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Renal Function Dependent Association of AGTR1 Polymorphism (A1166C) and Electrocardiographic Left-Ventricular Hypertrophy

Tom D.J. Smilde, Mike W. Zuurman, Hans L. Hillege, Dirk J. van Veldhuisen, Wiek H. van Gilst, Gerrit van der Steege, Adriaan A. Voors, Jan A. Kors, Paul E. de Jong, and Gerjan Navis

Background: The association of renin-angiotensin system (RAS) polymorphisms and left-ventricular hypertrophy (LVH) may depend on the presence of risk factors for LVH, such as renal dysfunction. We studied whether renal function modulates the association between RAS polymorphisms and LVH in a cross-sectional study of 8592 inhabitants of Groningen.

Methods: Left-ventricular hypertrophy was determined with electrocardiograms, using the Cornell voltage-duration product. The following RAS polymorphisms were determined: angiotensin II type-1 receptor (AGTR1 A1166C), angiotensin-converting enzyme (ACE) insertion/deletion (I/D), and angiotensinogen (AGT G-6A). The AGTR1 A1166C and ACE I/D polymorphisms were in Hardy-Weinberg equilibrium.

Results: Electrocardiographic LVH was present in 417 (5.0%) subjects. Subjects with LVH were older (53 *v* 49 years) and overall had more cardiovascular risk factors. Using logistic regression, creatinine clearance interacted with the relationship between the AGTR1 A1166C polymorphism and LVH (β, −0.19; $P = .033$). In subjects with the CC genotype, in contrast to carriers of an A allele, the prevalence of LVH increased with more pronounced renal dysfunction. Creatinine clearance also interacted with the relationship between the ACE I/D polymorphism and LVH (β, 0.12; $P = .037$), although less strongly, and the other way around. Creatinine clearance did not influence the association between the AGT G-6A polymorphism and LVH (β, −0.006; $P = .491$).

Conclusions: In this population-based study, the AGTR1 A1166C polymorphism was associated with LVH, dependent on concomitant renal dysfunction. A weaker renal function dependent association between the ACE I/D polymorphism and LVH was also observed. Renal function should be taken into account as a relevant environmental factor for the pathogenetic effects of RAS polymorphisms. Am J Hypertens 2007;20:1097–1103

Key Words: Left-ventricular hypertrophy, renal function, renin angiotensin system, polymorphism, PREVEND.

Left-ventricular hypertrophy (LVH) is a complex trait, with the possible involvement of multiple genetic and environmental factors. The renin-angiotensin system (RAS) is thought to be associated with LVH, because in various conditions with an activated RAS, the prevalence of LVH is high.[1,2] The RAS polymorphisms were shown to modulate RAS activity or responsiveness in several experimental and human settings,[3,4] and might therefore be involved in LVH. In subjects with hypertension, the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism was associated with LVH,[5,6] although not uniformly so.[7] However, in the general population, this relationship has not yet been demonstrated, suggesting that in the absence of other risk factors for LVH, RAS polymorphisms in themselves are not sufficient to induce a detectable risk for

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From the Thorax Center, Department of Cardiology (TDJS, HLH, DJVV, AAV), Department of Nephrology (MWZ, PEdJ, GN), Department of Clinical Pharmacology (WHvG), and Department of Medical Genetics (GvdS), University Medical Center Groningen, Groningen, and Department of Medical Informatics (JAK), Erasmus Medical Center, Rotterdam, The Netherlands.

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Address correspondence and reprint requests to Dr. Tom D.J. Smilde, Department of Cardiology, University Medical Center Groningen, Hanzeplein 1, P.O. Box 30001, 9700 RB Groningen, The Netherlands; e-mail: t.d.j.smilde@thorax.umcg.nl

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LVH.[8] However, the influence of polymorphisms in the risk for LVH may be dependent on the concomitant presence of pathophysiologic factors promoting LVH. Accordingly, in the presence of a condition promoting LVH, RAS polymorphisms may gain relevance.[8]

We recently demonstrated an association between renal function impairment and LVH.[9] The RAS may well be involved in this relationship, because RAS activation is associated with renal function impairment,[10] and is also known to stimulate myocardial hypertrophy.[11] In this context genetic variability in the RAS might influence the prevalence of LVH. In particular, the presence of renal dysfunction might modulate a possible relationship between RAS polymorphisms and LVH. Studies in renal patients so far do not support such an association, but were limited by very small population sizes.[12] In the present study, we investigated whether the association of RAS polymorphisms, such as ACE-I/D, angiotensin II receptor-type I (AGTR1) A1166C, and angiotensinogen (AGT) G-6A, with LVH is dependent on concomitant renal function impairment in a large, population-based cohort.

Methods

Study Design and Population

This study was performed in subjects participating in the Prevention of Renal and Vascular ENd-stage Disease (PREVEND) Study. The PREVEND Study is a prospective investigation of the natural course of albuminuria, and its relationship to renal and cardiovascular disease, in a large cohort drawn from the general population. Details of the study protocol were described elsewhere.[9,13] In total, 8592 subjects form the PREVEND baseline cohort were enrolled.

All subjects filled out a questionnaire concerning demographics and cardiovascular and renal history. Anthropometric measurements were performed, as were blood-pressure measurements during 10 min on 2 days with an automatic Dinamap XL Model 9300 Series device (Johnson-Johnson Medical, Inc., Tampa, FL).[13] Fasting blood samples were taken from the subjects, and subjects collected twice 24-h urine. We excluded 152 subjects because of the presence of macroalbuminuria, to rule out overt nephropathy. Furthermore, 88 subjects were excluded because of missing electrocardiographic data. In total, 8352 subjects were eligible for analysis. All subjects gave written, informed consent. The local medical ethics committee approved the PREVEND Study, and the study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Laboratory Methods

Urinary volume was measured in each collection.[13] Urinary albumin concentrations were determined by nephelometry (Dade Behring Diagnostics, Marburg, Germany). Serum glucose, cholesterol, creatinine, and urine creatinine were determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY).

Genotyping of RAS Polymorphisms

The AGT G-6A (rs5051) single-nucleotide polymorphism was analyzed using dual-labeled TaqMan probes, as described earlier.[14] The AGTR1 A1166C polymorphism was analyzed using TaqMan-MGB probes and polymerase chain reaction (PCR) primers, designed through the Assay-by-Design service (Applied Biosystems, Nieuwenkerk a/d Ijssel, the Netherlands), and with the followingsequences: AGTR1 A1166C (rs5186), forward primer 5′-ACATTCCTCTGCAGCACTTCACT, reverse primer 5′CGGTTCAGTCCACATAATGCATT,A-alleleprobe5′-FAM-ACCAAATGAGCATTAG, and C-allele probe 5′-VIC-ACCAAATGAGCCTTAG.

The TaqMan assays were performed according to the manufacturer's recommendations on an ABI 7900HT apparatus, and results were analyzed with SDS 2.0 genotype-calling software (Applied Biosystems).

The ACE I/D polymorphism (rs4340) was analyzed by PCR amplification. One of the primers had a fluorescent label, and subsequent determination of the lengths of PCR products was performed with a capillary sequencer. Primers were picked with the aid of online Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The ACE insertion/deletion site was amplified with flanking primers 5′-FAM-TCTCCCATTTCTCTAGACCTGCT and 5′-CATCACATTCGTCAGATCTGGTA. As a control reaction for the I allele, PCR was performed with insertion-specific primer 5′-ACTACGCCCGGCTAATTTTT and primer 5′-FAM-TGCCCATAACAGGTCTTCATATT, annealing downstream from the insertion site. The first PCR results in products of 451 and 165 base pairs for the I allele and D allele, respectively. The second PCR produces a 301-base-pair product if the I allele is present in the DNA sample. After PCR cycling, samples of the ACE I/D assays were combined and separated on a MegaBACE 1000 sequencer. Fragments were analyzed with Genetic Profiler 2.0 software (Amersham Biosciences, Buckinghamshire, United Kingdom). The AGTR1 A1166C polymorphism and the ACE I/D polymorphism were in Hardy-Weinberg equilibrium, whereas the AGT G-6A polymorphism was not.

Definitions

Urinary albumin excretion was measured as the mean of two 24-h urine collections. Normoalbuminuria was defined as a urinary albumin excretion of $<$15 mg per 24 h, high normoalbuminuria as a urinary albumin excretion of 15 to 29.9 mg per 24 h, microalbuminuria as 30 to 300 mg per 24 h, and macroalbuminuria as a urinary albumin excretion of $>$300 mg per 24 h. Urinary sodium excretion was measured as the mean of two 24-h urine collections. Creatinine clearance (CrCl) was calculated as the mean of two 24-h urine creatinine excretions divided by plasma

creatinine. Creatinine clearance was adjusted for body surface area (BSA), ie, BSA = $0.007184 \times \text{weight}^{0.425} \times \text{length}^{0.725}$, by dividing CrCl by BSA. To obtain body mass index (BMI), weight (kg) was divided by the square of height (m^2). Diabetes was defined as a fasting plasma glucose level of ≥7.0 mmol/L or a nonfasting plasma glucose level of ≥11.1 mmol/L, or by the use of oral antidiabetic drugs. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or by the use of antihypertensive medication.

Electrocardiography

Standard 12-lead electrocardiograms were recorded with Cardio Perfect equipment (Cardio Control, Rijswijk, The Netherlands), and were stored digitally using the computer program MEANS (Modular Electrocardiogram Analysis System). Infarct patterns, suggestive of myocardial infarction, were defined by Minnesota codes 1.1 and 1.2. Left-ventricular hypertrophy was identified using the Cornell voltage-duration product.[9,15]

Statistical Analysis

Differences between continuous variables were tested by a Student *t* test or Mann-Whitney U test when appropriate. Differences in proportions were tested using a chi-square test or Fisher's exact test. Because we were interested in the relationship of renal function and LVH, we performed a regression analysis to evaluate whether there was interaction between the polymorphisms and CrCl. Interaction terms were examined with linear regression. An interaction term of $P < .10$ in the multivariate analysis was considered significant. To evaluate whether a polymorphism was related to LVH, we compared the genotype of candidate genotypes for LVH to the rest of the population. For the ACE I/D, the candidate genotype was the DD genotype;[16,17] for the AGTR1 A1166C, it was the CC genotype;[18,19] and for the AGT G-6A, it was the AA genotype.[20,21] Analyses were performed using the statistical package SPSS 11.0 (SPSS Inc., Chicago, IL).

Results

Baseline Characteristics

We identified 417 (5.0%) subjects with electrocardiographic LVH. The baseline characteristics of subjects with or without LVH are presented in Table 1. Subjects with LVH were significantly older, more often had a history of myocardial infarction and diabetes, and had higher blood pressure. Also, the urinary albumin excretion (UAE) was higher and the CrCl was lower in subjects with LVH. Urinary sodium excretion was not increased in subjects with LVH.

Of all eligible subjects, genotypes were obtained in 91.8% for the ACE I/D genotype, 95.3% for the AGTR1 A1166C, and 93.0% for the AGT G-6A genotype. There

Table 1. Baseline characteristics of participants of PREVEND Study with and without LVH on electrocardiogram

	No LVH (*n* = 7935)	LVH (*n* = 417)	*P* value
Age (y)	49 (12)	53 (14)	<.001
Sex (male, %)	3929 (50)	212 (51)	.317
Medical history			
Diabetes (n, %)	165 (2)	15 (4)	.036
Myocardial infarction (n, %)	306 (4)	50 (12)	<.001
Hypertension (n, %)	2347 (30)	218 (53)	<.001
Medication			
Antihypertensive (n, %)	872 (11)	81 (20)	<.001
Physical examination			
Heart rate (bpm)	69 (10)	69 (12)	.945
Systolic BP (mm Hg)	128 (20)	140 (26)	<.001
Diastolic BP (mm Hg)	74 (10)	77 (11)	<.001
BMI (kg/m^2)	26.1 (4.2)	25.8 (4.4)	.257
Renal function			
Creatinine (μmol/L)	83 (15)	86 (31)	.076
Sodium excretion (mmol/24 h)	141 ± 51	140 ± 53	.636
Creatinine clearance ($mL/min/1.73\ m^2$)	93 (21)	89 (23)	<.001
UAE (mg/24 h)*	9.2 (6.2–16.7)	11.2 (7.4–26.2)	<.001
Microalbuminuria (n, %)	1030 (13)	94 (23)	
Polymorphism			
ATGR1 A1166C (AA/AC/CC)	3777/3116/669	198/161/39	.598
ACE I/D (II/ID/DD)	1793/3532/1961	96/178/108	.4825
AGT G-6A (GG/GA/AA)	1271/3337/2770	61/168/157	.220

ACE = angiotenin-converting enzyme; AGT = angiotensinogen; AT1 = angiotensin II-type 1; BMI = body mass index; BP = blood pressure; UAE = urinary albumin excretion.

All continuous variables are presented as mean ± SD.

* Continuous variables are presented as median values (25th to 75th).

Table 2. Interaction terms of polymorphisms and creatinine clearance in log regression explaining LVH

	Univariate β	*P* value	Multivariate β*	*P* value
AGTR1 A1166C (CC genotype) · CrCl	−0.19	.024	−0.19	.033
ACE I/D (DD genotype) · CrCl	0.14	.012	0.12	.037
AGT G-6A (AA genotype) · CrCl	−0.005	.490	−0.006	.417

* Adjusted for age, gender, systolic and diastolic blood pressure, antihypertensive medication, ln (urinary albumin excretion), creatinine clearance, diabetes, myocardial infarction, body mass index, and urinary sodium excretion.

were no differences in allele distribution between subjects with and without LVH.

Polymorphisms, Renal Function, and LVH Regression Analysis

We found significant interactions between CrCl and the AGTR1 CC genotype, and between CrCl and the ACE DD genotype. These interactions remained significant after adjustment for known risk markers of LVH, ie, age, gender, systolic and diastolic blood pressure, antihypertensive medication, log-transformed UAE, diabetes, myocardial infarction, BMI, and urinary sodium excretion. No such interaction was found for the AGT AA genotype. The β of the interaction terms is presented in Table 2. Thus, a lower CrCl positively modifies the relationship between the AGTR1 CC genotype and LVH, but negatively modifies the relationship between the ACE DD genotype and LVH. No significant interactions between the polymorphisms and other risk factors for LVH were encountered.

The interaction between CrCl and the AGTR1 CC genotype with the predicted prevalence of LVH is depicted in Figure 1. In subjects with mild renal function impairment (CrCl = 60 mL/min/1.73 m^2), the prevalence of LVH is approximately two times higher in AGTR1 CC homozygotes compared with subjects carrying an A allele, and that the prevalence elevated threefold in subjects with moderate to severe renal function impairment (a CrCl of 30 mL/min/1.73 m^2) (Fig 1). For a proper interpretation of this plot, it should be noted that it depicts the prevalence of LVH as predicted from the multivariate regression analysis, using centralized variables. Thus, these lines represent the predicted prevalence of LVH in a female of average age (49 years), systolic blood pressure (129 mm Hg), diastolic blood pressure (74 mm Hg), and UAE (9.3 mg/24 h), without a history of myocardial infarction and not using antihypertensive medication. Accordingly, in the presence of more risk factors for LVH, the prevalence of LVH would even be higher than shown here. For example, the predicted prevalence of a LVH in a female aged 65 years, using antihypertensive medication, with a blood pressure of 140/90 mm Hg, UAE of 30 mg/24 h, a history of myocardial infarction, a CrCl of 60 mL/min/1.73 m^2, and the AGTR1 CC genotype, would be 35.5%, which is again twice the prevalence in subjects carrying an A allele.

In subjects with the ACE DD genotype, the predicted prevalence of LVH decreased with lower renal function, but not very steeply. Thus the predicted prevalence differed only slightly between subjects with normal (CrCl = 90 mL/min/1.73 m^2) and severely (CrCl = 15 mL/min/1.73 m^2) impaired renal function (4.5% *v* 2.7%, respectively) (Fig 2). In subjects with an ACE I allele, the

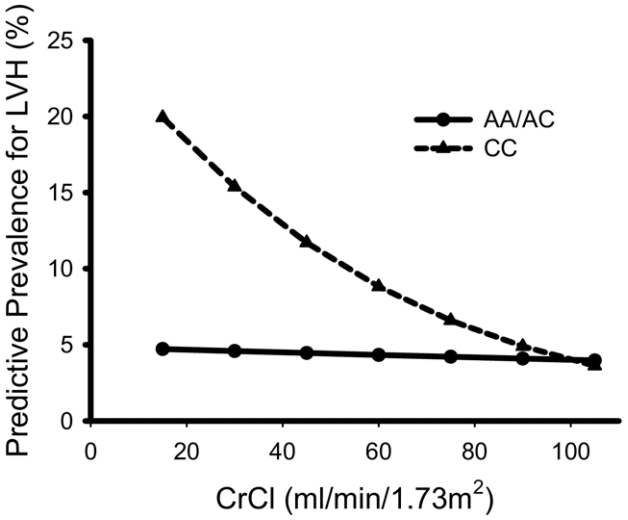

FIG. 1 Predictive prevalence of LVH (%) according to AGTR1 A1166C polymorphism and CrCl impairment. LVH, left-ventricular hypertrophy; CrCl, creatinine clearance.

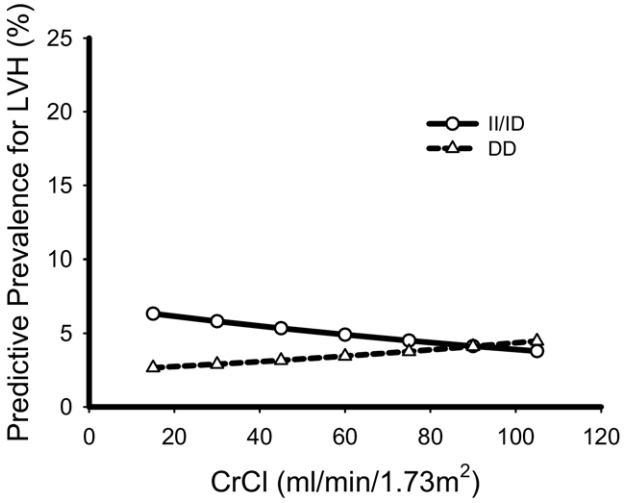

FIG. 2 Predictive prevalence of LVH (%) according to ACE I/D polymorphism and CrCl impairment. LVH, left-ventricular hypertrophy; CrCl, creatinine clearance.

predicted prevalence for LVH slightly increased (3.8% *v* 6.3%) when renal function was worse.

The interaction between renal function and the AGTR1 A1166C and ACE I/D polymorphisms was only present with the homozygote AGTR1 CC genotype and ACE DD genotype, but was absent for heterozygotes.

To test our hypothesis further, we evaluated the percentage of LVH over the different genotypes in subjects with impaired renal function. In total, 419 subjects were identified with a renal function <60 mL/min. In the subjects with the AGTR1 A1166C genotype CC, 17.1% had LVH, compared with 7.6% in all other subjects ($P = .05$). In subjects with the ACE I/D DD genotype, only 4% had LVH, compared with 9% of the other subjects ($P = .095$). In the AGT G-6A AA genotype, 4% had LVH, compared with 9% in the other subjects ($P = .175$).

Discussion

In this population-based study, we demonstrated a renal function dependent association of the AGTR1 A1166C polymorphism and the presence of LVH, resulting in a higher prevalence of LVH in subjects with the AGTR CC genotype, along with more pronounced renal function impairment. A less steep and reverse renal function dependent association was observed between the ACE I/D polymorphism and LVH.

The results of this study are in agreement with the concept proposed by Schunkert,[8] that common genotypes may only have adverse consequences for "cardiovascular" conditions in the presence of specific pathophysiologic conditions. According to this concept, in healthy subjects, a RAS polymorphism does not have enough biologic impact by itself to result in LVH. In line with this concept, in the present population-based study, we did not find a relationship between a single RAS polymorphisms and LVH, whereas studies that reported an association between RAS polymorphisms and LVH were generally performed in patient populations with a pathophysiologic condition carrying a risk for LVH, such as hypertension, diabetes, or chronic renal failure.[7,17,22] Taken together, these data support the concept that the risk for LVH conferred by RAS gene polymorphisms becomes mainly apparent in pathophysiologic conditions that carry a risk for LVH.[7,8]

Because renal function impairment is a risk factor for LVH, we investigated whether renal function influenced the association of several RAS polymorphisms and LVH. We found a strong interaction between AGTR1 A1166C and renal function. Thus, renal function modifies the association between AGTR1 A1166C and LVH, which can be considered a type of gene-environment interaction. Our study does not allow us to identify the mechanism underlying this gene-environment interaction, but several possibilities are conceivable. Angiotensin II levels are elevated in renal function impairment,[10] and angiotensin II has trophic effects on myocardial cells.[11] Furthermore, the potency of trophic responses to angiotensin II seems to be mediated through AGTR1.[23] Our data are compatible with the assumption that higher levels of (tissue) angiotensin II in renal function impairment preferentially induce LVH in CC subjects by altered sensitivity of the angiotensin II type I receptor in human myocardial cells. Although this has never been studied, Van Geel et al,[4] demonstrated that the C allele was associated with increased vasoreactivity to angiotensin II in human arteries, in line with this hypothesis.

The D allele of the ACE (I/D) genotype is associated with higher serum and tissue ACE activity, and possibly enhanced conversion of angiotensin I to angiotensin II in man, in vitro[3] and in vivo,[24,25] and is therefore a candidate gene for angiotensin II-associated organ damage. Several studies addressed the association between the ACE I/D polymorphism and LVH.[7,17,26] Together, these studies indicate that the D allele can be associated with LVH, and that the risk is context-dependent, because it can be different in different populations, and can be modulated by antihypertensive treatment and by sodium intake. In a small sample of the general population, the DD genotype was associated with LVH.[26] In end-stage renal disease, the ACE I/D polymorphism was either found to be neutral regarding LVH,[22,27] or to be a risk factor for LVH,[28] but these studies were limited by small sample size. In our study, the ACE I/D polymorphism was neutral, but renal function influenced the association with LVH. In remarkable contrast with the findings for the AGTR1 A1166C polymorphism, we observed a decreasing risk for LVH in DD subjects with more pronounced renal function impairment, compared with subjects carrying one or two I alleles. Whereas quantitatively the effect was very small, it nevertheless illustrates that the effect of AGTR1 A1166C on LVH cannot be attributed only to the higher prevalence of LVH in renal function impairment, but requires a specific explanation. The renal function dependent association between the ACE genotype and LVH could have several explanations. First, this is a cross-sectional study. As subjects with a D allele tend to have increased risk for mortality,[29] selection may have occurred. However, the ACE I/D polymorphism was in Hardy-Weinberg equilibrium, and was not different in subjects with renal dysfunction or hypertension, which does not support the selection hypothesis. Second, it could be that the generation of angiotensin II level is not the major effect modulator for the development of LVH, but this is more dependent on the activity and for the regulation of AGTR1 in response to angiotensin II.

The AGT G-6A polymorphism is a functional promoter polymorphism of AGT, and the AA genotype was associated with increased gene transcription.[30] It is postulated that increased levels of AGT result in an increase in angiotensin II. Some studies showed an association of the AGT G-6A polymorphism and hypertension,[30,31] but this is disputed.[32] We presume that in the presence of renal-function impairment, and consequently activated RAS,

subjects with the AA genotype would have an increased risk for LVH, but we could not confirm this hypothesis.

The AGT genotype distribution deviated from Hardy-Weinberg equilibrium. The reasons for this deviation are not entirely clear. We were unable to detect technical reasons. Some undetected form of population selection would be an alternative explanation. The PREVEND population was enriched for subjects with microalbuminuria.

Limitations

This study provides cross-sectional observational data, and therefore can only be used to generate new hypotheses. Because of the epidemiological nature of the study, no clinical data or data about known predictors of LVH, eg, valve disorders or the presence of myocardial ischemia, were obtained. We used electrocardiograms to identify subjects with LVH, and not echocardiograms. Therefore, the possibility exists that several subjects with LVH were not detected or were falsely identified. Finally, in this general population-based cohort, antihypertensive medication was given by the general practitioners as required, so that the use of ACE-inhibitors or diuretics may have influenced our results.

A strong point of this study involves the large size of the population, which is crucial in studying the effect of polymorphisms. Furthermore, we used computerized electrocardiogram analysis, thereby avoiding intra- and interobserver bias.

Conclusions

In the present population-based study, a strong, renal function dependent association between the AGTR1 A1166C polymorphism and LVH was observed, with an elevated risk in CC homozygotes with impaired renal function. A reverse, weaker association with LVH was found for the ACE I/D polymorphism and renal function. These data suggest that a genotype that is innocent under normal conditions can turn into a risk factor when renal function deteriorates or, inversely, can exert a protective effect. Renal function therefore should be taken into account as a relevant environmental factor for the pathogenetic effect of RAS polymorphisms. Further prospective studies should be conducted to support this assumption.

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