Lack of Association Between a Polymorphism of the Aldosterone Synthase Gene and Left Ventricular Structure

Heribert Schunkert, MD; Christian Hengstenberg, MD; Stephan R. Holmer, MD; Ulrich Broeckel, MD; Andreas Luchner, MD; Michael W. Muscholl, MD; Susanne Kürzinger, BS; Angela Döring, MD, PhD; Hans-Werner Hense, MD; Günter A.J. Riegger, MD

Background—Cardiac growth and function may be modulated in part by trophic effects of neurohormones. Specifically, aldosterone has been shown to stimulate the growth of cardiac myocytes and the accumulation of cardiac extracellular matrix proteins. Moreover, a variant of the aldosterone synthase gene (a cytosine/thymidine exchange at position \(2^{344}\) in the transcriptional regulatory region) has been associated with enlargement and disturbed filling of the left ventricle (LV) in a small sample of young white adults. The aim of the present study was to reinvestigate the implications of aldosterone synthase \(2^{344}C/T\) allele status for serum aldosterone levels, blood pressure, and LV structure and function in large population-based samples.

Methods and Results—Individuals who participated in the echocardiographic substudy of the third MONICA (MONitoring trends and determinants in CArdiovascular disease) survey (n=1445) or in the second follow-up of the first MONICA survey (n=562) were studied by standardized anthropometric, echocardiographic, and biochemical measurements as well as genotyping for aldosterone synthase \(2^{344}C/T\) allele status. In both surveys, the distribution of sex, age, arterial blood pressure, and body mass index was homogeneous in the aldosterone synthase genotype groups. Echocardiographic LV wall thicknesses, dimensions, and mass indexes were not significantly associated with a specific aldosterone synthase genotype. Likewise, no association was detectable with echocardiographic measures of LV systolic or diastolic function. Data were consistent in both samples and not materially different in subgroups defined by age, sex, or intake of antihypertensive medication. Finally, no significant association was observed for aldosterone synthase allele status and serum aldosterone levels in the group of 562 individuals.

Conclusions—The data are not in favor of a significant contribution of the C/T exchange at position \(2^{344}\) in the aldosterone synthase transcriptional regulatory region to the variability of serum aldosterone levels, blood pressure, or cardiac size or function as found in 2 white population-based samples. (Circulation. 1999;99:2255–2260.)

Key Words: aldosterone ■ genomics ■ hypertrophy

Aldosterone displays both myocardial and renal effects that may have profound implications for left ventricular morphology.1,2 Specifically, aldosterone has been shown to stimulate cardiac collagen synthesis and fibroblast proliferation via activation of local mineralocorticoid receptors in the heart. In addition, aldosterone may increase cardiac load indirectly via sodium and volume retention at the distal tubulus and the collecting duct of the kidney.

The clinical relevance of these cellular mechanisms was suggested by studies on hypertensive patients who demonstrated significant associations between serum aldosterone levels and left ventricular mass (LVM).2,3 Moreover, patients with aldosterone-secreting adenomas developed severe left ventricular hypertrophy that underwent substantial regression after resection of these tumors.4 Furthermore, in a population-based sample, we observed that serum aldosterone levels were independently correlated with left ventricular wall thickness and, in women, LVM.5 Taken together, these data document that aldosterone may be an important factor in the modulation of cardiac structure.

Aldosterone synthase (CYP11B2), a mitochondrial P450 oxidase mainly located in the zona glomerulosa of the adrenal cortex, is a key enzyme in aldosterone synthesis. Rare mutations of this gene accompany either markedly elevated aldosterone levels and arterial hypertension or insufficient aldosterone synthesis and sodium wasting.6–9 On the basis of this information, Kupari and coworkers10 analyzed the aldosterone synthase gene for more frequent genetic polymorphisms. The authors demonstrated 2 common genetic variants located either in the transcriptional regulatory region (a
cytosine/thymidine exchange at position −344, a putative SF-1 transcription factor binding site) or in the second intron (a gene conversion). The 2 polymorphisms occurred in linkage disequilibrium and were strongly associated with left ventricular dimensions and mass as well as echocardiographic parameters of diastolic filling in 84 young, healthy Finnish individuals. Given the enormous implications of these findings for the pathophysiology and potentially the treatment of left ventricular hypertrophy and failure, we reevaluated the associations of left ventricular size and function, as well as blood pressure and aldosterone serum levels, with the most informative of the 2 aldosterone polymorphisms in 2007 participants of 2 independently sampled Augsburg MONICA (MONItoring trends and determinants in CArdiovascular disease) surveys.

Methods

Study Population

The subjects of this study participated either in the echocardiographic substudy of the third MONICA Augsburg survey in 1995 and 1996 (n=1674) or in the second follow-up examination of the first MONICA Augsburg survey in 1994 (n=646). Subjects originate from a sex-age–stratified random sample of all German residents of the Augsburg study area. The third survey represents individuals 25 to 74 years of age and ~300 subjects for each 10-year increment. The subjects of the follow-up study of the first survey were 50 to 67 years of age with ~30% of subjects being 50 to 55 years of age, 50% 56 to 61 years of age, and 20% 62 to 67 years of age. All individuals who participated in this study gave written informed consent.

All subjects responded to a questionnaire on medical history, physical activities, medication, and personal habits. Body height and weight were recorded with subjects wearing light clothing, and body mass index was computed as weight in kilograms divided by height in meters squared (kg/m²). Resting blood pressure was measured after subjects had been in a sitting position for a minimum of 30 minutes. With a mercury sphygmomanometer, blood pressure was measured 3 times in the right arm. Hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or intake of antihypertensive medication during the 7 days preceding the examination.

Echocardiographic Measurements

A 2-dimensionally guided M-mode echocardiogram was performed on each subject by 1 of 3 expert sonographers using a single recorder (Sonos 1500, Hewlett Packard Inc). M-mode tracings were recorded on strip-chart paper at 50 mm/s. Only tracings that demonstrated optimal visualization of left ventricular interferences were used, a requirement that resulted in exclusion of 17% of potential subjects. The echocardiographers were blinded for clinical, biochemical, and molecular genetic data. Structures for M-mode– guided calculation of LVM (left ventricular internal end-diastolic [EDD] and end-systolic [ESD] dimensions, septal [sWth] and posterior [pWth] wall thickness) were measured according to the guidelines of the American Society of Echocardiography as previously reported in detail. LVM was calculated according to the formula described by Devereux et al:

\[ \text{LVM (in grams)} = (1.04 \times (\text{EDD} + \text{sWth} + \text{pWth})^2) - 13.6 \text{ g} \]

LVM was indexed to body surface area as LVM index in grams per square meter of body surface area. Left ventricular hypertrophy by M-mode criteria was considered when LVM index was >134 g/m² body surface area in men or >110 g/m² body surface area in women.

Biochemical Measurements

Blood was drawn from nonfasting subjects who were in a supine resting position for ≥30 minutes. Genotyping was performed according to the methods described by Kupari et al. After DNA purification from peripheral blood was accomplished by a standard protocol, 80 ng of genomic DNA was subjected to 35 rounds of specific amplification with 5′-CAG GAG GAC CCA TGT GAC-3′ (sense primer) and 5′-CCT CCA CCC TGT TCA GCCC-3′ (antisense primer). After a final extension for 7 minutes, PCR products were restricted with 10 U of HaeIII (Fermentas) for 2 hours at 37°C, separated on 2.5% agarose gels, and visualized under UV illumination. The T allele lacks 1 restriction site and results in a band of 273 bp; complete restriction (CC genotype) results in a main band of 202 bp. Both alleles display smaller fragments as well. Genotyping was successfully performed in 1445 participants of the third survey and 562 participants of the follow-up of the first MONICA survey. The reason for incomplete genotyping was lack of appropriate material (genomic DNA) in all cases. Aldosterone, renin, and prorenin serum concentrations were quantified in all participants of the follow-up of the first MONICA survey. Immunoreactive renin was measured in a 200-μL plasma sample by means of an immunoradiometric assay kit (Nichols Institute, according to the methods proposed by Derkx et al). Prorenin was activated nonproteolytically with the renin inhibitor remikiren. The concentration of prorenin was calculated by subtracting the results obtained before activation of prorenin (ie, active renin) from those obtained after activation (ie, total renin). Aldosterone levels were determined in 100 μL of serum by standard radioimmunoassays (Peninsula). ACE activity was measured by a fluorometric assay.

Statistical Analysis

Anthropometric and echocardiographic data were compared according to the aldosterone −344CT allele status by ANOVA for comparison of independent samples or χ² tests for comparison of classified values. Given the confounding effects of antihypertensive therapy, patients receiving such treatment were excluded from comparisons involving levels of renin, prorenin, aldosterone, and ACE activity. Multiple linear regression was used to compare LVM indexes; left ventricular EDD, fractional shortening, and isovolumetric relaxation period; and early to late diastolic filling (E/A) ratio in the −344CC, −344CT, and −344TT genotype groups after adjustment for age, sex, body mass index, systolic blood pressure, and antihypertensive therapy. In addition, the study samples were partitioned by sex, age (younger or older than 40 years), hypertension status, and presence or absence of left ventricular hypertrophy. With an α-error of 5%, the number of subjects in the present study samples provided a power of 90% to detect a 6.5 g/m² difference in LVM index and to detect a 30.0 pmol/L difference in serum aldosterone levels between respective genotype groups. P values are reported for each test and statistical model.

Results

Anthropometric and biochemical data of the 2007 participants of the 2 surveys are listed in Table 1. Overall, the frequencies of the −344C and −344T alleles were 0.46 and 0.54, respectively. The −344CC, −344CT, and −344TT genotypes were found in 20.4%, 49.5%, and 30.1% of the study population, respectively. These frequencies are in agreement with those predicted by Hardy-Weinberg equilibrium. Furthermore, the allele frequencies were similar in both surveys and similar to those reported in previous studies. There were no significant differences in sex distribution, body size, or blood pressure levels related to aldosterone synthase −344CT allele status. Furthermore, genotypes were related neither to hypertension status nor to intake of antihypertensive drugs (Table 1). Moreover, neither aldosterone nor renin serum levels were affected by −344C/T allele status.
TABLE 1. Anthropometric and Biochemical Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>TT Genotype</th>
<th>CT Genotype</th>
<th>CC Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants of the third MONICA survey</td>
<td>n=416</td>
<td>n=714</td>
<td>n=315</td>
</tr>
<tr>
<td>Age, y</td>
<td>52.1 (0.6)</td>
<td>51.9 (0.5)</td>
<td>52.3 (0.7)</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>48.9</td>
<td>47.9</td>
<td>49.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.4 (0.2)</td>
<td>26.9 (0.1)</td>
<td>26.5 (0.2)</td>
</tr>
<tr>
<td>Heart rate, min⁻¹</td>
<td>69 (0.6)</td>
<td>69 (0.4)</td>
<td>70 (0.6)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg⁻¹</td>
<td>135 (1.0)</td>
<td>134 (0.7)</td>
<td>133 (1.1)</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg⁻¹</td>
<td>80 (0.5)</td>
<td>80 (0.4)</td>
<td>79 (0.6)</td>
</tr>
<tr>
<td>Hypertensive, %</td>
<td>31.2</td>
<td>30.1</td>
<td>28</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>3.6</td>
<td>4.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Myocardial infarction, %</td>
<td>2.7</td>
<td>3.9</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Participants of the follow-up of the third MONICA survey

| Age, y                    | 57.4 (0.2)  | 58.1 (0.2)  | 57.5 (0.3)  |
| Sex, % male               | 56.1        | 53.0        | 46.9        |
| BMI, kg/m²                | 27.8 (0.3)  | 26.9 (0.2)  | 27.2 (0.3)  |
| Heart rate, min⁻¹         | 70.6 (0.9)  | 70.5 (0.7)  | 71.1 (1.0)  |
| Systolic BP, mm Hg⁻¹      | 148 (1.4)   | 147 (1.1)   | 145 (1.7)   |
| Diastolic BP, mm Hg⁻¹     | 91.6 (0.7)  | 89.8 (0.6)  | 89.6 (1.0)  |
| Hypertensive, %           | 53.2        | 47.9        | 41.1        |
| Diabetes mellitus, %      | 5.8         | 3.9         | 2.0         |
| Myocardial infarction, %  | 1.1         | 1.3         | 3.1         |
| Renin, mU/L*              | 15.3 (0.9)  | 16.1 (0.7)  | 15.0 (0.9)  |
| Prorenin, mU/L*           | 196 (10)    | 207 (7)     | 204 (11)    |
| Aldosterone, pmol/L*      | 121 (7)     | 125 (5)     | 131 (7)     |
| BNP, mU/L*                | 17.4 (1.7)  | 15.8 (1.0)  | 12.7 (1.3)  |
| cANP, pmol/L*             | 353 (2.4)   | 52.7 (1.7)  | 52.1 (3.4)  |
| ACE activity, mU/L*       | 2.4 (0.05)  | 2.5 (0.04)  | 2.5 (0.07)  |

TT genotype indicates homozgyosity for the aldosterone synthase T allele; CT, heterozygosity; CC, homozgyosity for the aldosterone synthase C allele; BMI, body mass index; BP, blood pressure; Rx, treatment; BNP, brain natriuretic peptide; and cANP, cyclic atrial natriuretic peptide. Values are expressed as mean ± SEM.

*After exclusion of patients using antihypertensive medication.

hypertension, diabetes mellitus, or myocardial infarction did not reveal any significant differences related to respective genotypes (data not shown). Likewise, partition of the samples by sex, age (younger or older than 40 years), hypertension status (normotensive or hypertensive), and presence or absence of left ventricular hypertrophy did not identify any subgroup in which any of the echocardiographic variables were associated with the aldosterone synthase CC genotype as previously observed.10

Subsequent multivariate analyses that adjusted for age, sex, body mass index, systolic blood pressure, and antihypertensive drug treatment came to similar conclusions, ie, no consistent significant association between −344C/T allele status and measures of left ventricular size and function was detectable (Figure). There was a trend toward higher E/A ratios in subjects with the CC genotype (1.29 versus 1.23 in the TT group; P=0.047). This minimal trend was not significant when the ratios of the integral of early and late atrial filling velocities were compared (data not shown). Compared with subjects with the TT genotype, the adjusted risk ratios to
present with left ventricular hypertrophy in the CT and CC genotypes were 1.12 (95% CI, 0.6 to 2.0; \( P = 0.44 \)) and 0.74 (95% CI 0.3 to 1.6; \( P = 0.7 \)), respectively.

**Discussion**

The main physiological determinants of serum aldosterone concentration are volume status, potassium levels, and activity of the renin-angiotensin system.\(^{19} \) In addition, mutations of the enzymes involved in aldosterone biosynthesis can result in elevated or suppressed aldosterone levels.\(^{6-9} \) Thus, genetic polymorphisms such as the cytosine/thymidine exchange found in the regulatory region of the aldosterone synthase gene might bear the potential to modulate the expression of the enzyme and thus contribute to the inherited variability of aldosterone levels, blood pressure, or other aldosterone-related phenotypes. Specifically, Kupari and co-workers\(^{10} \) studied 84 young, healthy individuals and observed strong associations between the \(-344C\) allele of the aldosterone synthase polymorphism and increased left ventricular size and impaired diastolic function. In contrast, we found no such association between echocardiographic measures of left ventricular size or function and aldosterone \(-344C/T\) allele status.

Previously, we\(^{5,20} \) had found that serum aldosterone levels are associated with the variability of LVM in healthy subjects as well as patients with arterial hypertension. Therefore, the negative result in the present study came quite unexpectedly. However, it should be mentioned that aldosterone \(-344C/T\) allele status thus far has not been consistently related to any intermediate phenotype, eg, serum aldosterone levels, that might explain the previously observed variabilities in LVM and diastolic function. Indeed, 1 recent study\(^{21} \) reported elevated serum aldosterone levels in 216 individuals with the \(-344CC\) genotype, whereas others\(^{22,23} \) reported elevated urinary or serum aldosterone levels in 92 and 117 individuals with the \(-344TT\) genotype, respectively. Interpolated between these discrepant observations, we found no association between serum aldosterone levels and aldosterone synthase \(-344C/T\) genotype groups in 562 individuals. Although discrepant results in smaller studies and a negative finding in a larger cohort suggest that the aldosterone synthase \(-344C/T\) polymorphism has no major effect on aldosterone levels, a cautious interpretation of such association studies with a given phenotype is advisable. One should specifically point to the possibility that other genetic variants of the same gene,\(^{6-9} \) specific environmental circumstances (eg, posture,
sodium or potassium intake and other dietary factors, and physical activity level), or populations with other genetic backgrounds may lead to other conclusions. Nevertheless, it remains to be established whether aldosterone levels are affected by aldosterone synthase −344C/T allele status and thereby offer a mechanism for differences in more complex (echocardiographic) phenotypes.

The present study as well as previous studies 10,21 concur in the absence of an association between −344C/T allele status and blood pressure levels. Whereas 1 investigation reported higher blood pressure levels in individuals with the −344CC genotype, 22 another reported higher blood pressure levels in individuals with the −344TT genotype. 23 The lack of a consistent association between −344C/T genotype status and hypertension might be of interest, because an elevation of blood pressure might be another plausible intermediate phenotype that could link a functionally active polymorphism in the aldosterone synthase gene and alterations in left ventricular size or function. Indeed, the known mutations of the aldosterone synthase gene have in common an effect on blood pressure. 6–9 For example, patients with glucocorticoid-suppressible hyperaldosteronism that is based on deregulated overexpression of the CYP11B2 gene are characterized by severe hypertension. 6 By contrast, other known mutations of this enzyme that accompany a loss of function result in hypoaldosteronism, salt wasting, and hypotension. 7–9 Moreover, in samples of the general population, including that in the present study, significant associations have been observed between aldosterone concentrations and blood pressure. 5,20 Thus, a lack of association between −344C/T allele status and blood pressure makes it unlikely that this polymorphism has a profound effect on aldosterone synthesis, as already suggested by our data on aldosterone measurements. One has to submit, therefore, that at present we have no information on the mechanism that could link this molecular polymorphism in the aldosterone synthase gene and increased left ventricular size or diastolic dysfunction.

Both the present negative and the previous positive study on the associations between aldosterone synthase −344C/T allele status and LVM or left ventricular function relied on echocardiographic assessments of adult men and women living in either Helsinki, Finland or Augsburg, Bavaria. 10 Given the geographic distance and different demographic histories between the northern and mid-European populations, differences in genetic background may account for the discrepant findings. 24 These differences may be of particular importance if a yet-unknown functional site is in linkage disequilibrium with the −344C/T genotype, at least in some populations. It is quite plausible that such a genetic variant adjacent to the chromosomal aldosterone synthase gene locus is only present in individuals with a given demographic or ethnic history and therefore accounts for variable associations between the −344C/T genotype and LVM or diastolic function.

Finally, Kupari and coworkers 10 excluded patients with hypertension or other cardiovascular conditions and limited their analysis to 84 individuals who were 36 or 37 years of age. In contrast, our aim was to study large representative samples of the entire population. Thus, respective experimen-

tal designs need to be addressed as an explanation for the discrepant results. First, with regard to the study population, the Helsinki study may allow a better estimate for an accurately studied but relatively narrow subgroup of individuals. By contrast, the present negative investigation may allow a better estimate of the implications of various aldosterone synthase genotypes for a mid-European population in general. This notion may be further confirmed by the finding that none of the relatively large subgroups defined by age, sex, or blood pressure status revealed a positive association between −344C/T allele status and the echocardiographic parameters under investigation. Second, although the statistical power increases with the size of the sample, the chance of a type A error tends to decrease. Thus, lack of statistical power is an unlikely explanation for the negative association between the aldosterone synthase −344C allele and left ventricular size and function reported here. This is reflected by the relative narrow confidence margin that diverges barely from 1 with respect to the risk ratio for left ventricular hypertrophy associated with the C allele. Third, with regard to the echocardiographic measurements, the present and previous studies of the Augsburg populations provide confirmation of the predictive value of known modulators of left ventricular hypertrophy. 5,11–14 Moreover, great care was taken to maximize the accuracy of echocardiographic readings. All measurements were performed by only 3 echocardiographers who underwent special training to minimize interobserver and intraobserver variability. 13 Consequently, the rank correlation for LVM between observers was 0.91. 14 Thus, we have no reason to believe that suboptimal echocardiographic measurements might have obscured the present lack of association.

Taken together, the present study of a large sample failed to associate −344C/T allele status of the aldosterone gene with echocardiographic measures of left ventricular size or function. In addition, we were unable to uncover potential intermediate phenotypes such as elevated aldosterone levels or elevated blood pressures in carriers of the aldosterone synthase C allele.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft (DFG Schu 6729-1, 672/10-1, 672/12-1, and Ho 1073/8-1), the Bundesministerium für Forschung und Technologie (Drs Schunkert, Döring, and Hense), and the Vaillant Stiftung. We thank A. Walgenbach and M. Wolff for excellent technical support.

References


Lack of Association Between a Polymorphism of the Aldosterone Synthase Gene and Left Ventricular Structure

Heribert Schunkert, Christian Hengstenberg, Stephan R. Holmer, Ulrich Broeckel, Andreas Luchner, Michael W. Muscholl, Susanne Kürzinger, Angela Döring, Hans-Werner Hense and Günter A. J. Riegger

_Circulation_. 1999;99:2255-2260
doi: 10.1161/01.CIR.99.17.2255

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/17/2255

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/