Genetic Variation in the Renin-Angiotensin System and Response to Endurance Training

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Key Words

Renin-angiotensin system · Physical training · Polymorphism

Abstract

Objective: To investigate the relationship between genetic variation in the renin-angiotensin system and the effect of 12-week endurance training in Korean women. **Materials and Methods:** Seventeen women who participated in an endurance training program for 12 weeks were genotyped for the angiotensinogen M235T polymorphism, angiotensin II type 1 receptor A1166C polymorphism, angiotensin-converting enzyme (ACE) T-3892C polymorphism, and angiotensin II type 2 receptor C3123A polymorphism. The following clinical parameters were measured before and after the endurance training program: blood pressure, body composition, ventilatory response, total cholesterol, triglyceride, and glucose. **Results:** Of the genetic markers investigated, the frequency of the T allele for the ACE T-3892C polymorphism was significantly associated with the response in body mass index and VO_{2max} after 12 weeks of endurance training ($p < 0.05$). None of the other polymorphisms were significantly associated with the effect of training. **Conclusion:** The significant association between ACE T-3892C and the change in body mass index and VO_{2max} in Korean women are attributed to training, suggesting that this genetic variation is a useful genetic marker for clarifying the interindividual response to endurance training. Copyright © 2007 S. Karger AG, Basel

Introduction

Data from many epidemiological studies have shown that endurance training has protective effects against cardiovascular risk factors in human health. Nevertheless, there is large variation in the physiological responses of participants when they perform the same endurance training. There is reasonable agreement regarding the suggestion that this variation in response is genetically mediated, at least in part. For example, Hagberg et al. [1] reported that systolic blood pressure (SBP) decreased with training in subjects who had the II or ID genotype of the angiotensin I-converting enzyme (ACE) gene or the E3 or E4 genotype of the apolipoprotein E gene. Recently, Delmonico et al. [2] also reported that resting blood pressure was reduced to a greater extent in C-allele carriers of angiotensinogen (AGT) A-20C and angiotensin II type 1 receptor (AT₁R) A1166C than in A homozygotes in response to strength training.

The renin-angiotensin system (RAS) has an important role in blood pressure homeostasis, and the component genes of the RAS include those encoding AGT, ACE, AT₁R, and the angiotensin II type 2 receptor (AT₂R). Angiotensin I is produced from AGT by renin, and it is subsequently converted into angiotensin II by ACE. Angiotensin II increases blood pressure by causing vasoconstriction, aldosterone secretion, and sodium/water reabsorption in the kidney. The cellular effects of angiotensin II are mediated by two structurally distinct receptor sub-

Table 1. Primer sequences used for PCR amplification of the candidate genes

Gene	Polymorphic site	Primer sequence	T _A °C ^a	Fragment size, bp	RE ^b	Reference
AGT	M235T	5'-CAGGGTGTCTGTCCACACTGGACCCC-3' 5'-CCCTTTGTGCAGGGCCTGGCTCTCT-3'	53	141/24	<i>Thl</i> III	7
ACE	T-3892C	5'-ATAGTGTATATAGGGCTTGGTAC-3' 5'-GAAGATATTTGCAAAGTATGTACTG-3'	55	114/90	<i>Pst</i> I	8
AT ₁ R	A1166C	5'-ATAATGTAAGCTCATCCACCAAGAAG-3' 5'-TCTCCTTCAATTCTGAAAAGTACTTAA-3'	50	139/27	<i>Afl</i> II	9
AT ₂ R	C3123A	5'-GGATTTCAGATTCTCTTTTGAA-3' 5'-GCATAGGAGTATGATTTAATC-3'	53	214/107	<i>Alu</i> I	10

^a Annealing temperature for PCR.^b Restriction endonuclease.

types, AT₁R and AT₂R. With respect to the ACE gene, several studies have reported that the I allele is associated with enhanced improvement in cardiovascular risk factors after endurance training and is more common in endurance athletes than in sedentary controls [3]. The I allele has been associated with improved capacity to perform repetitive elbow flexion after a physical training program [4]. The AGT M235T polymorphism is reported to be associated with the variability in left ventricular hypertrophy induced by endurance training, with athletes homozygous for the T allele having the largest hearts [5]. Until now, such studies have mainly examined Caucasian populations, and no study has attempted to correlate the genetic polymorphisms of the RAS genes with various physiological effects before and after endurance training in an ethnically homogeneous Korean population.

The association between the improvement in cardiovascular risk factors after endurance training and the presence of each of four common allelic polymorphisms in the RAS, namely ACE T-3892C, AGT M235T, AT₁R A1166C, and AT₂R C3123A, was investigated. The last two were included to determine whether these polymorphisms might be associated with the effects of an endurance training program.

Materials and Methods

Study Subjects

Seventeen unrelated individuals (age: 53 ± 6.52 years) from Seoul Hygiene Hospital, Seoul, Korea, were selected to participate in a 12-week endurance training program. None of the participants had a history of hypertension, coronary artery disease, diabetes mellitus, or obesity.

Endurance Exercise Training Program

During the first 2 weeks, training was carried out at a heart rate that corresponded to 55% of the baseline VO_{2max} for 30 min per session. The duration and intensity of the training sessions were gradually increased to 50 min and 75% of the heart rate associated with the baseline VO_{2max}, which were then sustained until the last week. The average training frequency was three times per week, and all training was performed on cycle ergometers under supervision in the laboratory [6].

Determination of Anthropometric and Biochemical Parameters

Blood samples were obtained in EDTA-tubes from subjects who had fasted for 12–16 h. A sphygmomanometer was used to measure systolic and diastolic blood pressures (SBP and DBP, respectively). The mean arterial pressure was calculated as [DBP-1/3(SBP - DBP)] mm Hg. The VO_{2max} was measured during motor-driven treadmill exercise tests. The body mass index (BMI) was calculated by dividing the body weight (kg) by the square of the height (m²). The concentrations of total cholesterol (TCH) and triglyceride (TG) were measured by enzymatic colorimetric methods using a commercial kit (Boehringer Mannheim, Germany) and a chemistry analyzer.

Genotype Analysis of the Four RAS Polymorphisms

Genomic DNA was isolated from buffy coats prepared from blood (5 ml). Polymerase chain reaction (PCR)-restriction fragment length polymorphism techniques were used to examine the AGT M235T, ACE T-3892C, AT₁R A1166C, and AT₂R C3123A gene polymorphisms. Briefly, each 50-μl reaction mixture contained 200–400 ng of extracted genomic DNA, 100 ng of each primer, 200 μM of each of four dNTPs, and the reaction buffer recommended by the manufacturer. The primer sequences, annealing temperatures for the PCR reaction, fragment sizes, and restriction endonucleases used for the four polymorphisms are shown in table 1. Each PCR reaction was performed in 50 μl containing 0.1 mM each of dCTP, dATP, dGTP, and dTTP; 10 pmol of each oligonucleotide primer; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM MgCl₂; 0.01% gelatin; 1.25 units of Taq DNA poly-

Table 2. Clinical characteristics of the study subjects at baseline and after a 12-week endurance training program

Variable	Baseline (mean $\pm$ SD)	12 weeks (mean $\pm$ SD)	t value	Probability
SBP, mm Hg	60.7 $\pm$ 10.2	59.3 $\pm$ 9.9	3.862	0.001*
DBP, mm Hg	80.2 $\pm$ 9.9	76.3 $\pm$ 8.7	2.104	0.051
Weight, kg	60.7 $\pm$ 10.0	59.3 $\pm$ 9.9	3.722	0.002*
BMI	25.6 $\pm$ 4.5	25.0 $\pm$ 4.3	3.877	0.001*
VO _{2max} , ml/kg/min	24.1 $\pm$ 4.6	27.7 $\pm$ 4.5	-5.179	0.000*
TG, mg/dl	163.7 $\pm$ 43.3	110.5 $\pm$ 29.6	5.672	0.000*
TCH, mg/dl	218.1 $\pm$ 57.6	194.1 $\pm$ 38.6	2.042	0.057
Glucose, mg/dl	115.9 $\pm$ 32.1	101.8 $\pm$ 27.3	2.812	0.012*

* p < 0.05.

Table 3. Clinical characteristics at baseline and in response to a 12-week endurance training program according to the genotype of the ACE T-3925C polymorphism

Variable	Genotypes	
	TT (5)	TC+CC (12)
SBP, mm Hg		
Baseline	126.8 $\pm$ 17.9	133.4 $\pm$ 7.0
Response	-4.8 $\pm$ 7.0	-9.6 $\pm$ 10.4
DBP, mmHg		
Baseline	77.2 $\pm$ 8.8	81.0 $\pm$ 10.8
Response	-1.0 $\pm$ 7.9	-3.4 $\pm$ 5.6
Weight, kg		
Baseline	56.8 $\pm$ 6.1	63.1 $\pm$ 11.0
Response	-0.3 $\pm$ 2.5	-1.9 $\pm$ 1.0
BMI		
Baseline	22.9 $\pm$ 3.6	27.1 $\pm$ 4.4
Response*	0.1 $\pm$ 1.0	0.9 $\pm$ 0.4
VO _{2max} , ml/kg/min		
Baseline	25.1 $\pm$ 5.8	23.8 $\pm$ 4.4
Response*	6.1 $\pm$ 3.5	2.6 $\pm$ 2.3
TG, mg/dl		
Baseline	160.6 $\pm$ 37.5	161.4 $\pm$ 50.2
Response	-59.4 $\pm$ 50.2	-51.3 $\pm$ 38.8
TCH, mg/dl		
Baseline	174.4 $\pm$ 90.2	237.3 $\pm$ 29.7
Response	10.8 $\pm$ 82.7	-36.9 $\pm$ 24.3
Glucose, mg/dl		
Baseline	122.8 $\pm$ 40.0	110.6 $\pm$ 29.7
Response	-18.6 $\pm$ 33.2	-12.8 $\pm$ 16.7

Values are expressed as mean $\pm$ SD. * p < 0.05.

Baseline signifies value before starting the 12-week endurance training. Response signifies difference between the pre- and post-training values.

merase (Perkin-Elmer/Cetus), and 100 ng genomic DNA. Amplification was carried out for 30 cycles of denaturation at 94°C for 1 min, annealing at the temperatures listed in table 1 for 1 min, and extension at 72°C for 1 min in a PTC-200 thermocycler (MJ Research, USA). After the PCR reaction, about 10 μ l of each PCR product was digested using the appropriate restriction enzyme, and the genotype was determined from ethidium bromide-stained agarose gels or polyacrylamide gels after electrophoresis. For the AGT gene, the M allele has a 165-bp PCR product, and the T allele has 141- and 24-bp fragments. For the ACE gene, the T allele has a 204-bp band, and the C allele has 114- and 90-bp fragments. For the AT₁R gene, the A allele has a 166-bp product, and the C allele has 129- and 27-bp fragments. For the AT₂R gene, the C allele has a 321-bp fragment, and the A allele has 214- and 107-bp fragments.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows version 11.0 (SPSS, Chicago, Ill., USA). The significance of differences in allele frequencies between the study groups was estimated using the χ^2 test. One-way ANOVA was used to compare the mean values of biochemical parameters among different genotypes. Allele frequencies were estimated using the gene counting method. Deviation in the genotype distribution from that expected for Hardy-Weinberg equilibrium was estimated using the χ^2 -fit test. An independent t test was used to compare baseline values and the responses to exercise training between genotype groups. Paired t tests were used to assess the significance of exercise training-induced changes in all variables within genotype groups. A p value of less than 0.05 was considered significant.

Results

The clinical characteristics of the study subjects at baseline and the response after finishing the 12-week endurance training program are shown in table 2. SBP, weight, BMI, VO_{2max}, TG, and glucose were significantly different after 12 weeks of endurance training (p < 0.05). Of these parameters, VO_{2max} was increased, whereas the

others were reduced. The observed genotype distribution of the four polymorphisms did not deviate from Hardy-Weinberg equilibrium. For the ACE T-3892C polymorphism, the observed genotype frequencies of TT, TC, and CC were 29.4, 58.8 and 11.8%, respectively, and the frequency of the T allele was 0.59. For the AT₂R C3123A polymorphism, the observed genotype frequencies of CC, CA, and AA were 50.0, 33.3 and 16.7%, respectively, and the frequency of the C allele was 0.68. Both the AGT M235T and ACE T-3892C polymorphisms showed monomorphic patterns. The AGT M235T polymorphism only showed the T allele, and the AT₁R C3123A polymorphism was always the A allele (data not shown). The response of the clinical parameters in the presence of the ACE T-3892T polymorphism after the 12-week endurance training program are shown in table 3. The T-3892C polymorphism of the ACE gene was significantly associated with the VO_{2max} and BMI responses. The TG concentration was reduced after training, but this was not statistically significant. There were no significant associations between the AT₂R C3123A polymorphism and any parameter after the 12-week endurance training program.

Discussion

Although there have been many reports on the association between the genetic variations in the RAS genes and physical performance and cardiovascular disease in many populations [3–5, 11–16], there are few reports on the effects of these genetic polymorphisms on the improvement of cardiovascular risk through endurance training [1, 2]. Nevertheless, several reports documented that genetic polymorphisms in the RAS genes, especially the ACE gene, were significantly associated with endurance performance in several populations. Gayagay et al. [17] first reported an association between I/D polymorphism in the ACE gene and athletic performance in an Australian population. However, other studies reported conflicting results about this issue among populations studied [3]. In this regard, our study investigated the effect of four polymorphisms of RAS genes on clinical parameters after a 12-week endurance training scheme in 17 Korean women. Of the genetic polymorphisms studied, we found a significant association between the ACE T-3892C polymorphism and the responses of VO_{2max} and BMI. Therefore, the ACE T-3892C polymorphism might modify the effect of endurance training on these parameters. Specifically, subjects with the TT genotype had a

greater increase in VO_{2max} than subjects with the TC or CC genotype. In addition, those with the TT genotype had a greater reduction in BMI than those with the TC or CC genotype. Therefore, the effect of endurance training might be maximally improved in women with the TT genotype. The molecular mechanism of these observations is unclear, but the effect might be the result of linkage disequilibrium between this polymorphic site and an unidentified functional allele. A promoter assay might clarify the function of this polymorphism. There was no association between the AT₂R C3123A polymorphism and the clinical parameters. However, our study must be regarded as preliminary because of the small sample size. Future studies with more subjects are needed to clarify the interindividual susceptibility of the ACE T-3892C marker on improving cardiovascular risk through endurance training.

Conclusion

The presence of both the ACE T-3892C and AT₂R C3123A polymorphisms resulted in reduced SBP, DBP, weight, BMI, TG, TCH and glucose, and increased VO_{2max} after endurance training. Specifically, the ACE T-3892C polymorphism was significantly associated with the changes in both VO_{2max} and BMI after a 12-week endurance training program.

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