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An aldosterone synthase gene variant is associated with improvement in left ventricular ejection fraction in dilated cardiomyopathy

Armindo D. Tiago[a], Danelle Badenhorst[a], Daniel Skudicky[b], Angela J. Woodiwiss[a], Geoffrey P. Candy[a,b,c], Richard Brooksbank[a], Karen Sliwa[b], Pinhas Sareli[b], Gavin R. Norton[a,*]

[a]*Cardiovascular Genomics and Heart Failure Research Units, Department of Physiology, Chris Hani-Baragwanath Hospital and the University of the Witwatersrand, Johannesburg, South Africa*

[b]*Cardiovascular Genomics and Heart Failure Research Units, Department of Cardiology, Chris Hani-Baragwanath Hospital and the University of the Witwatersrand, Johannesburg, South Africa*

[c]*Cardiovascular Genomics and Heart Failure Research Units, Department of Nuclear Cardiology, Chris Hani-Baragwanath Hospital and the University of the Witwatersrand, Johannesburg, South Africa*

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Abstract

Objective: To assess whether renin–angiotensin–aldosterone (RAA) system gene polymorphisms shown to be associated with alterations in the activity of the system, may predict cardiac function changes subsequent to initiating medical therapy in heart failure. **Methods:** The impact of RAA system genotypes on left ventricular ejection fraction (LVEF) following therapy to patients with idiopathic dilated cardiomyopathy (IDC) and class II–III heart failure was assessed. In 107 patients LVEF and LV dimensions were determined using radionuclide ventriculography and echocardiography prior to and subsequent to receiving furosemide, digoxin and angiotensin-converting enzyme (ACE) inhibitor therapy. Patients and controls were genotyped for variants of the ACE (insertion–deletion polymorphism), angiotensinogen (AGT; M235T polymorphism) and the aldosterone synthase (*CYP11B2*, C-344T polymorphism) genes. **Results:** RAA system genotypes were not significantly associated with LVEF prior to initiating medical therapy. However, the *CYP11B2* gene variant ($P=0.0064$ on covariate analysis [adjusted for multiple genotyping] with a 1–2% chance of false positive data), but neither the ACE, nor the AGT variants, predicted improvement in LV ejection fraction in patients on medical therapy. **Conclusion:** A *CYP11B2* gene variant predicts the variable improvement in LV ejection fraction that occurs subsequent to initiating medical therapy in IDC. These data suggest a role for the aldosterone synthase locus in regulating the progression of heart failure.

Keywords: Cardiomyopathy; Heart failure; Sequence (DNA/RNA/prot)

1. Introduction

Activation of components of the renin–angiotensin–aldosterone (RAA) system is important in contributing to congestive heart failure [1]. In heart failure, stimulation of renin release results in an increased secretion of aldosterone, a hormone which may alter cardiac function either indirectly by modifying loading conditions (through changes in vascular tone, body fluid balance [2] and subsequently cardiac dimensions) or directly through effects on the myocardium [3,4]. The importance of the RAA system in heart failure is underscored by the fact that those patients with the greatest elevations in plasma aldosterone concentrations have the lowest survival rate [5].

Activation of the RAA system has been linked to variants of the genes for angiotensin-converting enzyme (ACE) [6], angiotensinogen (AGT) [7] and aldosterone

*Corresponding author. Cardiovascular Genomics and Heart Failure Research Units, School of Physiology, University of the Witwatersrand Medical School, 7 York Road, Parktown, 2193, Johannesburg, South Africa. Tel.: +27-11-717-2363; fax: +27-11-717-2153.

E-mail address: 057nort@chiron.wits.ac.za (G.R. Norton).

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synthase (*CYP11B2*) [8–11] in some studies. Although recent studies have examined a potential association between loci encoding for substances involved in the RAA system, and heart failure, conflicting data have been found [12–15]. Presently there are no studies examining the relationship between genetic variants of the RAA system and the haemodynamic progression of heart failure. As the degree of left ventricular (LV) systolic dysfunction is a strong surrogate marker for survival in heart failure [16], the primary aim of the present study was to examine whether gene variants of the RAA system predict changes in LV systolic function in idiopathic dilated cardiomyopathy (IDC) subsequent to initiating medical therapy.

2. Methods

All patients and control subjects had provided verbal consent to undergo genetic testing as approved by the Institution's Committee for Research on Human Subjects (approval number M951122).

2.1. Case-control study subjects

To confirm the absence of a strong association between RAA system genotype and IDC in the population sampled, 157 unrelated Black South Africans with heart failure due to left ventricular dysfunction (LV ejection fraction LVEF $<40\%$ at the initial assessment) of unknown aetiology, in functional class II to IV heart failure according to the New York Heart Association classification and with a dilated LV (LV end diastolic diameter LVEDD >55 mm) as determined using echocardiography were included in this study. Patients were excluded from participation in the study if they had the following criteria: a history of hypertension or a blood pressure (BP)$\geq$170/105 mm Hg, the presence of a significant history of alcohol abuse, previous cardiac ischaemic events or the use of nitrates, wall motion abnormalities or pathological Q waves on electrocardiography, primary valvular disease, active myocarditis or a history of myocarditis, clinical or echocardiographic features consistent with an obstructive, hypertrophic or restrictive cardiomyopathy, pericardial disease, primary hepatic, renal, neurological, pulmonary or endocrine disease, and arrhythmias that could alter LVEF. Using these inclusion and exclusion criteria to select patients we have previously shown a normal coronary anatomy (on coronary angiography) in patients in whom, on history, a potential ischaemic contribution was uncertain [14].

A control group of 225 apparently healthy, unrelated Black South Africans were recruited from the general population of surrounding districts. These subjects had a clinical history taken, a general examination performed, and were screened for the presence of hypertension. The estimated sample size required for this study to avoid false negative results with 80% power for genotype frequency analysis was based on odds ratios calculated for the ACE gene deletion variant from original data provided in a previous study [13], and the genotype frequencies previously reported on for this population [14].

2.2. LV ejection fraction, dimensions and haemodynamics in patients with IDC

One hundred and seven newly diagnosed patients with functional class II–III heart failure had LVEF assessed using radionuclide ventriculography and LV end diastolic and end systolic diameters measured using echocardiography [14] in the first 2–3 weeks and at a mean time (final measurement) of 17.4 months (range=6–30 months) after medical therapy was initiated (furosemide, digoxin and an ACE inhibitor—either trandolapril at 4 mg except for 2 patients who could only tolerate 2 mg, or enalapril at 10 mg twice daily). These patients agreed to participate in a long-term study. A sample size of 34 in each genotype group had been estimated to provide statistical power at an 80% level ($\alpha=0.05$) to detect a 7.5 point (%) difference in improvement in LVEF (primary end point) from baseline between genotype groups with between-patient standard deviations of changes from baseline of 11 LVEF points (%). The 7.5 point difference in LVEF between genotype groups was predicted from 75% of the mean improvement normally noted in newly diagnosed patients receiving standard medical therapy in our Cardiology Unit. Follow-up LVEF measurements were made at 6 months (in all 107 patients, 83 of whom had ACE inhibitor therapy initiated within a few weeks of initial LVEF measurements) and between 2 and 2.5 years (50 patients who had ACE inhibitor therapy initiated within 6 months of initial LVEF measurements). In the 6 month follow-up of all 107 patients, no deaths or exclusions occurred. Initially 81 patients agreed to participate in a 2–2.5 year follow-up study, but 31 died ($n=7$), were lost to follow-up ($n=21$), or developed additional non-cardiac related pathology that required exclusion from the study ($n=3$). At the time of initiating this study β-blockers were not registered for routine use in heart failure in South Africa.

2.3. Genotyping

Deoxyribonucleic acid (DNA) was extracted from whole blood by lysing red blood cells and digesting the remaining white cell pellet with proteinase K. The ACE gene insertion/deletion (I/D) polymorphism was detected by a polymerase chain reaction (PCR) technique using oligonucleotide primers flanking the insertion sequence and insertion-specific primer pairs as previously described [14]. Genotyping of the T–C transition at nucleotide 704 in exon 2 of the AGT gene, where cytosine at position 704 corresponds to the 235T polymorphism and thymidine to

the M235 polymorphism [17], and the C-344T promoter region polymorphism of the *CYP11B2* gene [11] was undertaken using mismatch PCR-restriction fragment length polymorphism based techniques employing appropriate primer pairs and restriction enzymes.

2.4. Data analysis

The relative risk of the presence of IDC associated with risk alleles was estimated from an odds ratio calculation. Multiple logistic regression analysis was used to determine whether genotype was independently associated with IDC. Age, gender, and body mass index (BMI) were used as covariates. To determine whether improvement in LVEF, LV dimensions, and other haemodynamic parameters had occurred subsequent to initiating medical therapy a Wilcoxon matched-pairs signed rank test was employed. To assess the relationship between either LVEF, LV dimensions, or haemodynamics and genotype both prior to and subsequent to initiating medical therapy and the relationship between genotype and change in LVEF, LV dimensions and haemodynamics from baseline, analyses were performed in the total group of 107 patients, in the subgroup of 83 patients receiving ACE inhibitors in the first 6 months of follow-up, and in the subgroup of 50 patients followed for 2–2.5 years. In the analysis performed on the total group of 107 patients, 6 month follow-up data, and the last measurement of LVEF, LV dimensions, and haemodynamics were used to determine whether genotype predicted cardiac or haemodynamic changes. Analysis of covariance, adjusting for duration, type and dose of therapy, age, gender, body weight, change in body weight, heart rate, systolic BP, and baseline LVEF was used to determine the relationship between cardiac/haemodynamic values or change in cardiac/haemodynamic values and genotype. We adjusted α levels for multiple gene testing using Bonferroni's method. We calculated statistical power from the difference in LVEF between genotype groups, the standard deviation for the groups, the sample size employed in each genotype group, and the α value obtained. Continuous data are expressed as mean±S.D.

3. Results

3.1. Demographic and general clinical data of the whole group

Subjects in the case group were younger, consisted of more males and had a lower body mass index in comparison to controls (Table 1).

3.2. Demographic and general clinical data in patients with follow-up performance data

There were no differences in demographic or clinical characteristics between ACE, AGT, or *CYP11B2* genotype groups (data not shown). Importantly, there were no mean differences in type and dose of therapy in patients who had LVEF assessed subsequent to initiating medical therapy (Table 2).

Table 1
Demographic and clinical characteristics of patients with idiopathic dilated cardiomyopathy and controls

	Case (n=157)	Control (n=225)
Age (years)	53±11*	55±9
Gender (% male)	64*	52
Body mass index (kg.m^{-2})	25.5±5*	27.7±8
Blood pressure (mm Hg)	123±24/79±10	128±12/79±10
Functional class (II/III/IV)	68/81/8	0
LVEDD (cm)	6.5±0.8	–
LVESD (cm)	5.7±0.8	–
LVEF (%)	25.3±7.6	–

LVEDD, left ventricular end diastolic diameter; LVESD, LV end systolic diameter; LVEF, LV ejection fraction determined using radionuclide ventriculography. *P<0.05.

3.3. Association between RAA system genotype and IDC

None of the RAA system gene polymorphisms examined were noted to be risk factors for the development of IDC (Table 3). No association between genotype and exclusion during the 2–2.5 year follow-up period was noted.

3.4. Association between RAA system genotype and initial haemodynamics

Initial LVEF was lower in patients homozygous for the ACE gene D variant (22±6%, n=47) as compared to patients with the I/D and I/I genotypes (26±8%, n=60, P<0.04 versus DD genotype), but this failed to reach significance when adjusting for multiple genotyping (P=0.11). Moreover, neither the *CYP11B2* (Table 4), nor the AGT (MT genotype=24±7%, n=36; TT genotype=24±7%, n=71) gene variants were associated with initial LVEF. None of the gene polymorphisms examined were associated with baseline LV dimensions, heart rate, or BP (data not shown).

3.5. Improvement in haemodynamics after initiating medical therapy

At 6 months, 2 years, 2.5 years, and when considering the final LVEF in the group of 107 patients, irrespective of genotype, patients showed a significant improvement in LVEF (in %) as compared to baseline (24.0±0.7 at baseline to 31.1±1.1 at 6 months, to 32.8±1.3 at 2–2.5 years and to 31.8±1.2 at the final visits in all patients, P<0.0005 versus baseline at all follow-up time periods). Similarly LV end diastolic and end systolic diameters, and systolic BP were reduced at these time periods as com-

Table 2
Type and mean dose of medical therapy received by 107 patients with idiopathic dilated cardiomyopathy grouped according to renin–angiotensin–aldosterone system genotypes (ACEI therapy was at the same dose in all but 2 patients)

Gene	*CYP11B2*		ACE		AGT	
Genotype	CC+CT	TT	II+ID	DD	MT	TT
ACEI (enalapril/trandolapril)	9/36	17/45	13/47	13/34	10/26	16/55
Furosemide (mg.day^{-1})	159±40	160±39	156±38	164±41	169±41	155±42
Digoxin (mg.day^{-1})	0.19±0.08	0.19±0.08	0.17±0.08	0.21±0.07	0.22±0.09	0.17±0.08

ACE, angiotensin-converting enzyme; AGT, angiotensinogen; *CYP11B2*, aldosterone synthase; ACEI, ACE inhibitor; I and D, insertion and deletion alleles of ACE gene; T and M of AGT gene, T235 and 235M alleles of AGT gene; T and C of *CYP11B2* gene, -344T and C-344 alleles of the *CYP11B2* gene.

Table 3
Renin–angiotensin–aldosterone system genotype and allele frequencies of patients with idiopathic dilated cardiomyopathy and controls

	Genotype			Allele		OR	CI	*P* value[a]
Angiotensin-converting enzyme gene insertion (I)/deletion(D) polymorphism								
	DD	ID	II	D	I			
IDC (n=157)	71	60	26	202(64)	112(36)	0.82	0.61–1.12	0.80
Control (n=225)	102	105	18	309(69)	141(31)			
Angiotensinogen geneM235T polymorphism								
	TT	MT	MM	T	M			
IDC (n=157)	102	55	0	259(83)	55(17)	0.697	0.47–1.04	0.25
Control (n=225)	167	58	0	392(87)	58(13)			
Aldosterone synthase (*CYP11B2*) gene C-344T polymorphism								
	TT	CT	CC	T	C			
IDC (n=157)	100	49	8	249(79)	65(21)	0.84	0.58–1.21	0.52
Control (n=225)	151	67	7	369(82)	81(18)			

Numbers represent sample number (%). The odds ratios (OR) and 95% confidence intervals (CI) are calculated from allele frequencies and the risk alleles are assumed to be the D, 235T, and -344T alleles. IDC, idiopathic dilated cardiomyopathy.
[a] Determined from multiple logistic regression analysis.

pared to baseline values ($P<0.01$ at all times). However, neither diastolic BP nor heart rate were significantly reduced with therapy.

3.6. Association between RAA system genotype and improvement in haemodynamics

At 6 months, during subsequent measurements at 2–2.5 years after initiating medical therapy, and when considering the final LVEF measured in all 107 patients, the increase in LVEF from baseline was greater in patients with a *CYP11B2* C-344 allele (Table 4). A similar genotype effect on LV end diastolic and systolic diameters was noted, but this effect only reached statistical significance for the change in LV end diastolic diameter in all 107 patients before correcting for multiple genotyping (in

Table 4
Effect of an aldosterone synthase (*CYP11B2*) gene variant on left ventricular ejection fraction (LVEF) in patients with idiopathic dilated cardiomyopathy followed up for different time periods

LVEF (%) Genotype	Baseline		Final		Change in	
	CC+CT	TT	CC+CT	TT	CC+CT	TT
Follow-up period						
6 months [1]	23±5(34)	24±7(49)	32±13(34)	29±11(49)	9±8.9(34)	5.5±10(49)
6 months [2]	23±6(45)	25±7(62)	33±12(45)	30±11(62)	9.6±8(45)*	5.3±10(62)
2–2.5 years	25±6(20)	28±6(30)	42±12(20)**†	31±13(30)	17.7±10(20)**†	3.8±12.5(30)
Average final follow-up [3]	23±6(45)	25±7(62)	35±14(45)**†	29±12(62)	12.6±12(45)**†	5.6±13(62)

Numbers in parentheses=sample numbers; 6 months [1], refers to analysis performed only on patients receiving angiotensin-converting enzyme (ACE) inhibitor therapy in this time period; 6 months [2], refers to analysis performed on all patients irrespective of whether ACE inhibitor therapy was being administered; follow-up [3], refers to final data obtained in all patients (CC+CT=17.2±14.1 months, TT=17.5±14.2 months). *$P<0.03$; **$P<0.003$ versus TT group before correcting for multiple genotyping. $^{\dagger}P<0.01$ after correcting for multiple genotyping.

cm: CC+CT=−0.51±0.72, TT=−0.14±0.66, P=0.02). *CYP11B2* genotype failed to predict changes in either heart rate or BP (data not shown). Neither the ACE (change in LVEF in %: DD genotype=9±13; ID+II genotype= 8±13), nor the AGT (change in LVEF in %: MT genotype=9±12; TT genotype=8±13) gene variants predicted improvement in LVEF in patients receiving medical therapy. As a consequence of the greater improvement in LVEF in patients with the C-344 allele of the *CYP11B2* gene, *CYP11B2* genotype predicted final LVEF (Table 4). Moreover, when considering the final LVEF, 42% (19/45) of patients with a *CYP11B2* C-344 allele had a LVEF> 40% in comparison to only 18% (11/62) of patients without a *CYP11B2* C-344 allele (odds ratio=3.39, confidence interval=1.14–8.12, unadjusted P=0.008, and adjusting for multiple genotyping, P=0.024). None of the other genetic variants examined predicted the frequency of patients with a final LVEF >40%.

4. Discussion

The main finding of the present study is that a *CYP11B2* gene variant, but neither ACE, nor AGT gene variants examined predicted improvement in LVEF measured after initiating medical therapy with furosemide, digoxin and ACE inhibitors in patients of African ancestry with IDC.

A problem with reporting on associations between gene variants and complex disease traits has been the limited statistical power of many studies. In the present study we had a 1–2% chance of false positive results calculated from the final LVEF difference between *CYP11B2* genotype groups in all 107 patients, and the LVEF difference between genotype groups in the 50 patients assessed at 2–2.5 years. Hence, it is unlikely that our present results are spurious.

A potential explanation for the association between *CYP11B2* genotype and the change in LVEF from baseline in the present study is that the C-344T allele of this gene influences aldosterone production [8–11] from either the adrenal cortex or the myocardium [18,19]. Alterations in aldosterone production could influence cardiac performance through both changes in cardiac loading conditions (by modifying vascular tone, blood volume [2], and subsequently cardiac dimensions), or directly through effects on the myocardium [3,4]. Although LV dimensions were not a primary end-point evaluated in the present study, a significant association between *CYP11B2* genotype and changes in LV end diastolic diameter was noted before correcting for multiple genotyping. Consequently, the genetic association identified in this study, could be explained by gene variant effects on aldosterone production, and consequently preload changes or myocardial remodelling, a hypothesis which requires investigation with LV dimension measurements determined as a primary end point. However, *CYP11B2* genotype failed to predict either BP, or heart rate, and therefore it is unlikely that the genotype effects noted in the present study can be accounted for by alterations in loading conditions mediated through blood pressure and heart rate changes.

Whether the *CYP11B2* genotype predicts change in LVEF after medical therapy because of differences in therapeutic responses of patients grouped according to genotype, or because of differences in the natural history of the disease, was not determined in the present study. Moreover, we failed to assess the effect of these genes on changes in LVEF in the presence of alternative pharmacological agents, including β-blockers, which have been shown to be effective in heart failure. At the time of initiating the present study β-blockers were not registered for routine therapy in heart failure in South Africa. Clearly, further work is required to answer these questions.

In summary, we have shown that the C-344T polymorphism of the *CYP11B2* gene, although not associated with IDC, is an independent predictor of improvement in LVEF in patients of African ancestry with IDC subsequent to initiating furosemide, digoxin and ACE inhibitor therapy. These results provide evidence in support of a role for the aldosterone synthase locus in regulating the progression of heart failure.

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