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Original Articles

Study on Relationship between Iris Constitution and Apolipoprotein E Gene Polymorphism

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Iridology, a form of complementary and alternative medicine (CAM), is the diagnosis of medical conditions through noting irregularities of the pigmentation in the iris. Iridological constitution has a strong familial aggregation and is implicated in heredity. Apolipoprotein E (apoE) gene polymorphism is one of the most well studied genetic markers of vascular disease. I investigated the relationship between iridological constitution and apoE polymorphism. I classified 87 hypertensive patients with family 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 those in controls ($\chi^2=0.726$, $df=2$, $P=0.696$).

However, in a population with ε3/ε4 genotype, the frequency of neurogenic constitution was significantly higher in hypertensives than in controls (60% vs. 0%) ($\chi^2=5.265$, $df=1$, $P=0.022$). These results could imply that apoE ε3/ε4 genotype and neurogenic iris constitution are risk factors for hypertension. *(Korean J of Oriental Med 2003;24(4):25-33)*

Key Words: iridology, apolipoprotein E, hypertension, polymorphism

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[21] 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. Although iridology is a popular alternative medical treatment and it has been 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. I 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.

Apolipoprotein E (apoE) is a 299 amino-acid protein with a central role in cholesterol transport and

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lipoprotein metabolism. The gene for apoE is located on chromosome 19 in linkage with the genes encoding for other apolipoproteins: apo C-I and C-II and the low-density lipoprotein (LDL) receptor gene. It is polymorphic, with three common alleles, ε4, ε3, ε2 which code for three major isoforms in plasma designated apo E4, apo E3, and apo E2 respectively, resulting in six common genotypes[22]. ApoE is a key protein modulating the highly atherogenic apoB-containing lipoproteins[4] and is a candidate gene for the development of coronary artery disease (CAD), including hypertension. The ε2/ε2 genotype was the first to be implicated in premature coronary artery disease[4], which resulted in this polymorphism being extensively studied. Therefore, the aim of this study was to compare the prevalence of the three most frequent alleles of apoE in a defined group of hypertensives with those in a control group, and to investigate the association between apoE polymorphism and hypertension according to Iris constitution.

Materials and Methods

1. Subjects

Starting in 1999, 87 hypertensives between ages 28 and 62 years were enrolled from Oriental Medical Hospital, Won-Kwang University, Jeonju, Korea. Hypertension was defined as systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg. The control group 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 apoE genotypes

Blood was stored at -20 °C until it was ready to be extracted. Genomic DNA was extracted by inorganic procedure[17]. Concentration of DNA was estimated by absorbance at 260 nm. ApoE polymorphism was detected by PCR amplification[8].

Briefly, a PCR reaction was carried out in a 20 *l* volume containing 200 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 M of each dNTP, and 1 U of rTaq DNA polymerase (Takara, Japan), with 1 M of apoE F4/F6 (Bioneer, Korea). The primer pairs for each gene were as follows (Fig. 1):

F4: 5-ACAGAATTCGCCCCGGCCTGGTACAC-3,

F6: 5-TAAGCTTGGCACGGCTGTCCAAGGA-3 (5).

Amplification conditions were 5 min preincubation step at 95 °C, 40 cycles of denaturation at 94 °C for 40 sec, annealing at 67 °C for 40 sec, and extension at 72 °C for 40 sec. A final extension for 10 min at 72 °C was included (MJ Research). The PCR product was digested for 16h at 37 °C with 5.5 units HhaI in the presence of 2 g bovine serum albumin. PCR products were then separated electrophoretically through 8% polyacrylamide gel with a pGEM DNA marker (Promega, U.S.A.) and the products visualized by ethidium bromide staining (Fig. 2). The following fragments were obtained after restriction enzyme digestion: apo ε 2: 91, 81, 21, 18, 16, apo ε3: 91, 48, 21, 18, 16, apo ε4: 72, 48, 33, 21, 19, 18, 16 (Fig. 3). DNA of a subject with known apo ε2/ε2 genotype was included with each batch as a control to prevent inaccurate typing resulting from an incomplete digest. Genotypes were determined without reference to case or control status.

The amplified E4 nucleotide sequence (244bp, numbered to the right) is shown above the E4 amino acid sequence. The sequences of amplification primers

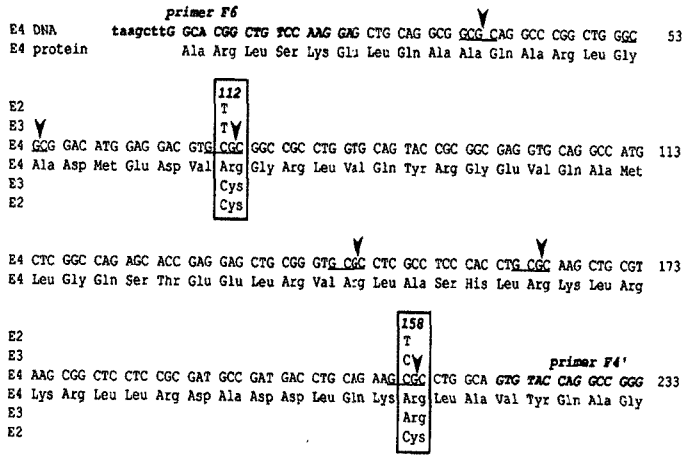

Fig. 1. DNA and protein sequences of amplified regions encoding common apoE isoforms and locations of *Hhal* cleavage sites.

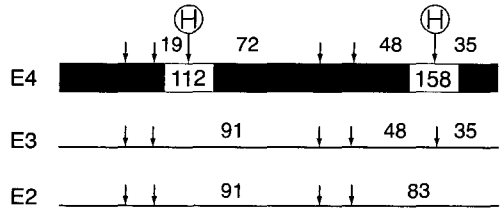

Fig. 2. HhaI cleavage maps.

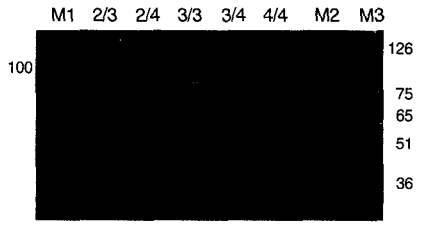

Fig. 3. Electrophoretic separation of HhaI fragments after gene amplification of DNA from subjects with known apoE isoforms.

(F6 and F4, the reverse complement of F4) are also shown (upper case italics are apoE sequences, lower case italics are synthetic cleavage sites). Nucleotide substitutions that distinguish E2 and E3 isoforms are shown above the E4 nucleotide sequences, and amino acid substitutions are shown below the E4 amino acid sequence (substitution sites at codons 112 and 158 are boxed). The sites for HhaI cleavage in the E4 nucleotide sequence are underlined and marked by arrows.

HhaI cleavage maps (downward arrows show sites) are given for amplified sequences (E4 is shown as a filled box containing codons 112 and 158, E3 and E2 maps are shown below E4). The distances (in bp) between polymorphic HhaI sites (circled H) that distinguish isoforms are shown for each cleavage map.

A polyacrylamide gel is shown after electrophoresis of HhaI fragments from an ε2/ε3 heterozygote (lane marked 2/2), ε2/ε4 heterozygote (lane marked 2/4), ε3/ε3 homoozygote (lane marked 3/3), ε3/ε4 heterozygote (lane marked 3/4), and ε4/ε4 homoozygote (lane marked 4/4). The fragment sizes (in bp) of a DNA standard (100bp ladder, ACE genotypes (86bp and 64bp), and pGEM DNA marker, lane marked M1, M2, and M3, respectively) are shown in the gel.

4. Statistical analysis

Comparisons of the allele frequencies of the apoE genotypes between the control and hypertensives were carried out using the Pearson chi-square test or linear-by-linear association chi-square test. All statistical analyses were performed using SPSS v9.00 (SPSS Inc.)

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

Results

1. Distribution of iris constitutions

I classified 87 hypertensives and 79 controls according to iris constitution, and determined apoE genotype. The distribution of iris constitution in hypertensives was significantly different from the distribution in controls (χ^2=40.244, *df*=3, *P*<0.001) (Table 1). The frequencies of iris constitutions in controls was as follows: neurogenic type, 16.5%; abdominal connective tissue weakness type, 39.2%; cholesterol + cardiorenal connective tissue weakness type, 16.5%; other types, 27.8%. This was significantly different from the distribution in hypertensives: neurogenic type, 32.2%; abdominal connective tissue weakness type, 25.3%; cholesterol + cardiorenal connective tissue weakness type, 42.5%; other types, 0%.

2. Distribution of apoE genotypes

The genotype distribution in patients and controls did not deviate significantly from Hardy-Weinberg equilibrium. The distribution of apoE genotype in 87 patients with hypertension were as follows: ε2/ε2, 0 (0%); ε2/ε3, 13 (14.9%); ε2/ε4, 0 (0%); ε3/ε3, 68 (78.2%); ε3/ε4, 6 (6.9%); and ε4/ε4, 0 (0%), which was not different from the distribution in the 79 control subjects: ε2/ε2, 0 (0%); ε2/ε3, 8 (10.1%); ε2/ε4, 0 (0%); ε3/ε3, 66 (83.5%); ε3/ε4, 5 (6%); and ε4/ε4, 0 (0%) (χ^2=0.726, *df*=2, *P*=0.696) (Table 2).

3. Distribution of apoE alleles

Table 3 shows the association between apoE allelic frequencies and hypertension. The apoE allelic frequencies of the individuals with hypertension were as follows: ε2, 13 (7.5%); ε3, 155 (89.1%); and ε4, 6 (3.4%). This was not significantly different from the distribution in control subjects: ε2, 8 (5.1%); ε3, 145 (91.8%); and ε4, 5 (3.2%) (χ^2=0.846, *df*=2, *P*=0.655).

4. Association between apoE polymorphism and hypertension

Table 4 shows the association between apoE genotypes and iris constitutions in our population. The frequency of ε3/ε4 genotype in neurogenic type was 9.4% compared with 6.3% and 2.4% in abdominal connective tissue weakness type and in cholesterol + cardiorenal connective tissue weakness type,

Table 1. Distribution of Iris Constitutions

	Iris Constitutions				
	neurogenic	abdominal connective tissue weakness	cardiorenal + cholesterol	others	Evidence for association(*P*-value*)
Control, n(%) (n=79)	13(16.5)	31(39.2)	13(16.5)	22(27.8)	χ^2=40.244
Patients, n(%) (n=87)	28(32.2)	22(25.3)	37(42.5)	0(0)	*P*<0.001

*: Statistical tests by Pearson's χ^2-test (2-sided)

Table 2. Distribution of ApoE Genotypes in Normal Controls and Hypertensive Patients

	Genotypes				
	ε2/ε3	ε3/ε3	ε3/ε4	ε4/ε4	Evidence for association(*P*-value*)
Control, n(%) (n=79)	8(10.1)	66(83.5)	5(6.0)	0(0)	0.696
Patients, n(%) (n=87)	13(14.9)	68(78.2)	6(6.9)	0(0)	

*: Statistical tests by Pearson's χ^2-test (2-sided)

Table 3. Distribution of ApoE Alleles in Normal Controls and Hypertensive Patients

	Alleles			
	ε2	ε3	ε4	Evidence for association (*P*-value*)
Control, n(%) (n=158)	8(5.1)	145(91.8)	5(3.2)	0.655
Patients, n(%) (n=174)	13(7.5)	155(89.1)	6(3.4)	

*: Statistical tests by Pearson's χ^2-test (2-sided)

Table 4. Relationship between Iris Constitutions and ApoE Genotypes in Whole Population

	Iris constitutions, %				
	neurogenic	abdominal connective tissue weakness	cardiorenal+ cholesterol	others	Evidence for association(*P*-value*)
ε2/ε3	18.8	12.5	14.6	4.5	
ε3/ε3	71.9	81.3	82.9	86.4	0.648
ε3/ε4	9.4	6.3	2.4	9.1	

*: Statistical tests by Pearson χ^2-test (2-sided)

Table 5. Association between Iris Constitution and ApoE Genotype in Hypertensives

	Iris Constitutions, %				
	neurogenic	abdominal connective tissue weakness	cardiorenal+ cholesterol	others	Evidence for association(*P*-value*)
ε2/ε3 Controls	22.2	33.3	33.3	11.1	0.314
Patients	40.0	30.0	30.0	0	
ε3/ε3 Controls	16.1	38.7	14.5	30.6	0.046
Patients	24.5	28.3	47.2	0	
ε3/ε4 Controls	0	25.0	25.0	50.0	0.022
Patients	60.0	40.0	0	0	

*: Statistical tests by Linear-by-Linear Association χ^2-test (2-sided)

respectively. However, the higher frequency of ε3/ε4 genotype in neurogenic constitution was not significant in the statistics (χ^2=4.210, *df*=6, *P*=0.648).

The hypertensives and controls were stratified as apoE genotypes and the association with iris constitutions was investigated (Table 5). Neurogenic type was more prevalent in hypertensives with ε2/ε3 and ε3/ε4 genotypes than in the remaining iris constitutions: 40.0% in patients with ε2/ε3 genotype; 60.0% in patients with ε3/ε4 genotype. Especially, the frequency of neurogenic constitution was significantly higher in patients with ε3/ε4 genotype than in the remaining iris constitutions (60% vs. 40%, 0%, and 0%) (χ^2=5.265, *df*=1, *P*=0.022).

Discussion

Iridology, developed more than 100 years ago, is the diagnosis of medical conditions through noting irregularities of the pigmentation in the iris. Iridology assumes that all bodily organs represented on the surface of the iris via intricate neural connections and that dysfunction of most organs is marked on the iris, usually as a pigmentary change.

In the study of iridology constitution, 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.

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 brown, not bright. The stroma is lighter near the autonomous wreath. Lacunas are in the projections of heart and kidneys. People with such subtype have increased lymphoid tissue reactivity and connective tissue weakness. They are predisposed to cardiac and renal diseases.

In childhood such persons may suffer from lymphatic hyperplasia of nasopharynx and inclination to rhinitis and sinusitis. Weakness of connective tissue of internal organs may manifest itself by forming valvular heart disease failure (congenital and acquired), early myopia, vascular disturbances and debility of spinal column ligaments and articulations. Kidneys are often subjected to inflammatory processes, which may cause nephritis, pyelonephritis and urodynamics disturbances with calculi forming.

In middle age, people with this subtype have increased predisposition to rheumatism (causing valvular heart disease) and collagenosis. Myopia may progress; varicosis, hemorrhoids, hernias, and radiculitis can develop. Risk of chronic renal disease with secondary renal hypertension is very high.

In old age, stable arterial hypertension and chronic ischemic heart disease with metabolic disturbances (osteochondrosis, arthrosis, lithogenesis) are very probable.

Pneumosclerosis, lungs insufficiency and diabetes take place rather often.

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

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

In childhood this is manifested by predisposition to rhinitis, sinusitis, and lymphatic nodes enlargement. Increased reactivity of the nervous system leads to heightened irritability, unpredictable behavior, non-motivated actions, and neurosis.

In middle age for people with such subtype diligence, purposefulness and high capacity for work is typical, which, however, may result in strain, nervous system exhaustion and nervous frustration. Migraine like headaches, irritation, fatigue, nervous and asthmatic attacks are frequent in such people; gastric and duodenum ulcer of nervous etiology is possible. According to our data, such iris subtype is extremely rare in pilots, astronauts, sailors and the military.

In the old age risk of hypertension, chronic ischemic cardiac disease, Parkinson' s disease, and senile neurasthenic reactions is increased.

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

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

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

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

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

Although the notion that iridology is a valid diagnostic tool has been criticized, many iridologists exist and are practicing on patients in many areas6). Indeed, in Germany, 80% of Heilpraktikers (non-medically qualified health practitioners) practice iridology.

Yang reported a 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[25].

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 (χ^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 (χ^2=15.425, P<0.001). I did not find any association between ACE polymorphism and CI in Koreans[9].

In this study, I investigated apoE genotypes of the hypertensives classified by iris constitution. As a result, 74.7% of hypertensives were of neurogenic or cholesterol + cardiorenal 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. These results are consistent with the reports that apoE ε4 allele was associated with the occurrence of myocardial infarction and coronary atherosclerosis in relation to hypertension[8,11,23] However, these have produced mainly contradictory results[2,3,7,14,15,16,18,20]. Different ethnic groups can also affect the results of these studies[19]. The apoE ε2 allelic frequency of our Korean controls was similar to that in Japanese controls (0.05 vs. 0.05)[10,26] and Europeans (0.05 vs. 0.06)[1,21,13], but lower than that in Taiwanese (0.05 vs. 0.08)[24]. In addition, the apoE ε4 allelic frequency of our controls was lower than that in Japanese controls (0.03 vs. 0.11)[10,26], and higher than in Taiwanese (0.03 vs. 0.08)[24]. Even among Europeans there are geographic differences, with an ε4 frequency as high as 0.20 in Norway[13] and as low as 0.07 in Turkey[1]. These differences indicate that ethnicity should be carefully considered in the studies on the association between apoE genotype and disease aetiology.

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

Conclusion

I investigated the relationship between iridological constitution and apoEpolymorphism. I classified 87 hypertensive patients with familial history of cerebral infarction and 79 controls according to iris constitution, and determined apoE genotype.

1. Neurogenic type in hypertensives was 32.2% compared with 16.5% in controls (P<0.001). This result suggests that neurogenic type is related to hypertension.
2. No differences in the apoE genotypes frequencies were observed in patients compared with those in controls (χ^2=0.726, df=2, P=0.696). However, in the population with ε3/ε4 genotype, the frequency of neurogenic constitution was significantly higher in hypertensives than in controls (χ^2=5.265, df=1,

P=0.022).

These results imply that apoE ε3/ε4 genotype and neurogenic iris constitution are risk factors for hypertension.

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