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RAS genes influence exercise-induced left ventricular hypertrophy: an elite athletes study

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ABSTRACT

FATINI, C., R. GUAZZELLI, P. MANETTI, B. BATTAGLINI, F. GENSINI, R. VONO, L. TONCELLI, P. ZILLI, A. CAPALBO, R. ABBATE, G. F. GENSINI, and G. GALANTI. RAS genes influence exercise-induced left ventricular hypertrophy: an elite athletes study. *Med. Sci. Sports Exerc.*, Vol. 32, No. 11, pp. 1868–1872, 2000. **Purpose:** The association of ACE I/D polymorphism with changes in LV mass in response to physical training has been observed, but no association has been found with AT1R A1166C polymorphism. We investigated the ACE I/D, AT1R A1166C, and AT1R CA microsatellite polymorphisms genotype distribution in elite athletes and whether the presence of AT1R C1166 variant, in addition to ACE D allele affects the training-induced LV mass alterations in elite trained athletes. **Methods:** The study population comprised 28 healthy players recruited from an Italian elite male soccer team and 155 healthy male subjects. LV mass, LV mass adjusted for body surface area, septal thickness, posterior wall, end-diastolic and end-systolic ventricular dimension, and ejection fraction were determined by echocardiography in pretrained period, at rest and 7 months later during the training. All subjects were genotyped for ACE I/D, AT1R A1166C, and CA microsatellite polymorphisms. **Results:** Training induced an LV mass increase in all but six athletes. The percentage of athletes in whom an increase of LV mass was found after training was statistically different in relation to the ACE D allele: no increase was observed in three of 24 D allele carriers and in three of four II genotype players (Fisher's exact test, $P = 0.02$). As AT1R is concerned, no increase was observed in 4 of 15 C allele carriers and in 2 of 13 AA genotype athletes (Fisher's exact test, $P > 0.05$). The contemporary presence of ACE D and AT1R C allele did not affect the changes after training. No difference has been observed in the CA microsatellite marker allele frequencies between athletes and controls ($P = 0.46$). **Conclusion:** In this study, we provide the evidence that soccer play does not select athletes on genotype basis. Training-induced LV mass changes in male elite athletes are significantly associated with the presence of ACE D allele, but not of AT1R C allele. **Key Words:** RAS POLYMORPHISMS, LEFT VENTRICULAR MASS

The endocrine renin angiotensin system (RAS) plays an important role in controlling the circulatory system. Genes encoding components of RAS regulate angiotensin-converting enzyme (ACE) levels, as well as angiotensin II expression and function. In intron 16 of the ACE gene, a polymorphism consisting in the presence (insertion) or absence (deletion) of the 287-bp Alu sequence has been detected. This insertion/deletion (I/D) polymorphism accounted for 47% of the total phenotypic serum ACE variance (12). The D allele of ACE gene I/D polymorphism is associated with high ACE levels in ventricular tissue as well as in the circulation (12). As the presence of each allele had an additive effect on serum ACE and two alleles had twice the effect of one, each allele was codominant. High ACE levels could determine their effects through changes either in angiotensin II levels or in kinin degradation. The I/D polymorphism is associated also with concentric left ventricular (LV) remodeling in hypertension (4), cardiomyopathy (11), and LV hypertrophy (6). The association of ACE I/D polymorphism with change in LV mass in response to physical training has been observed in male subjects recruited in the Army Training Regiment Bassingbourn (7). Moreover, ACE II genotype has been found to be associated with improved endurance in a limited group of climbers (9). The final effector of RAS is angiotensin II, a highly vasoactive and aldosterone-stimulating octapeptide, which may modulate or induce the growth of vascular smooth muscle cells (SMC) and cardiomyocytes. In LV tissue, angiotensin II paracrine and autocrine action modulates myocardial growth through the angiotensin II type 1 receptor (AT1R) (4). ACE is a zinc metallo protease that inactivates bradykinin by cleaving two carboxy-terminal dipeptides and suppressing its biological effects. Bradykinin is a potent vasodilator and inhibitor of SMC proliferation,

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TABLE 1. Genotype distribution and allele frequency in athletes and controls.

	Athletes (N = 28)	Controls (N = 155)	P
ACE genotype			
II	4 (14%)	20 (13%)	
ID	12 (43%)	74 (48%)	
DD	12 (43%)	61 (39%)	
χ^2 = 0.23			0.89
ACE allele frequency			
I	36%	37%	
D	64%	63%	
χ^2 = 0.02			0.88
AT1R genotype			
CC	2 (8%)	9 (6%)	
AC	13 (46%)	70 (45%)	
AA	13 (46%)	76 (49%)	
χ^2 = 0.11			0.94
AT1R allele frequency			
C	31%	28%	
A	69%	72%	
χ^2 = 0.09			0.76
ACE DD+ID with AT1R AC+CC	11 (46%)	63 (47%)	
ACE DD+ID with AT1R AA	13 (54%)	72 (53%)	0.94

which could act through the release of endothelial factors, including nitric oxide and prostacyclin (16). In the AT1R gene, within the 3′ untranslated region, a polymorphism consisting in a transversion A→C in position 1166 (A1166C) has been identified; this C1166 variant has been associated with hypertension in humans (1), but no association between AT1R A1166C polymorphism and exercise-induced LV changes has been observed (8). In the 3′ flanking region of the AT1R gene, a fragment comprising highly informative dinucleotide repeat (CA) was established (2). A total of 10 alleles were detected (ranging in size from 132 to 146 bp).

Few studies evaluated the role of this highly polymorphic microsatellite located near the human AT1R gene[1] in human essential hypertension and in athletic performance (5). The aims of this study were to investigate: 1) the ACE and AT1R genotype and AT1R gene microsatellite markers distribution in elite athletes; and 2) whether the presence of AT1R C1166 variant, in addition to ACE D allele, affects the LV mass training-induced alterations in elite trained athletes.

METHODS

Subjects. Twenty-eight healthy players recruited from an Italian elite male soccer team aged between 17 and 29 yr (mean 20.2 ± 4.4) were studied during pretraining period and after seven months of training (36 h after the last training section). As controls, we studied 155 healthy male subjects (mean age 23 ± 2.3), untrained students of the Medical School of the University of Florence. In 28 control subjects, echocardiographic study was also performed. All the subjects were Caucasian, nonconsanguineous, and gave informed consent to genotyping.

Echocardiographic methods. Mono-two-dimensional echocardiographic examinations were performed using an Esaote device (AU3–3.5 2.5 MHz probes Esaote Biomedica Florence, Italy).

Parasternal long axis, short axis, apical four chambers with aorta and when necessary subcostal views were used. The interventricular septum and posterior wall thickness, systolic and diastolic LV dimensions and ejection fraction were measured, by means of leading edge technique, with monodimensional echocardiography under two dimensional guide (15).

Measurements were performed on four to six cycles by means of a computer-aided method and averaged. Recordings were made entirely on videotape and evaluated by two expert echocardiographers. The intraobserver and interobserver variability for repeated measurements was assessed by unpaired Student's *t*-test.

LV mass, according to Devereux formula (3), and LV mass index corrected for the body surface area were calculated. Ejection fraction was estimated by formula of Teicholz et al. (18).

DNA analysis. Genomic DNA was extracted from peripheral blood and ACE I/D polymorphism was determined by primers and PCR conditions previously described by Rigat et al. (14). DNA was amplified in presence of 5% dimethylsulfoxide (DMSO) in the reaction mixture at an annealing temperature of 60°C, for reducing the incidence of mistyping ID as DD. Fragments of ≈190 bp (deletion allele) and ≈490 bp (insertion allele) were separated on a 2% agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator. Moreover, each DD genotype was subjected to a second PCR amplification without the 5% DMSO at an annealing temperature of 67°C and using a primer pair that recognizes an insertion-specific sequence, to reduce underestimation of heterozygotes. The AT1R C1166 variant was analyzed by PCR and restriction fragment length polymorphism (RFLP) analysis, using primers and PCR conditions as described by Katsuja et al.

TABLE 2. LV mass, LV mass index, septal thickness, posterior wall, and end-diastolic dimension in controls and in athletes before and after training.

			Athletes (N = 28)		
	Controls (N = 28)	P^a (Unpaired t-Test)	Pretraining	P^b (Paired t-Test)	Post-Training
LV mass (g)	172.5 ± 35.6	0.0039	206.03 ± 36.7	0.002	229.4 ± 47.2
LV mass index (g·m^{-2})	95.1 ± 18.4	0.00009	109.5 ± 17.6	0.004	120.8 ± 22.8
Septal thickness (mm)	8.7 ± 1.0	0.08	9.2 ± 0.8	0.009	9.8 ± 1.1
Posterior wall (mm)	8.7 ± 0.8	0.017	9.2 ± 0.7	0.82	9.3 ± 1.1
End-diastolic dimension (mm)	49.5 ± 4.1	0.016	52.2 ± 3.8	0.03	53.7 ± 3.4
End-systolic dimension (mm)	30.3 ± 3.9	0.37	31.2 ± 4.1	0.19	32.2 ± 3.1
Ejection fraction (%)	68 ± 5.1	0.65	69.5 ± 6.1	0.86	69.7 ± 5.7

[a] P controls vs pretraining athletes.
[b] Pretraining vs post-training.
Values are mean ± SD.

TABLE 3. LV mass, LV mass index, end-systolic dimension, and ejection fraction before and after training in athletes in relation to ACE and AT1R genotype.

	Pretraining ($N = 28$)	Posttraining ($N = 28$)	Change	P (Paired t-Test)
LV mass (g)				
All	206 ± 36.7	229.5 ± 47.2		0.002
ACE genotype				
II	201.5 ± 7.5	195.5 ± 30.3	−6	0.71
ID+DD	206.8 ± 39.6	235.1 ± 47.6	28.3	<0.0001
AT1R genotype				
AA	207.7 ± 36.4	230.7 ± 42.6		0.0002
AC+CC	204.5 ± 38.1	228.3 ± 52.4		0.04
DD+ID/AA	208.2 ± 35.2	230.8 ± 42.6		0.002
DD+ID/AC+CC	205.6 ± 44.9	240.3 ± 54.5		0.005
LV mass index ($g \cdot m^{-2}$)				
All	109.5 ± 17.6	120.8 ± 22.8		0.005
ACE genotype				
II	107.8 ± 5.1	104.2 ± 15.2	−3.5	0.71
ID+DD	109.8 ± 18.7	123.8 ± 22.6	11.9	0.005
AT1R genotype				
AA	111.3 ± 19.2	122.7 ± 22.6		0.0007
AC+CC	108.0 ± 16.7	119.5 ± 22.7		0.06
DD+ID/AA	114.1 ± 19.2	122.8 ± 22.6		0.0008
DD+ID/AC+CC	108.7 ± 19.8	126.8 ± 23.4		0.006
End-systolic dimension				
All	31.2 ± 4.1	32.2 ± 3.1		0.19
ACE genotype				
II	31.6 ± 3.2	33.9 ± 3.02	−2.4	0.48
ID+DD	31.2 ± 4.3	31.9 ± 3.1	−0.7	0.31
AT1R genotype				
AA	31.4 ± 4.7	32.1 ± 3.3		0.50
AC+CC	31.1 ± 3.7	32.3 ± 3.1		0.49
DD+ID/AA	31.4 ± 4.7	32.1 ± 3.3		0.49
DD+ID/AC+CC	30.8 ± 3.9	31.7 ± 2.9		0.49
Ejection fraction				
All	69.5 ± 6.1	69.7 ± 5.7		0.86
ACE genotype				
II	68.2 ± 4.4	65.7 ± 5.7	−2.5	0.6
ID+DD	69.7 ± 6.4	70.4 ± 5.5	−0.7	0.65
AT1R genotype				
AA	69.6 ± 6.5	69.8 ± 5.9		0.94
AC+CC	69.3 ± 5.9	69.7 ± 5.6		0.57
DD+ID/AA	69.6 ± 6.5	69.8 ± 5.9		0.94
DD+ID/AC+CC	69.7 ± 6.6	71.1 ± 5.05		0.57

Values are mean ± SD.

(9). PCR products were digested by 5 U Dde I at 37°C for 2 h, and the digestion products were separated on 2% agarose gel and visualized by ethidium bromide. The C1166 variant was identified if digestion of the PCR products was observed.

Genotyping of a dinucleotide repeat (CA alleles) at the AT1R locus was performed by primers and PCR conditions as described by Davies et al. (2). The ATCA1 primer was end-labeled with (γ^{32} P) dATP using T4 polynucleotide kinase. Amplification by PCR was carried out in a total volume of 25 μL. A thermal cycler was used for 30 cycles of 30 s at 95°C and 20 s at 56°C. The length of each allele was determined on a 6% denaturing polyacrylamide gel.

Statistical analysis. Allele frequencies were estimated with the gene counting method. A chi-square test was used to assess the fit of the observed allele frequencies to the agreement of the Hardy-Weinberg equilibrium and the difference in genotype distribution. Differences between pre- and post-training measurements of LV dimensions and mass were calculated for each subject. Data were analyzed for the study population as a whole, and differences in response between different genotype groups were compared. Pre- and post-training data were compared using two-tailed paired *t*-test. Fisher's exact test was used to evaluate whether LV mass and LV mass index after training were associated to ACE and AT1R genotypes. Values of $P < 0.05$ were considered to be statistically significant. Paired and unpaired *t*-tests were used as appropriate.

RESULTS

Genotype distribution and allele frequency in athletes were comparable to those found in controls (Table 1). Pretraining LV mass was significantly higher in athletes respect to the controls (Table 2).

The training induced LV mass increase in 22 of 28 athletes. The changes in LV mass, LV mass index, septal thickness, posterior wall, and end-diastolic dimension after training are shown in Table 2. A significant difference between pre- and post-training in LV mass, LV mass index, septal thickness, and end-diastolic dimension, but not in posterior wall, end-systolic dimension, and LV function in relation to ACE genotype, was found (Tables 3 and 4). A significant difference between pre- and post-training in relation to AT1R genotype was found only in LV mass and LV mass index (Tables 3 and 4). The entity of changes after training was statistically different among ACE D allele carriers in LV mass, LV mass index, septal thickness, and end-diastolic dimension (Table 3 and 4). The evaluation of mean of difference between ACE II and ACE D allele carriers was statistically significant in LV mass (unpaired

TABLE 4. Septal thickness, posterior wall, and end-diastolic dimension in relation to ACE and AT1R genotype.

	Pretraining ($N = 28$)	Posttraining ($N = 28$)	Change	P (Paired t-Test)
Septal thickness (cm)				
All	9.2 ± 0.8	9.8 ± 1.1		0.81
ACE genotype				
II	9.1 ± 0.4	8.6 ± 0.5	−0.4	0.07
ID+DD	9.2 ± 0.8	10.0 ± 1.2	0.79	0.004
AT1R genotype				
AA	9.2 ± 0.9	9.9 ± 1		0.09
AC+CC	9.1 ± 0.7	9.7 ± 1.3		0.06
DD+ID/AA	9.2 ± 0.9	9.9 ± 1		0.09
DD+ID/AC+CC	9.1 ± 0.8	10.1 ± 1		0.014
Posterior wall (cm)				
All	9.2 ± 0.7	9.3 ± 1.1		0.81
ACE genotype				
II	9.2 ± 0.9	8.5 ± 0.9	−0.7	0.15
ID+DD	9.2 ± 0.7	9.4 ± 1	−0.2	0.44
AT1R genotype				
AA	9.3 ± 0.7	9.2 ± 1		0.90
AC+CC	9.2 ± 0.8	9.3 ± 1.3		0.86
DD+ID/AA	9.3 ± 0.7	9.2 ± 1		0.90
DD+ID/AC+CC	9.2 ± 0.8	9.6 ± 1.2		0.20
End-diastolic dimension				
All	52.2 ± 3.8	53.7 ± 3.4		0.08
ACE genotype				
II	52.2 ± 2.6	53.6 ± 2.9	−1.3	0.64
ID+DD	52.1 ± 3.9	53.7 ± 3.5	1.6	0.04
AT1R genotype				
AA	52.1 ± 4.3	53.7 ± 3.6		0.14
AC+CC	52.3 ± 3.7	53.9 ± 3.4		0.21
DD+ID/AA	52.1 ± 4.3	53.7 ± 3.6		0.13
DD+ID/AC+CC	52.2 ± 3.9	53.9 ± 3.4		0.16

Values are mean ± SD.

TABLE 5. Mean of differences between ACE II and ID+DD genotypes (unpaired t-test).

	II	ID+DD	*P*
LV mass			
ACE genotype	−6 ± 14.9	28.3 ± 5.2	0.02
LV mass index			
ACE genotype	−3.5 ± 8.7	11.9 ± 3.8	0.13
Septal thickness			
ACE genotype	−0.37 ± 0.1	0.79 ± 0.2	0.07
Posterior wall			
ACE genotype	−0.2 ± 0.5	0.17 ± 0.2	0.52
End-diastolic dimension			
ACE genotype	1.37 ± 2.6	1.36 ± 0.7	0.99
End-systolic dimension			
ACE genotype	2.37 ± 2.9	0.60 ± 0.7	0.42
Ejection fraction			
ACE genotype	−2.5 ± 4.3	0.71 ± 1.5	0.44

Values are mean ± SD.

t-test) (Table 5). The percentage of athletes in whom an increase of LV mass and LV mass index after training was found was significantly higher (Fisher's exact test, $P = 0.02$) in ACE DD and ID genotype in comparison with ACE II genotype: an increase was observed in 21 of 24 DD+ID, and in one of four II genotype carriers (Fig. 1).

As AT1R genotype is concerned, no increase was observed in 4 of 15 AC+CC and in 2 of 13 AA genotype carriers (Fisher's exact test = 0.64). In athletes with the contemporary presence of ACE D and AT1R C allele, the increase in LV mass after the training period was more elevated, although it did not reach the statistical significance ($P = 0.063$) in comparison with DD+ID/AA athletes. The genotype distribution and allele frequency were in agreement with the Hardy-Weinberg equilibrium.

No significant difference in the distribution of the AT1R gene microsatellite marker frequencies between athletes and controls has been found ($\chi^2 = 7.703$, $P = 0.46$) (Fig. 2).

DISCUSSION

This is the first study investigating RAS genes in athletes, which suggests that the soccer play does not select athletes on a genotypic basis. In fact, our data show that ACE and AT1R genotype distribution and allele frequency observed in elite athletes are comparable to those observed in the general population. These results seem at variance with the findings obtained in high altitude mountaineers in whom the observed high percentage of II genotype has been implicated in the increase of the cardiac output and muscle capillary density (10). However, these two physical performances are quite dissimilar.

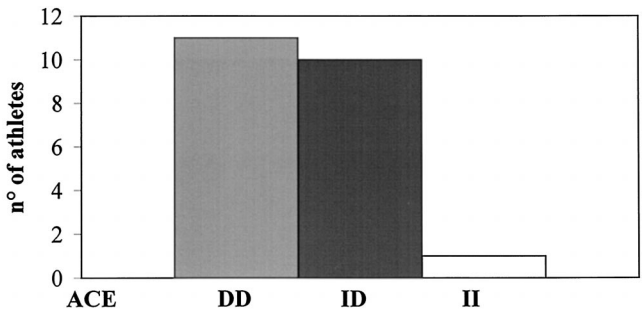

Figure 1—Athletes with training-induced left ventricular mass increase in relation to ACE genotype.

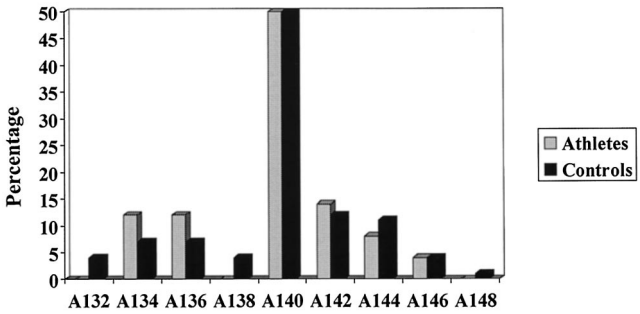

Figure 2—AT1R gene microsatellite markers allele frequencies.

This report indicates that ACE genotype influences the exercise-induced ventricular growth in elite athletes, as previously found in untrained young male subjects (11). The role of the ACE I/D polymorphism on the LV growth may be mediated through ACE via alterations in tissue kinin metabolism or effects on angiotensin II synthesis. Increased cardiac ACE activity has been associated with increased local generation of angiotensin II when isolated hypertrophied rat hearts were perfused with angiotensin I (17), and increased angiotensin II levels have been associated with ACE D allele (13). However, ACE is not the only rate-limiting step in basal angiotensin II generation; in fact, a discovered non-ACE chymase peptidase, found in the cardiac membrane preparations from human hearts, has been shown to generate angiotensin II from angiotensin I in an *in vitro* system (19). Angiotensin II might become a rate-limiting step in hypertrophic stimulation and ACE genotype might be correlated with physiological and pathological hypertrophy. The exercise-related LV growth is associated with improved myocardial function and the ACE association with physiological hypertrophy is consistent with a role for paracrine renin-angiotensin system in the control of LV growth.

Regarding to the association between AT1R genotypes and LV hypertrophy, we found no effect from AT1R alleles on exercise-induced ventricular mass; however, our results, although not statistically significant due to the sample size, suggest that the contemporary presence of ACE D and AT1R C alleles affects the exercise-related LV hypertrophic response. This study did not identify the AT1R CA microsatellite as a genetic marker for soccer play, according to Gayagay et al. (5)

Larger studies addressed not only to ACE, but also to AT1R polymorphisms, which mediate the angiotensin II effects, may offer new insights in the knowledge of the mechanism involved in the physiology and pathophysiology of myocardial exercise-induced hypertrophy.

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