J. ENVIRON. TOXICOL.
Vol. 18, No. 1, 27~32 (2003)

Lack of Association of the Mitochondrial DNA 5178 A/C Polymorphism with Hypertension in a Korean Population

Byung Yong Kang, Seon Jeong Kim*, Dai Ho Jang*, Hyun Hee Kim* and Kang Oh Lee*,*

Research Institute for Life Science, Sahmyook University, Seoul 139-742, Korea
**Dept. of Life Science, Sahmyook University, Seoul 139-742, Korea*

한국인 집단에서 사립체 DNA에 존재하는 5178 A/C 다형성과 고혈압과의 관련성에 관한 연구

강병용, 김선정*, 장대호*, 김현희*, 이강오*,*

삼육대학교 생명과학연구소, *삼육대학교 생명과학과

요 약

고혈압은 다양한 유전적 요인과 환경적 요인이 상호작용하는 다인자성 질환으로 알려져 있으며, 최근의 연구에 의하면 사립체 DNA에 존재하는 유전적 다형성이 고혈압과 유의한 관련성을 나타낸다는 보고가 있다. 이에 본 연구에서는 한국인 집단을 대상으로 하여 사립체 DNA의 5178번째 위치의 염기서열에 존재하는 A/C 다형성이 고혈압과 관련성을 나타내는 지를 분석하였다. 환자-대조군 연구를 수행한 결과, 사립체 DNA의 5178번째 위치에 존재하는 다형성의 대립 유전자 빈도는 한국인에서 고혈압군과 정상 혈압군 사이에 유의한 차이를 나타내지 않았다. 따라서, 이 다형성은 적어도 한국인에 대해서는 고혈압에 유의하게 영향을 미치는 유전적 소인은 아닌 것으로 사료된다.

주요어 : 고혈압, 사립체 DNA, 다형성

INTRODUCTION

Hypertension, a major independent risk factor for stroke, myocardial infarction, and end-stage renal failure (Bae *et al.*, 2002), affects 15~20% of the adult population in industrialized societies (Lifton, 1996; Shin *et al.*, 2001). The recognition that genetic factors are involved in the pathogenesis of hypertension is derived from studies comparing the blood pressures of monozygotic and dizygotic twins (Ward, 1990), from epidemiologic studies of familial aggregation of hypertension (Longini *et al.*, 1984), and the adoptive siblings (Biron *et al.*, 1976). Some genetic variants, such as polymorphisms in the angiotensinogen (Jeunemaitre *et al.*, 1992; Inoue *et al.*, 1997) and α-adducin genes (Casari *et al.*, 1995; Cusi *et al.*, 1997), can increases the risk for hypertension, but the full spectrum of genes that contribute to this condition are poorly defined.

Shoji *et al.* (2002) performed a case-control study using genetic variation in the mtDNA as genetic markers, and suggested that the genetic variation in the mtDNA may be one of the genetic susceptibility fac-

※ To whom correspondence should be addressed.
Tel: 82-2-3399-3561, E-mail: leeko@syu.ac.kr

tors for hypertension. However, to our knowledge, there were few reports about relationship between genetic variation of mtDNA and hypertension in other ethnic groups including Korean population.

In the present study, we investigated an association between mt5178 A/C polymorphism in the mtDNA and hypertension in an ethnically homogeneous Korean population.

MATERIALS AND METHODS

Study subjects

We obtained 179 blood samples from the outpatients of Seoul Hygiene Hospital, Seoul, Korea. Of these, 90 hypertensive Korean individuals were defined as having a blood pressure above 140/90 mmHg. Subjects with secondary forms of hypertension were excluded from this study.

Determination of clinical phenotypes

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12~16 hour. Systolic and diastolic blood pressures were measured by mercury sphigmomanometer. The body mass index (BMI) value was calculated by the body weight (kg) devided by the square of the height (m^2). Concentrations of serum total cholesterol (TC) and triglyceride were measured by enzymatic colorimetry methods with commercial kits (Boehringer Mannheim, Germany) and chemistry analyzer. Serum HDL-cholesterol concentration was determined by measuring cholesterol in the supernatant after precipitation of the serum with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Serum LDL-cholesterol concentration was calculated by using the formular of Fridewald *et al.* (1972).

DNA analysis

Total genomic DNA was isolated from whole blood by using QIAamp Blood Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) techniques were used for mt5178 A/C polymorphism of mtDNA (Kokaze *et al.*, 2001). Briefly, total 50 μl of the reaction mixture contained 200~400 ng of genomic DNA, 100 ng of each primer, 200 μM of each dNTP, and buffers recommended by the manufacturer. The sequences of the primer for mt5178 A/C polymorphism studied were:
sense, 5′-CTTAGCATACTCCTCAATTACCC-3′,
anti-sense 5′-CTGAATTCTTCGATAATGGCCCA-3′ (Kokaze *et al.*, 2001).

Amplification was carried out with automated thermocycler: one cycle at 94°C for 5 min, 40 cycles at 94°C for 30 sec, at 60°C for 1 min and at 72°C for 1 min 30 sec with a final polymerization at 72°C for 10 min. Amplified PCR products were digested with the restriction enzyme *Alu* I (Promega, Co., Ltd., Madison, WI, USA), and electrophoresed in 1.5% agarose gel. Gels were stained with ethidium bromide, visualized under UV light, and photographed. The absence of the *Alu* I site was designated as mt5178 C allele, and the presence of this restriction enzyme cutting site was designated mt5178 A allele (Fig. 1).

Statistical Analysis

Allele frequencies were estimated by gene counting method. The heterozygosity and polymorphism information content (PIC) values were estimated by the method of Bostein *et al.* (1980). The significance of differences in allele frequencies between popu-

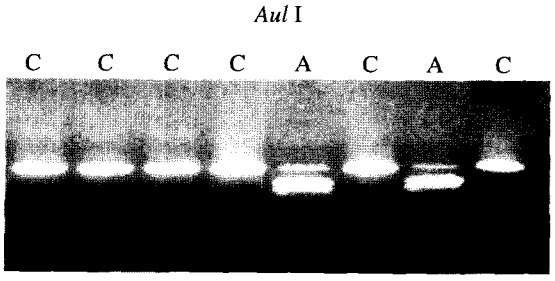

Fig. 1. Polymorphic patterns of mt5178 A/C. Mt5178 A allele has an *Alu* I restriction cutting enzyme site, while mt 5178 C allele does not have this site.

lations was also estimated by 2 × 2 contingency table. Student's t-test was performed to compare the mean levels of clinical phenotypes between different alleles. Statistical significance was accepted at the P = 0.05 level. All statistical analysis was performed using the computer program of SPSS for windows (version 11).

RESULTS

Allele distribution of mt5178 A/C

In the present study, we attempted to clarify the allele frequency of mt 5178 A/C polymorphism in Korean population. Table 1 displays the allele frequencies and the values of heterozygosity and PIC for mt5178 A/C polymorphism in Korean normotensives and hypertensives, respectively. The allele frequencies of A and C were 64 and 32% in normotensives, and 62 and 38% in hypertensives, respectively. There was no significant difference in allele frequency between normotensives and hypertensives (P > 0.05). The heterozygosity and PIC values of mt5178 A/C polymorphism represented the values of 0.4605 and 0.3545 for normotensives, and 0.4701 and 0.3596 for hypertensives, respectively. According to heterozygosity and PIC values, mt5178 A/C polymorphism indicated a relatively high degree of polymorphism in the both groups.

Table 1. Allele frequencies of the mt5178 A/C polymorphisms in Korean normotensives and hypertensives

	Allele No. (%)		H[1]	PIC[2]
	A	C		
Normotensives	57(64)	32(36)	0.4605	0.3545
Hypertensives	56(62)	34(38)	0.4701	0.3596
Chi-square	0.0096			
Probability	0.9221			
Odds ratio(CI)[3]	1.08(0.59~1.99)			

[1]Heterozygosity was calculated as $H = 1 - \Sigma pi^2$ (p; allele frequency).
[2]Polymorphism Information Content was calculated as
$PIC = 1 - \Sigma pi^2 - \Sigma \Sigma^2 pi^2 pj^2$ (p; allele frequency).
[3]95% Confidence Interval.
Frequency is given as a percentage in parenthesis.

Table 2. The comparison of the clinical phenotypes according to mt 5178 A/C polymorphism in total subjects

Variable	Allele	
	A (No.)[6]	C (No.)
Age (year)	60.8±10.6 (111)	58.2±11.1 (66)
BMI (kg/m^2)[1]	23.6±2.3 (104)	24.0±2.9 (57)
TG (mg/dl)[2]	127.5±79.3 (89)	132.2±70.7 (53)
TC (mg/dl)[3]	155.4±32.3 (89)	147.8±34.3 (53)
LDL-chol (mg/dl)[4]	102.6±33.7 (89)	94.9±34.8 (53)
HDL-chol (mg/dl)[5]	27.6±10.0 (89)	25.5±8.4 (53)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number.
Value are mean±SD (standard deviation).

Table 3. The comparison of the clinical phenotypes according to mt 5178 A/C polymorphism in normotensives

Variable	Allele	
	A (No.)[6]	C (No.)
Age (year)	57.6±9.3 (56)	54.2±9.3 (32)
BMI (kg/m^2)[1]	23.2±1.8 (56)	23.8±3.1 (32)
TG (mg/dl)[2]	127.5±79.3 (46)	132.2±70.7 (31)
TC (mg/dl)[3]	153.1±32.8 (46)	151.5±36.7 (31)
LDL-chol (mg/dl)[4]	99.5±36.5 (46)	97.3±37.7 (31)
HDL-chol (mg/dl)[5]	28.8±9.7 (46)	27.9±9.0 (31)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number.
Value are mean±SD (standard deviation).

Association with clinical phenotypes

Table 2 presented the comparison of clinical phenotypes across mt5178 A/C polymorphism in total subjects. Mt5178 A/C polymorphism was not significantly associated with any clinical phenotypes (P > 0.05). When stratified by blood pressure status, there were also no significant differences in any clinical phenotypes across this polymorphism in the both groups (Table 3 and 4).

DISCUSSION

Hypertension is a multifactorial disease with a

Table 4. The comparison of the clinical phenotypes according to mt 5178 A/C polymorphism in hypertensives

Variable	Allele	
	A (No.)[6]	C (No.)
Age (year)	64.0±10.8 (55)	62.0±11.7 (34)
BMI (kg/m^2)[1]	24.0±2.6 (48)	24.2±2.5 (25)
TG (mg/dl)[2]	128.1±66.7 (43)	144.5±64.3 (22)
TC (mg/dl)[3]	157.9±31.8 (43)	142.7±30.8 (22)
LDL-chol (mg/dl)[4]	106.0±30.5 (43)	91.6±30.8 (22)
HDL-chol (mg/dl)[5]	26.3±10.2 (43)	22.3±6.4 (22)

[1]Body Mass Index, [2]Triglyceride, [3]Total cholesterol, [4]LDL-cholesterol, [5]HDL-cholesterol and [6]Number.
Value are mean±SD (standard deviation).

substantial genetic component (Lifton and Jeunemaitre, 1993). Between 30% and 50% of blood pressure variation in the population is determined by genetic factors (Ward, 1990). To search for genetic factor of hypertension, association studies using the candidate gene approach may provide important clues regarding the etiology of hypertension and define a basis for further genetic investigation (Kurtz and Spence, 1993; Soma *et al.*, 1999). Thus, we performed a candidate gene study of case-control type in order to investigate the relationship between the mt5178 A/C polymorphism as genetic marker and hypertension in Korean population.

Mitochondria are present in the cytoplasm of all eukaryotic cells of animals and higher plants and also in some microorganisms (algae, fungi and protozoa) (Dahl and Thorburn, 2001). They play a pivotal role in protecting the rest of the cell from the damaging effects of the reactive oxygen species created during the oxidative phosphorylation process by harnessing and inactivating these highly reactive and potentially damaging byproducts as well as oxidative phosphorylation and energy production. Also, mitochondria play a central role in necrosis and apoptosis, which are so important in normal development and in the etiology of many diseases (Dahl and Thorburn, 2001; Raha and Robinson, 2001).

The human mitochondrial genome is a small (16,568 bp) (Niemi *et al.*, 2003), circular, double-stranded, maternally inherited DNA molecule containing 37 genes (Anderson *et al.*, 1981). Of these, 24 genes (2 ribosomal RNAs and 22 transfer RNAs) are needed for mtDNA translation, and 13 genes encode subunits of the respiratory chain (seven subunits of complex I, one subunit of complex III, three subunits of complex IV, and two subunits of complex V) (Dimauro and Schon, 2001). Until now, it has been reported that many human diseases are due to mtDNA mutations or polymorphisms (Dimauro and Schon, 2001; Orth and Schapira, 2001; Thorburn and Dahl, 2001).

Mt5178 A/C polymorphism is located in the NADH dehydrogenase subunit 2 (ND2) coding region of mitochondrial DNA, causing Leu-to-Met re-placement (Tanaka *et al.*, 1998). Some studies have reported that this polymorphism was associated with various clinical phenotypes such as serum lipid levels (Kokaze *et al.*, 2001), longevity (Tanaka *et al.*, 1998), the mean intima-media thickness (IMT) in type 2 diabetic patients (Matsunaga *et al.*, 2001) and serum protein fraction levels in healthy women (Kokaze *et al.*, 2002).

In the present study, we failed to demonstrate the significant association between the mt5178 A/C polymorphism and other clinical phenotypes as well as hypertension in Korean population. Therefore, it is unlikely that this genetic polymorphism is significantly associated with the etiology of hypertension among Koreans. It should not excluded, however, that this polymorphism could have small effect for the etiology of hypertension because a small effect may expected in the case of a disease as complex as hypertension. Furthermore, these types of study design (association studies of case-control type) are prone to type II errors. In other word, negative findings generated by retrospective case-control studies can in no way be advocated to rule out gene effects in clinical phenotypes under investigation (Frossard *et al.*, 1998). Finally, these limitations will be overcome by large-scale cohort study.

A negative finding between a genetic marker of mtDNA and hypertension in our subjects was not

agreed with the result performed in a Japanese population (Shoji *et al.*, 2002). This discrepancy may be at least in part, explained by the differences in study design, marker selection and sample size between two studies.

Cann *et al.* (1987) reported that among 147 samples from the world, only five Asians and one European individual have mt5178 A allele. This observation indicates that mt5178 A allele is relatively rare among the global population. On the other hand, Tanaka *et al.* (1998) reported that the frequency of mt5178A allele in Japanese population is relatively high (0.45) among populations studied, and this allele associated with longevity. They also proposed that high life expectancy of Japanese population might at least in part, be characterized by this allele (Tanaka *et al.*, 1998). Nowadays, life expectancy of Korean population is lower than that of Japanese population. Nevertheless, mt5178 A allele frequency in Korean populations (0.64) was rather higher than those of Japanese populations (0.42～0.45) (Tanaka *et al.*, 1998; Kokaze *et al.*, 2001). The reason for this contradiction is unclear, but may be due to complexity of life span. In other word, life span is influenced by various environmental and genetic factors, but mt5178 A allele may be one of multiple interactive genetic factors for longevity. Further studies are needed to clarify the relationship between mt5178 A allele and longevity in Korean population. It will also be interesting to investigate whether other polymorphisms in the mtDNA are susceptible to hypertension in Korean population.

REFERENCES

Anderson S, Bankier AT, Barrel BG, DeBruijin M, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith A, Staden R and Young IG. Sequence and organization of the human mitochondrial genome, Nature 1981; 290: 457-465.

Bae JS, Kang BY, Lee KO, Yoon TJ, Kim JH and Kim KT. Haplotype distribution of the β_2-adrenergic receptor gene in Korean essential hypertensives. J. Toxicol. Pub. Health. 2002; 18: 233-240.

Biron P, Mongeau JG and Bertrand D. Familial aggregation of blood pressure in 558 adopted children, Can. Med. Assoc. J. 1976; 115: 773-774.

Bostein D, White RL, Skolnick M and Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms, Am. J. Hum. Genet. 1980; 32: 314-331.

Cann RL, Stoneking M and Wilson AC. Mitochondrial DNA and human evolution. Nature 1987; 325: 31-36.

Casari G, Barlassina C, Cusi D, Zagato L, Muirhead R, Righetti M, Nembri P, Amar K, Gatti M, Macciardi F, Binelli G and Bianchi G, Association of the α-adducin locus with essential hypertension, Hypertension 1995; 25: 320-326.

Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, Glorioso N, Lanzani C, Manunta P, Righetti M, Rivera R, Stella P, Troffa C, Zagato L and Bianchi G. Polymorphisms of α-adducin and salt sensitivity in patients with essential hypertension, Lancet 1997; 349: 1353-1357.

Dahl H-HM and Thorburn DR. Mitochondrial diseases: beyond the magic circle, Am. J. Med. Genet. 2001; 106: 1-3.

Dimauro S and Schon EA. Mitochondrial DNA mutations in human disease, Am. J. Med. Genet. 2001; 106: 18-26.

Fridewald WT, Levy RI and Friedrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge, Clin. Chem. 1972; 18: 499-502.

Frossard PM, Obineche EN, Elshahat YI, Lestringant GG, John A and Parvez SH. Association study of mutation G^{-75} to A in the promoter of the human apolipoprotein AI gene and essential hypertension, Biogenic Amines 1998; 14: 91-100.

Inoue I, Nakajima T, Williams CS, Quackenbush J, Puryear R, Powers M, Cheng T, Ludwig EH, Sharma AM, Hata A, Jeunemaitre X and Lalouel JM. A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription *in vitro*, J. Clin. Invest. 1997; 99: 1786-1797.

Jeunemaitre X, Soubrier F, Kotelevtsev Y, Lifton R, Williams C, Charru A, Hunt S, Hopkins P, Williams R, Lalouel JM and Corvol P. Molecular basis of human hypertension: the role of angiotensinogen, Cell 1992; 71: 169-180.

Kokaze A, Ishikawa M, Matsunaga N, Yoshida M, Sekine

Y, Teruya K, Takeda N, Satoh M, Sumiya Y, Uchida Y and Takashima Y. Association of the longevity-associated mitochondrial DNA 5178 A/C polymorphism with serum protein fraction levels in healthy Japanese women, Exp. Gerontol. 2002; 37: 931-936.

Kokaze A, Ishikawa M, Matsunaga N, Yoshida M, Sekine Y, Teruya K, Takeda N, Sumiya Y, Uchida Y and Takashima Y. Association of the mitochondrial DNA 5178 A/C polymorphism with serum lipid levels in the Japanese population, Hum. Genet. 2001; 109: 521-525.

Kurtz TW and Spence MA. Genetics of essential hypertension, Am. J. Med. 1993; 94: 77-84.

Lifton RP and Jeunemaitre X. Finding genes that cause human hypertension, J. Hypertens. 1993; 11: 231-236.

Lifton RP. Molecular genetics of human blood pressure variation, Science 1996; 272: 676-680.

Longini IM Jr, Higgins MW, Hinton PC, Moll PP and Keller JB, Environmental and genetic sources of familial aggregation of blood pressure in Tecumseh, Michigan. Am. J. Epidemiol. 1984; 120: 131-144.

Matsunaga H, Tanaka Y, Tanaka M, Gong J-S, Zhang J, Nomiyama T, Ogawa O, Ogihara T, Yamada Y, Yagi K and Kawamori R. Antiatherogenic mitochondrial genotype in patients with type 2 diabetes, Diabetes Care 2001; 24: 500-503.

Niemi A-K, Hervonen A, Hurme M, Karhunen PJ, Jylhä M and Majamaa K. Mitochondrial DNA polymorphisms associated with longevity in a Finnish population, Hum. Genet. 2003; 112: 29-33.

Orth M and Schapira AHV. Mitochondria and degenerative disorders, Am. J. Med. Genet. 2001; 106: 27-36.

Raha S and Robinson BH. Mitochondria, oxygen free radicals and apoptosis, Am. J. Med. Genet. 2001; 106: 62-70.

Shin JH, Kang, BY, Lee KH, Lee CC and Kim KT. Association between genetic variation in the human factor VII gene and essential hypertension in Korean population. Environ, Mutagens Carcinogens 2001; 21: 106-112.

Shoji M, Tsutaya S, Kasai T and Yasujima M. Implication of single nucleotide polymorphisms in association study: mitochondrial variations as another genetic markers for hypertension, Rinsho. Byori. 2002; 50: 497-501.

Soma M, Nakayama T and Katuo K. Nitric oxide synthase gene polymorphism and its influence on cardiovascular disease, Curr. Opin. Nephrol. Hypertens. 1999; 8: 83-87.

Tanaka M, Gong J-S, Zhang J, Yoneda M and Yagi K. Mitochondrial genotype associated with longevity, Lancet 1998; 351: 185-186.

Thorburn DR and Dahl H-HM. Mitochondrial disorders: genetics, counseling, prenatal diagnosis and reproductive options, Am. J. Med. Genet. 2001; 106: 102-114.

Ward R, Familial aggregation and genetic epidemiology of blood pressure. In: Laragh JH, Brenner BM (eds). Hypertension, Pathophysiology, Diagnosis and Management. Raven Press: New York, 1990; 81-100.