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원 저

Study on relationship between "cholestrol · cardio-renal connective tissue weakness type" and TNF-alpha gene polymorphism in Iris constitution analysis

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국문초록

홍채체질 분석에서 콜레스테롤 · 심신이 약한 체질과 TNF-alpha 유전자 다형성과의 상관성 연구

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홍채학은 대체의학의 한 분야로서 홍채 침착의 불규칙성을 주시함으로써 의학적 상태를 진단한다. 홍채학적 분류에 의한 체질은 가족력을 보이고 있으며 이는 홍채체질의 유전성을 의미한다. 강력한 면역조절자이며 전 염증성 사이토카인인 종양괴사인자(tumor necrosis factor-a, TNF-α)는 많은 병리적 과

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정에서 중요한 역할을 한다. 따라서 본 연구자는 고혈압환자에서 홍채체질과 TNF-a 유전자 다형성과의 관련성을 조사하였다. 87명의 고혈압 환자와 79명의 정상인을 홍채체질에 따라 분류하였으며 이들의 TNF-α 유전자형을 분석하였다. 결과적으로 정상인에 비하여 TNF-α GA 이형접합체의 빈도가 고혈압 환자군에서 감소하였다. 이 같은 결과는 TNF-α 다형성이 고혈압에 대한 저항성과 관련 있음을 의미한다. 또한 고혈압환자에서 콜레스테롤 침착체질과 심신 결합조직 허약 체질은 42.5%로서 정상인 16.5%에 비하여 현저하게 증가하였다 (P<0.001). GG TNF-α 유전자형을 갖고 있는 군에서 심신 결합조직 허약 체질과 콜레스테롤 침착체질의 빈도는 정상인보다 환자에서 유의하게 높았다 (P<0.001). 본 연구에서 저자는 홍채체질과 고혈압사이의 관련성을 발견함과 동시에 TNF-α 유전자 다형성과 고혈압, 그리고 홍채체질과의 관련성을 최초로 입증하였다.

Key words : TNF-a gene, hypertension, Iris constitution.

I. Introduction

Iridology, developed more than 100 years ago, assumes that all bodily organs are represented on the surface of the iris via intricate neural connections[17] and that dysfunction of most organs is marked on the iris, usually as a pigmentary change; the right half of the body is represented in the right iris, the left half in the left iris. Each iris is divided into 60 sectors, and each segment is related to an inner organ or bodily function.

Iridology is based on the study of the iris and its links with the nervous system. The organs of the body are connected by nerves to the spinal cord, which is divided into separate segments, each corresponding to a segment of the body[9].

Although iridology is a popular alternative medical treatment and it was very often reported on favorably by patients, there is little evidence in favor of this treatment. Up to date, more than 80 publications on the subject of iridology have been reported. However, most of the papers were review articles, comments, and descriptions of the technique. We hypothesized that the predisposition to disease by Iris constitution may be due to genetic factors. Then I focused to evaluate the diagnostic validity of iridology in terms of genetic factors.

Atherosclerosis can be viewed as an inflammatory process[7,12,15-16]. Hypertension is a multifactorial disease caused by the interactions of several genetic and environmental factors, and is pathologically based on atherosclerosis. Inflammatory mediators not only can contribute to atheroma formation, but may also be involved in the rapid evolution of the

atheromatous injury, leading to rupture of the plaque and intraluminal thrombosis[15]. In this sense, it is worth noting that several cytokines may play a role in determining the degree of inflammation and contributing to atherothrombosis.

Tumor necrosis factor-α (TNF-α) is a macrophage- and lymphocyte-derived immune mediator that regulates the inflammatory response, modulates growth and cellular differentiation, and activates blood coagulation[2]. In general, increased TNF-a plasma levels and activity are also associated to increased production of other interleukins[7]. Previous studies using in situ hybridization techniques showed increased levels of TNF-a messenger RNA in the atherosclerotic plaque of symptomatic patients[1]. These data pointed to a local rise in the expression of this inflammatory mediator, which may therefore contribute to arterial thrombosis.

The TNF-a 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-a 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)[19]. The A allele has been shown to be associated with increased TNF-a expression after in vitro stimulation[3,20-21]. Therefore this genetic variation might result in an altered TNF-a expression and thereby affect susceptibility and clinical severity of inflammatory diseases. Indeed, the A allele of TNF--a 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[6,18].

Therefore, the aim of this study was to compare the distribution of TNF-a in a defined group of hypertensives with those in a control group, and to investigate the association between TNF-a polymorphism and hypertension according to Iris constitution.

II. Materials and Methods

1. Subjects

Starting in 1999, 87 hypertensives between ages 28 and 62 years were enrolled from Oriental Medical Hospital, Wonkwang University, Jeonju, Korea. Hypertension was defined as systolic blood pressure of 140nmm Hg or diastolic blood pressure of 90 mm Hg. The control groups consisted of 79 healthy adults without hypertension. All patients and controls (all Korean) gave informed consent before participating in the research protocol, which was approved by the ethics committee

of each hospital.

2. Diagnosis of Iris constitutions

All subjects including patients and controls were diagnosed by automatic Iris analysis system, Bexel Irina (Korea).

3. Determination of TNF-a genotypes

The blood was stored at -20℃ until it was ready to be extracted. The genomic DNA was extracted by inorganic procedure[14]. The concentration of DNA was estimated by absorbance at 260 nm. A single base pair polymorphism at position -308 in the promoter region of the TNF-a gene was examined by the *Nco*I (Takara, Shiga, Japan) restriction fragment length polymorphism (RFLP) method described elsewhere[4]. The following primers were used:

5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-TCCTCCCTGCTCCGATTCCG-3′. Briefly a PCR reaction was carried out in a 20 ml volume containing 200 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 250 mM of each dNTP, and 1 U of r*Taq* DNA polymerase (Takara, Japan), with 0.2 mM of each primer. Cycling conditions for TNF-a 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. Product of 107 bp was generated and the primers were designed to incorporate a polymorphic site at a position -308 bp of the TNF-a gene into an *Nco*I restriction site. The PCR product was digested for 2h at 37℃ with 5.5 units *Nco* I. Restriction digests generated products of 87 bp and 20 bp for G allele and 107 bp for A allele. PCR products were then separated electrophoretically through 8% polyacrylamide gel with a 100 bp DNA marker (Promega, U.S.A.) and the products visualized by ethidium bromide staining (Fig. 1).

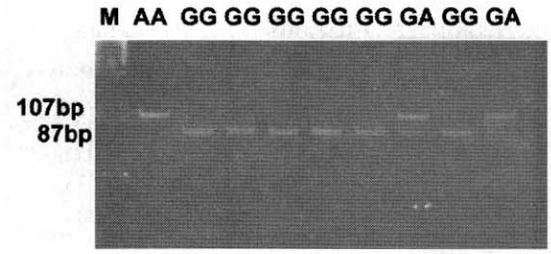

Fig. 1. TNF-a genotype. Restriction digests were separated electrophoretically through 8% polyacrylamide gel with a 100 bp DNA marker (Promega, U.S.A.) and the products visualized by ethidium bromide staining: 87 bp and 20 bp for G allele; 107 bp for A allele

4. Statistical analysis

Comparisons of the genotypic or allelic frequencies of the TNF-a genotypes between controls and hypertensives were carried out using the Pearson chi-square test. All statistical analyses were performed using SPSS v10.00 (SPSS Inc.) statistical analysis

software. A *P*-value less than 0.05 was considered statistically significant.

Ⅲ. Results

1. Distribution of Iris constitutions

The author classified 87 hypertensives and 79 controls according to Iris constitution, and determined TNF-a genotype. The distribution of Iris constitution in hypertensives was significantly different from the distribution in controls (c^2=40.244, *df*=3, *P*<0.001) (Table 1). The frequencies of Iris constitutions in controls as follows: neurogenic type, 16.5%; abdominal connective tissue weakness type, 39.2%; cholesterol+ cardio-renal connective tissue weakness type, 16.5%; the others type, 27.8%. It was significantly different from the distribution in hypertensives: neurogenic type, 32.2%; abdominal connective tissue weakness type, 25.3%; cardio-renal connective tissue weakness type, 42.5%; the others type, 0%.

Table 1. Distribution of Iris constitutions

	neurogenic	abdominal connective tissue weakness	cardio-renal weakness + cholesterol	the others	Evidence for association (*P*-value[a])
Control, n(%) (n=79)	13(16.5)	31(39.2)	13(16.5)	22(27.8)	c^2= 40.244 P<0.001
Patients, n(%) (n=87)	28(32.2)	22(25.3)	37(42.5)	0(0)	

[a] Statistical tests by Pearson c^2 -test (2-sided).

Table 2. Distribution of TNF-a genotypes in normal controls and hypertensives

	Genotypes			Evidence for association (*P*-value[a])
	GG	GA	AA	
Control, n(%) (n=79)	63(79.7)	16(20.3)	0(0)	0.080
Patients, n(%) (n=87)	78(89.7)	9(10.3)	0(0)	

[a]Statistical tests by Pearson c^2 -test (2-sided).

Table 3. Distribution of TNF-a alleles in normal controls and hypertensives

	G allele	A allele	Evidence for association (P-value[a])
Control, n(%) (n=158)	142(89.9)	16(10.1)	0.094
Patients, n(%) (n=174)	165(94.8)	9(5.2)	

[a]Statistical tests by Pearson c^2 -test (2-sided).

2. Distribution of TNF-a genotypes

The genotype distribution in hypertensives and controls did not deviate significantly from Hardy-Weinberg equilibrium. The distribution of TNF-a genotype in 87 patients with hypertension were as follows: GG, 78 (89.7%); GA, 9 (10.3%); and AA, 0 (0), which was different from the distribution in 79 control subjects: GG, 63 (79.7%); GA, 16 (20.3%); and AA, 0 (0), although the statistical significancy was marginal (c^2=3.056, df=1, P=0.080) (Table 2).

3. Distribution of TNF-a alleles

Table 3 shows the association between TNF-a allelic frequencies and hypertension. The TNF-a allelic frequencies of the individuals with hypertension were as follows: G, 165 (94.8%); and A, 9 (5.2%). It was different from the distribution in control subjects: G, 142 (89.9%); and A, 16 (10.1%), although the statistical significancy was marginal. (c^2=2.801, df=1, P=0.094).

4. Association between TNF-a polymorphism and hypertension

Table 4 shows the association between TNF-a genotypes and Iris constitutions in whole population. The distribution of TNF-a genotype in the individuals with choleserol+cardio-renal connective tissue weakness type was as follows: GG, 86.0%; GA, 14.0%; and AA, 0%, which was not different from the distribution in the other Iris constitutions. Therefore, the author could not find the relationship between TNF-a genotypes and Iris constitutions. Also, the author stratified the individuals as the affection of hypertension and investigated the distribution of TNF-a genotype in each Iris constitution (Table 5). However, I did not find a certain association between TNF-a genotypes and Iris constitutions, not only in controls but also in hypertensives.

In addition, the hypertensives and controls were stratified as TNF-a genotypes and the association with Iris constitutions was investigated (Table 6). Cholesterol+cardio-renal connective tissue weakness type was more prevalent in hypertensives with GG genotype than in the remaining Iris constitutions: 42.2% in patients with cardio-renal connective tissue weakness type; 0-29.7% in patients with the remaining Iris constitutions.

Table 4. Relationship between Iris constitutions and TNF-a genotypes in whole population

	neurogenic	abdominal connective tissue weakness	cardio-renal + choleserol	the others	Evidence for association (*P*-value[a])
GG	82.4	87.5	86.0	86.4	
GA	17.6	12.5	14.0	13.6	0.930
AA	0	0	0	0	

[a]Statistical tests by Pearson c^2 -test (2-sided).

Table 5. Distribution of TNF-a genotype in Iris constitution

	neurogenic	abdominal connective tissue weakness	cardio-renal + choleserol	the others	Evidence for association (*P*-value[a])
Controls GG	76.9	82.1	76.9	86.4	0.869
GA	23.1	17.9	23.1	13.6	
Patients GG	85.7	95.0	90.0	0	0.608
GA	14.3	5.0	10.0	0	

[a]Statistical tests by Pearson c^2 -test (2-sided).

Table 6. Prevalence of Cardio-renal connective tissue weakness type in TNF-a genotype carriers

	Iris Constitutions, %				Evidence for association (*P*-value[a])
	neurogenic	abdominal connective tissue weakness	cardio-renal + choleserol	the others	
GG Controls	16.1	37.1	16.1	30.6	<0.001
Patients	28.1	29.7	42.2	0	
GA Controls	21.4	35.7	21.4	21.4	0.290
Patients	42.9	14.3	42.9	0	

[a]Statistical tests by Pearson c^2 -test (2-sided).

IV. Discussion

The eyes are the mirror of a soul, clear eyes of a child, the eyes, sparkling with happiness and the eyes, extinct because of grief - these and the others descriptions, associated with the expression of human eyes, or, to be more precise, with the ability of the iris to absorb and to reflect the light, exist in almost all languages of peoples on the Earth. Human brain receives more than 90 % information about the surrounding world by the visual analyzer. The injury or absence of iris, violation of the motor or associated reactions of pupils lead to the pathology of vision, disturbance of the light-regulation functions, causing the considerable limitation of the physical and social activity of a person. The brightens, colour saturation of iris, dynamics of pupil reactions and bottomless deepness of the healthy crystalline is inherent to the healthy youth, while dim, low-lively eye are the destiny of the disease and old age. The essence and reasons of these phenomenon were always of great interest for the investigators.

Iris markings have fascinated people throughout time, and many have searched for their true meanings. Certain markings have already been identified, while others are now close to being identified. So the mystery of the iris is gradually being unravelled. For example, one study in America revealed that athletes with brown eyes performed differently in various sports from blue-eyed people, and concluded that thee difference was due to reaction time. Athletes with brown irises responded faster than those with blue irises and performed better in sports such as boxing and squash, while blue-eyed people proved superior when a somewhat steadier concentration was needed, such as in golf and pool. Further research in laboratory conditions confirmed that these reaction times were due to reflex differences between light and dark-eyed people.

Iridology is based on the study of the iris and its links with the nervous system. The organs of the body are connected by nerves to the spinal cord, which is divided into separate segments, each corresponding to a segment of the body. Impulses from the organs or glands enter the spine via these nerves, and they can be 'read' in the iris, but an inherent weakness will-and it is these inherent weakness with which iridology is concerned[9].

Iris has the complex histological structure. It consists of several layers. Two main layers, mesoderm and ectoderm, are, in their turn, subdivided into more thin ones. Mesoderm part consists of anterior epithelium, superficial mesoderm layer and deep mesoderm layer. The ectoderm part consists of two pigment layers.

Anterior epithelium is formed by the squamous cells. Collagen fibers of the

boundary superficial layer look like the narrow strip. More deep connective tissue contains radial vessels, and its fibers concentrate around these vessels. There are plasmatic, pigment cells and macrophages. Pigment cells are called melanocytes (according to the old terminology-chromatophores); they define the color of an iris, containing goldish xanthophores and silvery guanophores, which provide the light colour of eyes. The cells of posterior epithelium are highly pigmented in all irises, independently of the color, due to the pigment fuscin.

Different authors distinguish in the iris stroma up to 7 types of stromal cells. Besides fibroblasts and melanocytes there are mast cells, macrophages, melanophages, lymphocytes and lump cells. Fibroblasts, which are the most common cells, are concentrated along the vessels and nervous fibers. Collagen fibers support the cellular structure of iris. The particular feature of iris tissue is the arrangement of melanocytes around the adventitia of the blood vessels.

In the study of iridology constituion, arterial hypertension, chronic ischemic heart disease with metabolic disturbances (osteochondrosis, arthrosis, lithogenesis) are very probable in constitution of cardiorenal connective tissue weakness, neurogenic and cholesterosis[22].

Cardiorenal connective tissue weakness is a pigment saturated cardiorenal subtype with the weakness of the connective tissue in Asia.

Cardiorenal connective tissue weakness has the color of the iris is brown, not bright. The stroma is more light near the autonomous wreath. Lacunas are in the projections of heart and kidneys[22].

People with such subtype have the increased lymphoid tissue reactivity and connective tissue weakness. They are predisposed to cardiac and renal diseases.

Pigment saturated neurogenic has the color of the iris is from light-brown to reddish-brown, 'tiger' color of the iris[22].

People with such iris subtype have increased reactivity of nervous system and lymphoid tissue.

Pigment saturated subtype with the signs of cholesterosis has the color of the iris from turbid-brown to reddish-brown. The lipid-sodium ring near the limb[22].

This genetic subtype is distinguished because cholesterol ring points to the violation of cholesterol metabolism. It should be emphasized that in old people cholesterol ring is indicative of the metabolic disturbances with atherosclerosis development, connected with the age, and in the young people it is the symptom of hyper-lipidemia and associated metabolic violations.

In childhood it is manifested by the lymphatic hyperplasia of nasopharynx, lymphatic nodes enlargement, allergic reactions, hyperlipidemia with predisposition to obesity.

In the middle age susceptibility to cholesterol metabolic disturbances reveals itself (seborrhea, furunculosis, folliculitis, xanthoma etc).

In the old age general atherosclerosis with concomitant diseases (encephalopathy, chronic ischemic disease, arterial hypertension) and metabolic disturbances by the type of arthrosis, spinal osteochondrosis are typical.

Kang[11] reported the relationship between iridological constitution and apoE polymorphism. The author classified 87 hypertensives patients with familial history of cerebral infarction and 79 controls according to Iris constitution, and determined apoE genotype. Neurogenic type in hypertensives was 32.2% compared with 16.5% in controls ($P<0.001$). No differences in the apoE genotypes frequencies were observed in patients compared with that in controls ($c^2=0.726$, $df=2$, $P=0.696$). However, in population with ε 3/ε4 genotype, the frequency of neurogenic constitution was significantly higher in hypertensives than in controls (60% vs. 0%) ($c^2=5.265$, $df=1$, $P=0.022$). These results imply that apo E ε3/ε4 genotype and neurogenic Iris constitution are risk factors for hypertension.

Kang investigated apoE genotypes of the hypertensives classified by Iris constitution. As a result, 74.7% of hypertensives were neurogenic or cardio-renal connective tissue weakness type. Also, the frequency of neurogenic constitution was significantly higher in patients with ε3/ε4 genotype than in the remaining Iris constitutions.

Yang reported the relationship between iridological constitution and ACE polymorphism. We classified 87 hypertensives and 79 controls according to Iris constitution, and determined ACE genotype. DD genotype was more prevalent in patients with neurogenic constitution than in controls. This finding supports the hypothesis that D allele is a candidate gene for hypertension and demonstrates the association among ACE genotype, Korean hypertensives and Iris constitution.[22]

Joo reported that he investigated the association among ACE genotypes, CI and Sasang constitutional classification. The frequencies of D allele were 0.32 in subjects with CI and 0.40 in without CI ($c^2=0.128$, $P=0.720$). The frequency of Taeumins, one type of Sasang constitutional classification, in patients with CI was significantly higher than that in controls ($c^2=15.425$, $P<0.001$). I did not find any association between ACE polymorphism and CI in Koreans[10].

Although it has been criticized the notion that iridology is a valid diagnostic tool, many iridologist exist and are practicing on patients in many areas[8]. Indeed, in Germany, 80% of *Heilpraktiker* (nonmedically qualified health practitioners) practice iridology. In this study, I investigated TNF-a genotypes of the hypertensives classified by Iris constitution. As a result, 74.7% of hypertensives were neurogenic or cardio-renal connective tissue weakness type. Also, the frequency of cardio-renal connective tissue weakness type was significantly higher in hypertensives with GG genotype than in the remaining Iris constitutions. These results imply that the

Iris constitution was related to hypertension, and then this study is a first attempt to find an association among the TNF-a gene polymorphism, hypertension and Iris constitution.

In addition, the author found that the frequency of the TNF-a GA heterozygote was decreased in hypertensives compared to controls. This result implies that the TNF-a polymorphism is associated with resistance against hypertension. The mechanism by which the TNF-a gene polymorphisms might have a protective role for hypertension is probably related to a different TNF-a synthesis, secretion and activity. Tumor necrosis factor is a pleiotropic cytokine that promotes inflammation and signals leading to cell death. Contrary to the preponderance of evidence that TNF-a is toxic to neurons, it also exerts neuro-protective effects, particularly in conditions of excitotoxic death[5]. In addition, Wilson et al. reported that the AA genotype significantly increased the transcriptional activity of the TNF-a -308 gene with respect to the GG genotype, and that a slight increase of the protein levels was also observed in the plasma[20]. Therefore, G allele carriers are regarded to be low producers of TNF-a, whereas A allele carriers are high producers of TNF-a. The increased TNF-a in A allele carriers might induce the neuro-protective effects, particularly in conditions of excitotoxic death, and thereby affect susceptibility of hypertension.

This is the first report to have examined the association of TNF-a genetic polymorphism with hypertension according to Iris constitutional classifications. These results suggest the apparent relationship between TNF-a genotypes and Iris constitutions, as well as the novel possibility of molecular genetic understanding of iridology.

V. Conclusion

The author compared the distribution of TNF-a in a defined group of hypertensives with those in a control group, and investigated the association between TNF-a polymorphism and hypertension according to Iris constitution.

1. Compared to controls, the frequency of the TNF-a GA heterozygote was decreased in hypertensives, although the statistical significancy was marginal (P=0.08). This result implies an association with resistance to the disease.

2. Choleserol+Cardio-renal connective tissue weakness type in hypertensives was 42.5% compared with 16.5% in controls (P<0.001).

3. The frequency of cholesterol + cardio-renal connective tissue weakness type was significantly higher in hypertensives with GG genotype than in the remaining Iris constitutions (P<0.001).

The author found the relationship between

the Iris constitution and hypertension, and first attempted to find an association between TNF-a gene polymorphism, hypertension and Iris constitution.

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