cytokine IL4 as a molecular signal that controls myoblast fusion with myotubes.

Pritchard et al[22] localized the IL4R gene to 16p12.1-p11.2 by in situ hybridization and Southern blot analysis of DNA from a panel of mouse-human somatic cell lines. By interspecific backcross analysis, they assigned the mouse homolog to the distal region of chromosome 7. Thus, the IL4R locus is unlinked to other members of the hematopoietin receptor family. Suzuki et al[23]. demonstrated that the murine IL4R gene is closely linked to the homolog of the LFA1 (Lymphocyte Function-Associated Antigen 1) gene on chromosome 7.

Khurana Hershey et al[24]. noted that the IL4R is composed of 2 subunits: a 140-kd alpha subunit, which binds IL4 and transduces its growth-promoting and transcription-activating functions, and a gamma-c subunit, common to several cytokine receptors, that amplifies signaling of IL4R alpha. The central role played by IL4R alpha in regulating production of IgE prompted, Khurana Hershey et al[24] to investigate possible mutations in the gene that would enhance receptor signaling and hence precipitate atopy. Using single-strand conformation polymorphism(SSCP) analysis and DNA sequencing, they searched for mutations in the alpha-subunit of the IL4R that might predispose persons to atopy. They examined the prevalence of alleles among patients with allergic inflammatory disorders and among 50 prospectively recruited adults. Subjects with atopy were identified on the basis of elevated serum IgE level or a positive radioimmunosorbent test in response to standard inhalant allergens. An arg576 allele of IL4R was found to be strongly associated with atopy. It had previously been demonstrated that gain-of-function polymorphisms in the IL4 gene are associated with increased output of IL4, which in turn is associated with asthma, skin-test positivity, and higher total concentrations of serum IgE.

Besides, there are many association studies of IL4R alleles with atopy or asthma[25-32], though Patuzzo et al[33] could find no evidence of linkage or association of atopic asthma with this mutation in 851 Italian subjects with atopic asthma.

Recently, it was reported that IL4 serum levels were higher in the patients with cerebral infarction than in the normal groups[3]. IL4 levels were also found similar in patients with or without neurological worsening in the patients with cerebral infarction[2].

In cerebral infarction many polymorphism were investigated and some polymorphism such as 1-

antichymotrypsin gene was associated[34]. However some polymorphism such as promoter of lipopolysaccharide receptor CD14 was not related[35].

About the association of IL4R polymorphism with stroke it was reported that the combination of TNF (-308) G/G homozygosity and the IL4R 503P variant carrier status was associated with a particularly strong predisposition to large-vessel stroke[36].

In our study the observed genotype frequencies of cerebral infarction patients (P=0.147) and the control group (P=0.207) did not show significant difference predicted by the Hardy-Weinberg equation. As for IL4R genotypes distribution of A/A, A/G, and G/G genotypes there was no significant difference between control and cerebral infarction group (P=0.919). About allele frequencies distribution, there was no significant difference between control and cerebral infarction group (P=0.908).

Han et al[37] performed genetic analysis about IL-4R polymorphisms in stroke patients. In that study, there was no significant difference between control and stroke group. However, that study was for not only cerebral infarction but also cerebral hemorrhage patients, and the population was too small.

This is the report to have shown the association of IL4R gene polymorphisms with cerebral infarction by use of CT or MRI findings. The IL4R is composed of multiple chains, including a specific chain and a γ c chain. In the IL4R α-chain gene, an A→G transition at nucleotide 1902, causing a change from glutamine to arginine at codon 576, has been described and the presence of this rare allele has been associated with familial hyper-IgE syndrome and atopy[11].

Zee et al[38] performed population-based, prospective genetic analysis about IL-4 polymorphisms to prospectively determine whether candidate gene polymorphisms contribute to stroke risk. They genotyped IL-4 polymorphisms among 319 individuals who subsequently developed ischemic stroke and among 2092 individuals who remained free of reported cardiovascular disease over a mean follow-up period of 13.2 years. After adjustment for multiple comparisons, a C582T polymorphism in the IL-4 gene were found to be independent predictors of thrombo-embolic stroke (OR=1.40, 95% CI 1.13-1.73, P=0.003). That study was not for A→G transition at nucleotide 1902 in the IL-4R, but C582T polymorphism in the IL-4.

There is increasing evidence implicating immune system dysfunction in the pathogenesis of hypertension in both hypertensive human subjects and experimental animal models[39-40]. In hypertensive patients, elevated plasma levels of immunoglobulins[41], and increased incidence of autoreactive antibodies to arterial wall antigens and antinuclear antigens have been reported[41-42].

Cytokines secreted by T lymphocytes play a key role in the induction and development of the immune response. In mice, two distinct subtypes of T helper cells have been identified: T helper1 (Th1) cells producing more IFN-g, IL-2, TNF b and T helper 2 (Th2) cells producing IL-4, IL-5, IL-6, IL-10 and IL-13[43].

IL-4 is one of the important regulators of T-lymphocyte function. IL-4 drives the development of Th2 cells and thereby regulates antibody production or humoral immunity.

Association studies with candidate genes involved in immune responses, such as those encoding elements of the T cell activation pathway, represent one approach to finding type 1 Diabetes Mellitus (TID) disease genes. Among these genes, IL4 have been shown to protect against diabetes development in rodent models of TID[44], suggesting the possibility that cytokines involved in the Th1 and/or Th2 pathways may play a role in TID pathogenesis[45].

IL4 is one of the key components in the induction of the Th2 lymphocyte phenotype and the downregulation of the Th1 lymphocyte phenotype. In humans, IL4 transcript levels are greatly reduced in new-onset TID[46].

The effects of IL4 on Th1/Th2 balance and on other immune phenotypes are mediated via

binding to a receptor containing the IL4R chain[47].

Therefore, we investigated the effect of IL4R gene on the development of hypertension or Diabetes Mellitus in cerebral infarction patients by case-control genetic association study.

In the IL4R genotypes distribution, there was no significant difference between control and cerebral infarction group (P=0.919). The comparison of genotypes distribution between controls and hypertension or diabetes mellitus patients there was no significant difference (P=0.954, P=0.919 respectively). In addition, about allele frequencies distribution there was no significant difference between control and all cerebral infarction group, hypertension patients or diabetes mellitus patients (P=0.908, P=0.756, P=0.692 respectively).

Our results suggest that the investigated IL4R polymorphisms might not be the susceptibility factors in the etiology of cerebral infarction. Genetic factors and environmental factors are both critical in the development of cerebral infarction.

So far, it is very difficult to apply the results from genetic studies to the patients. With regard, to cerebral infarction risk additional epidemiologically based studies of the relationship between IL4R or other genes and lifestyles are required. Establishment of more systemic approach and high quality of prospective cohorts will be necessary for the good prediction of genetic markers.

V. Conclusions

In this thesis the IL4R genotype might not be the risk factor or a good predictive genetic marker for Korean Patients with cerebral infarction. Further studies including different cytokine genes will be necessary for the exact genetic markers.

VI. References

1. Lou HC, Nielsen JD, Bornholt A, Gormsen J. Platelet hyperaggregability in young patients with completed stroke. Acta Neurol Scand 1977 ; 56 : 326-334.
2. Vila N, Castillo J, Davalos A, Esteve A, Planas AM, " Chamorro A. Levels of anti-inflammatory cytokines and neurological worsening in acute ischemic stroke. Stroke 2003 ; 34 : 671-675.
3. Shin HY, Jeong HJ, Lee JH, Joo JC, Kim KY, Song HJ, Lee SG, Chae HJ, Kim HR, Kim JJ, Kim HM. Regulatory effect of cytokine production in patients with cerebral infarction by Yulda-Hanso-Tang. Immunopharmacol Immunotoxicol 2000 ; 22 : 183-193.
4. Miossec P, Chomarat P, Dechanet J, Moreau J-F, Roux J-P, Delmas P, Banchereau J. Interleukin 4 inhibits bone resorption through an effect on osteoclasts and proinflammatory cytokines in an ex vivo model of bone resorption in rheumatoid arthritis. Arthritis Rheum 1994 ; 37 : 1715-1722.
5. Joosten LA, Lubberts E, Durez P, Helsen MM, Jacobs MJ, Goldman M, van den Berg WB. Role of interleukin-4 and interleukin-10 in murine collagen-induced arthritis. Protective effect of interleukin-4 and interleukin-10 treatment on cartilage destruction. Arthritis Rheum 1997 ; 40 : 249-260.
6. Bugawan TL, Mirel DB, Valdes AM, Panelo A, Pozzilli P, Erlich HA. Association and interaction of the IL4R, IL4, and IL13 loci with type 1 diabetes among Filipinos. Am J Hum Genet 2003 ; 72 : 1505-1514.
7. Mirel DB, Valdes AM, Lazzeroni LC, Reynolds RL, Erlich HA, Noble JA. Association of IL4R haplotypes with type 1 diabetes. Diabetes 2002 ; 51 : 3336-3341.
8. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, McNicholl J, Pociot F, Hardt C,

D'Alfonso S. Cytokine gene polymorphism in human disease: on-line databases. Genes Immun 1999 ; 1 : 3-19.

9. Olavesen MG, Hampe J, Mirza MM, Saiz R, Lewis CM, Bridger S, Teare D, Easton DF, Herrmann T, Scott G, Hirst J, Sanderson J, Hodgson SV, Lee J, MacPherson A, Analysis of single-nucleotide polymorphisms in the interleukin-4 receptor gene for association with inflammatory bowel disease. Immunogenetics 2000 ; 51 : 1-7.
10. Buchs N, Silvestri T, di Giovine FS, Chabaud M, Vannier E, Duff GW, Miossec P. IL-4 VNTR gene polymorphism in chronic polyarthritis. The rare allele is associated with protection against destruction. Rheumatology 2000 ; 39 : 1126-1131.
11. Hershey GK, Friedrich MF, Esswein LA, Thomas ML, Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. New Engl J Med 1997 ; 337 : 1720-1725.
12. LeschKP, Bengel D, Heils A. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996 ; 274 : 1527-1530.
13. http://www.pyrosequencing.com.
14. ruber JD, Colligan PB, Wolford JK. Estimation of single nucleotide polymorphism allele frequency in DNA pools by using Pyrosequencing. Hum Genet 2002 ; 110 : 395-401.
15. Kim YS, Clinical Chungpung, Seowondang, Seoul, 1997, 47-61.
16. Idzerda RL. March CJ. Mosley B. Lyman SD. VandenBos T. Gimpel SD. Din WS, Grabstein KH. Widmer MB. Park LS. Cosman D. Beckmann MP. Human interleukin-4 receptor confers biological responsiveness and defines a novel receptor superfamily. J. Exp. Med. 1990 ; 171 : 861-873.
17. Zurawski SM. Vega FJr. Huyghe B. Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. EMBO J. 1993 ; 12 : 2663-2670.
18. Ihle JN. Kerr IM. Jaks and Stats in signaling by the cytokine receptor superfamily. Trends Genet. 1995 ; 11 : 69-74.
19. Kotanides H. Reich NC. Interleukin-4-induced STAT6 recognizes and activates a target site in the promoter of the interleukin-4 receptor gene. J. Biol. Chem. 1996 ; 271 : 25555-25561.
20. Kelly-Welch AE. Hanson EM. Boothby MR. Keegan AD. Interleukin-4 and interleukin-13 signaling connections maps. Science 2003 ; 300 : 1527-1528.
21. Horsley V, Jansen KM, Mills ST, Pavlath GK, IL-4 acts as a myoblast recruitment factor during mammalian muscle growth. Cell 2003 ; 113 : 483-494.
22. Pritchard MA. Baker E. Whitmore SA. Sutherland GR. Idzerda RL. Park LS. Cosman D. Jenkins NA. Gilbert DJ. Copeland NG. Beckmann MP. The interleukin-4 receptor gene (IL4R) maps to 16p11.2-16p12.1 in human and to the distal region of mouse chromosome 7. Genomics 1991 ; 10 : 801-806.
23. Suzuki H. Chung F. Palmer E. Sasaki T. Ohara N. Taylor BA. Ohara J-I. Gene mapping of mouse IL-4 receptor : the loci of interleukin 4 (IL-4) receptor gene and lymphocyte function associated antigen 1 (LFA-1) gene are closely linked on chromosome 7. Immunogenetics 1991 ; 34 : 252-256.
24. Khurana Hershey GK. Friedrich MF. Esswein LA. Thomas ML. Chatila TA. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. New Eng. J. Med. 1997 ; 337 : 1720-1725.
25. Caggana M, Walker K, Reilly AA, Conroy JM, Duva S, Walsh AC. Population-based studies reveal differences in the allelic frequencies of two functionally significant

human interleukin-4 receptor polymorphisms in several ethnic groups. Genet. Med. 1999 ; 1 : 267-271.

26. Ober C. Tsalenko A. Willadsen S, Newman D. Daniel R. Wu X. Andal J. Hoki D. Schneider D. True K. Schou C. Parry R. Cox N. Genome-wide screen for atopy susceptibility alleles in the Hutterites. Clin. Exp. Allergy 1999 ; 29 (suppl. 4) : 11-15.
27. Ober C. Leavitt SA. Tsalenko A. Howard TD. Hoki DM. Daniel R. Newman DL. Wu X. Parry R. Lester LA. Solway J. Blumenthal M. King RA. Xu J. Meyers DA. Bleecker ER. Cox NJ. Variation in the interleukin 4-receptor alpha gene confers susceptibility to asthma and atopy in ethnically diverse populations. Am. J. Hum. Genet. 2000 ; 66 : 517-526.
28. Deichmann KA, Heinzmann A, Forster J, Dischinger S, Mehl C, Brueggenolte E, Hildebrandt F, Moseler M, Kuehr J, Linkage and allelic association of atopy and markers flanking the IL4-receptor gene. Clin. Exp. Allergy 1998 ; 28 : 151-155.
29. Galizzi J-P, Zuber CE, Harada N, Gorman DM, Kastelein R, Banchereau J, Howard M, Miyajima A, Molecular cloning of a cDNA encoding the human interleukin 4 receptor. Int. Immun. 1990 ; 2 : 669-675.
30. Mitsuyasu H. Izuhara K. Mao XQ. Gao PS. Arinobu Y. Enomoto T. Kawai M. Sasaki S. Dake Y. Hamasaki N. Shirakawa T. Hopkin JM. Ile50val variant of IL4R-alpha upregulates IgE synthesis and associates with atopic asthma. (Letter) Nature Genet. 1998 ; 19 : 119-120.
31. Kruse S. Japha T. Tedner M. Sparholt SH. Forster J. Kuehr J. Deichmann KA. Abello A. The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. Immunology 1999 ; 96 : 365-371.
32. Howard TD. Koppelman GH. Xu J. Zheng SL. Postma DS. Meyers DA, Bleecker ER. Gene-gene interaction in asthma : IL4RA and IL13 in a Dutch population with asthma. Am. J. Hum. Genet. 2002 ; 70 : 230-236.
33. Patuzzo C. Trabetti E. Malerba G. Martinati LC. Boner AL. Pescollderungg L. Zanoni G. Pignatti PF. No linkage or association of the IL-4R-alpha gene Q576R mutation with atopic asthma in Italian families. (Letter) J. Med. Genet. 2000 ; 37 : 382-384.
34. Nicolas V, Victor O, Marian R, Rafael O, Angel C. 1-Antichymotrypsin gene polymorphism in patients with stroke, Stroke 2000 ; 31 : 2103-2105.
35. Daisuke I, Mitsuru M, Norio T, Hideki S, Akira S, Ikuo S, Kiyoaki W, Yasuo F. Polymorphism in the promoter of lipopolysaccharide receptor CD14 and ischemic cerebrovascular disease. Stroke 2000 ; 31 : 2661-2664.
36. Hoppe C, Klitz W, Cheng S, Apple R, Steiner L, Robles L, Girard T, Vichinsky E, Styles L. Gene interactions and stroke risk in children with sickle cell anemia. Blood 2004 ; 103 : 2391-2396.
37. Han SW, Lim SC, Jung TY, Shin DH, Kim MJ, Seo JC. Interleukin 4 receptor(IL4R) gene polymorphism in Korean stroke patients by using pyrosequencing. Kor J Acup Moxi 2004 ; 21(2) : 21-29.
38. Zee RY, Cook NR, Cheng S, Reynolds R, Erlich HA, Lindpaintner K, Ridker PM. Polymorphism in the P-selectin and interleukin-4 genes as determinants of stroke : a population-based, prospective genetic analysis. Hum Mol Genet. 2004 Feb 15 ; 13(4) : 389-396.
39. Dzielak DJ. Immune mechanisms in experimental and essential hypertension. Am J Physiol. 1991 ; 260(3 Pt 2) : R459-R67.
40. Dzielak DJ. The immune system and hypertension. Hypertension. 1992 ; 19(1 Suppl) : I36-144.

41. Martinez Amenos A, Buendia E, Carreras L, Font I, Mestre M, Rama H, Sarrias X, Alsina J. Humoral and cellular immunological abnormalities in hypertensive patients. J Clin Hypertens. 1985 ; 1(2) : 153-160.
42. Hilme E, Herlitz H, Soderstrom T, Hansson L. Increased secretion of immunoglobulins in malignant hypertension. J Hypertens. 1989 ; 7(2) : 91-95.
43. Mosmann TR, Schumacher JH, Street NF, Budd R, O'Garra A, Fong TA, Bond MW, Moore KW, Sher A, Fiorentino DF. Diversity of cytokine synthesis and function of mouse CD4+ T cells. Immunol Rev. 1991 ; 123 : 209-229.
44. Zaccone, P, Phillips J, Conget I, Gomis R, Haskins K, Minty A, Bendtzen K, Cooke A, Nicoletti F. Interleukin-13 prevents autoimmune diabetes in NOD mice. Diabetes 1999 ; 48 : 1522-1528.
45. Glimcher LH, Murphy KM. Lineage commitment in the immune system : the T helper lymphocyte grows up. Genes Dev 2000 ; 14 : 1693-1711.
46. Berman MA, Sandborg CI, Wang Z, Imfeld KL, Zaldivar F Jr, Dadufalza V, Buckingham BA. Decreased IL-4 production in new onset type I insulin-dependent diabetes mellitus. J Immunol 1996 ; 157 : 4690-4696.
47. Kawakami, K, Taguchi J, Murata T, Puri RK. The interleukin-13 receptor a2 chain : an essential component for binding and internalization but not for interleukin-13-induced signal transduction through the STAT6 pathway. Blood 2000 ; 97 : 2673-2679.