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ARTICLE

Effect of Genetic and Nongenetic Factors on the Clinical Response to Mineralocorticoid Receptor Antagonist Therapy in Egyptians with Heart Failure

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This prospective cohort study evaluated the association between the renin angiotensin aldosterone system genotypes and response to spironolactone in 155 Egyptian patients with heart failure with reduced ejection fraction (HFrEF). Genotype frequencies for *AGT* rs699 were: CC = 16%, CT = 48%, and TT = 36%. Frequencies for *CYP11B2* rs1799998 were: TT = 33%, TC = 50%, and CC = 17%. After 6 months of spironolactone treatment, change in the left ventricular ejection fraction (LVEF) differed by *AGT* rs699 (CC, 14.6%; TC, 7.9%; TT, 2.7%; *P* = 2.1E-26), and *CYP11B2* rs1799998 (TT, 9.1%; TC, 8.7%; CC, 1.4%; *P* = 0.0006) genotypes. Multivariate linear regression showed that the *AGT* rs699 and *CYP11B2* rs1799998 polymorphisms plus baseline serum potassium explained 71% of variability in LVEF improvement (*P* = 0.001), 63% of variability in serum potassium increase (*P* = 2.25E-08), and 39% of the variability in improvement in quality of life (*P* = 2.3E-04) with spironolactone therapy. These data suggest that *AGT* and *CYP11B2* genotypes as well as baseline serum K are predictors of spironolactone response in HFrEF.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Response to mineralocorticoid receptor antagonist (MRA) therapy in patients with heart failure with reduced ejection fraction (HFrEF) is subject to interindividual variability.

☑ There are differences in frequencies of polymorphisms for genes encoding proteins in the renin angiotensin aldosterone system (RAAS) by ethnicity and between healthy patients and those with HFrEF.

☑ *AGT* rs699 and rs5051, *CYP11B2* rs1799998, and *NR3C2* rs2070950 polymorphisms have been associated with blood pressure response to angiotensin-converting enzyme inhibitors, but their association with response to MRAs is unknown.

WHAT QUESTION DID THE STUDY ADDRESS?

☑ Is variability in response to MRA therapy among patients with HFrEF correlated with variability in the *AGT*, *CYP11B2*, and/or *NR3C2* genes?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ This study investigated associations between four single nucleotide polymorphisms in RAAS-related genes with response to MRAs in HFrEF.

☑ Our data suggest that *AGT* and *CYP11B2* genotypes could be predictors of response to MRAs among Egyptians with HFrEF.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ RAAS genetic profiling may be used as a valuable tool for therapeutic optimization in HFrEF.

Chronic heart failure is a serious clinical disease in the modern world with a 5-year mortality rate exceeding 50% and affecting ~ 1–2% of the adult population.[1,2] Heart failure with reduced ejection fraction (HFrEF) is characterized by a multifactorial interplay of inflammatory, neurohormonal, metabolic, and genetic factors.[3,4] The activation of compensatory mechanisms to maintain physiological functioning results in increased sympathetic activity, vasoconstriction, volume overload, and ventricular remodeling with deleterious effects on cardiac function.[5]

The renin angiotensin aldosterone system (RAAS) is a key stimulator of the compensatory mechanisms that drive the progression of heart failure.[6] Therapies that target the RAAS, such as angiotensin-converting enzyme inhibitors (ACEIs), β-blockers, and mineralocorticoid receptor antagonists (MRAs), collectively inhibit the pathological

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compensatory mechanisms, including remodeling and apoptosis of cardiomyocytes and improve clinical outcomes, and, thus, are considered the foundation of heart failure (HF) therapy.[6,7]

According to the 2017 American College of Cardiology/American Heart Association guidelines, MRAs should be prescribed in addition to standard therapy to all stable patients with HFrEF (classes II–IV), provided there are no contraindications,[8] such as decreased creatinine clearance (< 30 mL/minute) or hyperkalemia (serum potassium > 5 mEq/L) and that renal function and potassium can be carefully monitored. Although studies have shown that MRAs as add-on therapy significantly reduce mortality and HF hospitalization,[9] a considerable proportion of MRA-treated patients do not respond effectively, with a mortality rate in clinical trials exceeding 35% in spironolactone-treated patients[10] and 12.5% in eplerenone-treated patients.[11] Although interpatient variability in response to MRAs has been documented in the literature, few studies have been conducted to evaluate underlying causes of this variability.[12,13] Such variability could be due to clinical and/or genetic factors, including genetic polymorphisms in the RAAS pathway. Identifying clinical and genetic markers affecting response to MRA therapy in patients with HFrEF may facilitate the development of effective and personalized therapies for those patients, which could eventually improve clinical outcomes in this population.

Genes involved in the RAAS pathway have not been well interrogated for their effects on response to MRA therapy. Angiotensinogen (AGT) is released by the liver and cleaved by renin to angiotensin I, which is subsequently converted to angiotensin II, a potent vasoconstrictor and simulator of aldosterone release.[14] The *AGT* rs699 p.M268T polymorphism has been associated with higher *AGT* expression and plasma AGT levels,[15,16] and linked to multiple diseases, including hypertension, coronary heart disease, and atrial fibrillation.[17]

Aldosterone synthase catalyzes the final reaction to generate aldosterone. The aldosterone synthase gene (*CYP11B2*) consists of nine exons and is localized to chromosome 8q24.[18] A common single nucleotide polymorphism (SNP) in the promoter region of the *CYP11B2* gene, rs1799998 c.-344T > C, occurs in ~ 30% of African Americans and 46% of Europeans,[19] with an increased aldosterone excretion and a higher plasma aldosterone to renin ratio reported with the -344T allele.[20,21]

The nuclear receptor subfamily 3 group C member 2 (*NR3C2*) genes span over 400 kb, contain 9 exons, and codes for the target protein of MRAs. SNPs in *NR3C2*, rs2070950 c.-2-358C > G, and rs2070951 c.-2C > T, have been associated with blood pressure (BP) response to ACEI therapy and changes in serum potassium with spironolactone for HF, respectively.[22,23] The two SNPs are reportedly in high linkage disequilibrium.[22]

We aimed to investigate the association among SNPs in the *AGT*, *CYP11B2*, and *NR3C2* genes and response to MRA therapy in Egyptian patients with HFrEF. We also aimed to identify clinical factors affecting response to MRA in this population.

METHODS

Study design and population

This was a prospective observational cohort study aimed at investigating the association between SNPs in genes within the RAAS system and clinical response to the addition of the MRA spironolactone to standard treatment (β-blocker and ACEI or angiotensin receptor blocker therapy) among Egyptian patients with HFrEF. Between April 2017 and June 2018, 156 patients were enrolled from Ain Shams University Hospitals, Cairo, Egypt. Eligible patients were those with established HFrEF, defined as a left ventricular ejection fraction (LVEF) < 40% and New York Heart Association (NYHA) functional classes II–IV, who were candidates for add-on treatment with MRAs. We included patients who had MRAs added to baseline therapy with a β-blocker plus ACEI or angiotensin receptor blocker. Patients with an acute coronary syndrome within the past 3 months, valvular disease (defined as mild-to-severe valvular stenosis or severe (grades III/IV) valvular regurgitation), or valve surgery within 3 months were excluded. The study was registered in ClinicalTrials.gov; Identifier: NCT03122834. The study protocol was approved by the Research Ethics Committee at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, and all participating patients provided written informed consent.

Procedures

At baseline, patients who met the eligibility criteria were subjected to full laboratory, echocardiographic evaluation, and assessment of quality of life (QOL). Spironolactone was initiated at a dose of 25 mg/day, which was maintained throughout the study for all but two patients, for whom the dose was up-titrated to 100 mg/day, as per the cardiologist's discretion. Spironolactone was supplied by the study team. All patients were followed monthly to monitor potassium and renal function.

Clinical and medical information were abstracted from the electronic medical record and included age, sex, body weight, NYHA class, cigarette smoking status, medical history, and medications. Whole blood samples (7 mL) were collected for DNA extraction and genotyping. After 6 months, echocardiographic evaluation, serum K, and QOL assessment were repeated.

Standard transthoracic 2D and Doppler echocardiographic studies were performed using standard equipment (Siemens ACUSON SC2000). Left ventricular volumes derived from apical four chamber and apical two chamber views and LVEF were calculated by the Simpson biplane method indexed to body surface area. All images were analyzed by the same cardiologist using the same equipment at baseline and at the end of the study to avoid equipment and reader variability.

QOL was assessed using the Minnesota Living with Heart Failure Questionnaire (MLHFQ), which addresses 21 physical, emotional, and socioeconomic ways HF can adversely affect a patient's life. After receiving brief standardized instructions, patients marked a zero to five score to indicate how much each itemized adverse effect of HF prevents them from living as they desire. The questionnaire was scored by summation of all 21 responses. The MLHFQ scores increase with the adverse impact of HF on the respondent's life.

Evidence supports the validity of using the MLHFQ physical, emotional, and total scores in patients with HF, for clinical practice and research.[24]

Genotyping procedure

Genomic DNA was extracted from peripheral blood leukocytes using the automated QIAcube device (QIAGEN, Cairo, Egypt) according to the manufacturer's guidelines. Polymorphisms were selected from three candidate genes: *AGT* (rs699, rs5051), *CYP11B2* (rs1799998), and *NR3C2* (rs2070950). The selected polymorphisms were genotyped by TaqMan allelic discrimination method using four TaqMan genotyping probes (C_1985481_20, C_9616191_10, C_8896484_10, and C_1594391_1, respectively) according to the manufacturer's recommendations (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA).

Data analysis

Of the 156 subjects enrolled, 155 (99%) completed the 6 months of follow-up and were included in the analysis. The remaining patient was lost to follow-up. The phenotypes of interest were change in LVEF, left ventricular end systolic volume (LVESV), left ventricular end diastolic volume (LVEDV), diastolic dysfunction grade, serum potassium (K), and QOL from baseline to after 6 months of MRA therapy. Diastolic dysfunction was defined as the change in the E/A ratio grade, which is the ratio of peak velocity blood flow from gravity in early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave).

Continuous variables were presented as mean ± SD. Categorical variables were presented as numbers and percentages. Changes in the evaluated clinical measures were compared between baseline and post-spironolactone treatment using the Wilcoxon signed rank for continuous variables and the χ^2 test for categorical variables. For the genotype data, Hardy–Weinberg equilibrium was tested for each SNP using the χ^2 test with one degree of freedom. The frequency of the evaluated variant allele was reported for the Egyptian population under study and compared with published data of other populations.

The distribution of baseline clinical measures across genotype groups of the four SNPs (*AG*T rs699, *AG*T rs5051, *CYP11B2* rs1799998, and *NR3C2* rs2070950) was compared using the χ^2 or Fisher's exact test for categorical variables and one-way analysis of variance or Kruskal–Wallis, as appropriate, for continuous variables.

Changes in the outcome of interest (LVEF, LVESV, LVEDV, K, and MLHFQ) were determined by subtracting the pre-spironolactone treatment value from the post-treatment value for each outcome. We then tested the effect of SNPs on each outcome with adjusting for clinical factors and genetic covariates that may influence the tested outcome, in addition to other SNPs.

A step-wise multiple linear regression analysis was conducted to assess the contribution of genetic variability and clinical predictors to each outcome of interest. We included genotypes at the four SNPs, relevant clinical covariates, and other variables that were associated with the outcome at a P value < 0.2 on a univariate analysis including age, sex, diabetes, hypertension, HF etiology, ACEI use, and the relevant baseline parameter (baseline LVEF, LVEDV, LVESV, K level, QOL, or diastolic dysfunction grade). The final model included significant clinical predictors ($P < 0.05$) and genotypes at a Bonferroni corrected $P = 0.0125$ (0.05/4 SNPs). For categorical outcomes, such as diastolic dysfunction grade, a logistic regression analysis was performed.

We tested the effect of combined genotypes at *AGT* rs699 and *CYP11B2* rs1799998 on each outcome with adjusting for clinical factors. All statistical analyses were carried out using SPSS software version 22.0 for Windows (SPSS, Chicago, IL) and SAS version 9.3 SAS Institute.

Sample size calculation

A sample size of 152 patients provided over 90% power to detect an effect size f2 of 0.15, assuming a two-sided hypothesis and alpha-level of 0.0125 after correction for multiple testing (0.05/4).

RESULTS

Baseline clinical characteristics of 155 study patients are summarized in **Tables 1 and 2**. Differences in baseline characteristics across genotype groups of each SNP are shown in **Table S1**. The majority of study patients was men (78%) and had HFrEF of an ischemic origin (74%). The mean daily spironolactone dose was 25 mg. Serum electrolytes, renal function, BP, echocardiographic parameters, and QOL

Table 1 Baseline demographic and clinical characteristics of study participants

Baseline characteristic	
Age, years	51.7 ± 11.6
Male sex	121 (77.6%)
Diabetes mellitus	71 (45.5%)
Hypertension	93 (59.6%)
HF cause	
Ischemic origin	115 (73.7%)
Dilated cardiomyopathy	41 (26.3%)
Current or former cigarette smoker	113 (72.4%)
CBC	
RBC count (10^6cells/μL)	4.7 ± 0.6
Hemoglobin (gm/dL)	14.0 ± 1.7
WBC count (10^3cells/μL)	10.2 ± 2.7
Platelet count (10^3cells/μL)	263 ± 76
NYHA functional class	
Class II	79 (50.6%)
Class III	62 (39.7%)
Class IV	15 (9.6%)
Medications	
β-Blocker	127 (81.9%)
ACEI	102 (65.8%)
ARB	24 (15.5%)
Statin	110 (71.0%)
Ivabradine	24 (15.5%)

Mean ± SD or No. (%).
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CBC, complete blood count; HF, heart failure; NYHA, New York Heart Association; RBC, red blood cell; WBC, white blood cell.

Table 2 Clinical measures at baseline and after 6 months of spironolactone therapy

Variable	Baseline	Post-spironolactone therapy	*P* value for change from baseline*
Serum K, mEq/L	3.7 ± 0.3	4.5 ± 0.5	2.49E-25
Serum Na, mmol/L	139 ± 2	140 ± 2	0.75
BUN, mg/dL	20 ± 12	18 ± 8	0.065
Serum creatinine, mg/dL	1.1 ± 0.4	1.1 ± 0.2	0.66
SBP, mmHg	131 ± 11	121 ± 9	5E-11
DBP, mmHg	78 ± 9	73 ± 7	2.04E-06
LVESV, mL	104 ± 41	88 ± 38	1.64E-22
LVEDV, mL	148 ± 53	140 ± 50	2.25E-18
LVEF, %	30 ± 7	38 ± 10	7.31E-26
Diastolic dysfunction grade			
Normal diastolic function	13 (8.4%)	17 (11.0%)	1.33E-08
Grade 1	66 (42.6)	69 (44.5%)	
Grade 2	69 (44.5%)	52 (33.5%)	
Grade 3	7 (4.5%)	17 (11.0%)	
QOL, points	48.4 ± 12	40.3 ± 13	7.3E-26

BUN, blood urea nitrogen; DBP, diastolic blood pressure; K, potassium; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; Na, sodium; QOL, quality of life; SBP, systolic blood pressure.
Mean ± SD or no. (%).
*Data were analyzed using Wilcoxon signed rank and χ^2 tests.

after 6-month treatment with spironolactone are shown in **Table 2**. Patients had a higher K level ($P = 2.5 \times 10^{-25}$) and lower BP (systolic blood pressure $P = 5 \times 10^{-11}$; diastolic blood pressure $P = 2.0 \times 10^{-6}$) compared with pre-MRA treatment. There was a significant improvement in echocardiographic parameters as indicated by a higher LVEF ($P = 7.3 \times 10^{-26}$), lower LVESV ($P = 1.6 \times 10^{-22}$) and LVEDV ($P = 2.3 \times 10^{-18}$), and a higher percentage of patients with improved diastolic function ($P = 1.3 \times 10^{-8}$) post-spironolactone treatment. Additionally, there was a significant improvement in QOL ($P = 7.3 \times 10^{-26}$).

Genotype frequencies are shown in **Table 3**. None of the SNP frequencies deviated from the Hardy–Weinberg equilibrium. As expected, for an admixed population from Egypt, we observed differences in SNP frequencies among Egyptians and Africans, Hispanics, Asians, and Europeans (**Table S2**). For example, the frequency of the *AGT* rs699 C and rs5051 T alleles in Egyptians was lower than that reported in Africans, Hispanics, and Asians, but similar to Europeans. On the other hand, the *CYP11B2* rs1799998 C allele frequency in Egyptians was similar to Europeans and Hispanics, but higher than in African and Asian populations. The *NR3C2* rs2070950 C allele frequency differed from that reported in all other populations assessed. Although the frequencies of the rs699 and rs5051 SNPs seemed similar, according to genotyping data from the Egyptians, the SNPs were not in linkage disequilibrium ($R^2 = 0.19$, $D' = 0.47$).

Change in echocardiographic parameters by genotype

We observed a greater improvement in LVEF and greater reduction in LVESV, but not LVEDV, with the *AGT* rs699

Table 3 Distribution of the studied genetic polymorphisms among study participants

SNP	Minor allele frequency	Genotype	Number of patients	Genotype frequency	HWE[a]
AGT rs699	0.40	TT	56	36%	0.96
		TC	74	48%	
		CC	24	16%	
AGT rs5051	0.37	CC	18	12%	0.90
		CT	76	50%	
		TT	59	38%	
CYP11B2 rs1799998	0.42	TT	51	33%	0.74
		TC	77	50%	
		CC	26	17%	
NR3C2 rs2070950	0.33	GG	68	45%	0.33
		GC	70	45%	
		CC	16	10%	

Genotypes were missing for one patient for rs699, rs1799998, and rs2070950 and two patients for rs5051.
AGT, angiotensinogen; *CYP11B2*, aldosterone synthase; HWE, Hardy–Weinberg equilibrium; *NR3C2*, nuclear receptor subfamily 3 group C.
[a]HWE *P* value corresponds to the χ^2 goodness-of-fit test results, assuming one degree of freedom.

Table 4 Change in clinical measures with spironolactone by genotype

SNP	Genotype	Change in LVEF (%)	Change in LVESV (mL)	Change in LVEDV (mL)	Change in MLHFQ (points)	Change in K (mEq/L)
AGT rs699	TT	2.7%	–6.8 mL	–5.2 mL	–3.3 points	0.4 mEq/L
	TC	7.9%	–12.4 mL	–7.9 mL	–5.3 points	0.7 mEq/L
	CC	14.6%	–19.2 mL	–10.2 mL	–15.1 points	1 mEq/L
	Adjusted *P* value	2.1E-26	3.04E-07	0.065	1E-06	4.36E-10
AGT rs5051	CC	9.6%	–15.5 mL	–10.4 mL	–10.2 points	0.68 mEq/L
	CT	7.7%	–11.2 mL	–8.4 mL	–8.4 points	0.70 mEq/L
	TT	7.9%	–11.7 mL	–6.4 mL	–7.6 points	0.74 mEq/L
	Adjusted *P* value	0.123	0.255	0.277	0.887	0.826
CYP11B2 rs1799998	TT	9.1%	–16 mL	–10.9 mL	–8.9 points	1 mEq/L
	TC	8.7%	–15 mL	–9.1 mL	–7.1 points	0.9 mEq/L
	CC	1.4%	–7.4 mL	1.2 mL	–2.1 points	0.3 mEq/L
	Adjusted *P* value	0.0006	8.6E-05	0.012	0.002	1.07E-10
NR3C2 rs2070950	GG	9.5%	–17.1 mL	–9.9 mL	–8.4 points	0.60 mEq/L
	GC	10.1%	–18.1 mL	–9.3 mL	–8.5 points	0.77 mEq/L
	CC	5.6%	–3.3 mL	5.6 mL	–6.8 points	0.75 mEq/L
	Adjusted *P* value	0.189	0.002	0.008	0.199	0.216

AGT, angiotensinogen; *CYP11B2*, aldosterone synthase; K, potassium; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; MLHFQ, Minnesota Living with Heart Failure Questionnaire; *NR3C2*, nuclear receptor subfamily 3 group C.
Change in LVEF was adjusted for baseline potassium, platelets, red blood cells count, LVEF, LVESV, and LVEDV as well as ischemia, diabetes, smoking, New York Heart Association (NYHA) class and all tested single nucleotide polymorphisms (SNPs). Change in LVESV was adjusted for age, baseline potassium, LVEF, LVESV, and LVEDV, as well as ischemia, diabetes, hypertension, NYHA class, and all tested SNPs. Change in LVEDV was adjusted for age, baseline potassium, LVEF, LVESV, and LVEDV, as well as ischemia, diabetes, hypertension, and all tested SNPs. Change in K was adjusted for age, baseline potassium, platelets, white blood cells count, and renal functions, as well as sex, diabetes, smoking, NYHA class, and all tested SNPs. Genotype-phenotype associations with $P < 0.0125$ were considered significant after Bonferroni correction.

CC genotype compared with the TC and TT genotypes (**Table 4**, **Figure 1a**, **Figures S1A and S2A**). The *CYP11B2* rs1799998 TC and TT genotypes were also associated with greater improvement in LVEF, in addition to greater reductions in LVESV and LVEDV at 6 months compared with the CC genotype (**Table 4**, **Figure 1b**, **Figures S1B and S2B**). Compared with the *NR3C2* rs2070950 CC genotype, the GC and GG genotypes were associated with greater reductions in LVESV and LVEDV, but were not associated with changes in LVEF (**Table 4**, **Figures S1D and S2D**). There was no association between *AGT* rs5051 genotype and changes in any echocardiographic parameter (**Table 4**, **Figures S1C** and **S2C**).

Additionally, we observed that 83% of patients with the *AGT* rs699 CC genotype had significant improvement in diastolic dysfunction grade compared with 24% and 7% of patients with TC and TT genotypes, respectively (adjusted $P = 2.4E-05$; **Figure S3A**). For *CYP11B2* rs1799998, 51% of patients with TT genotype had improvement diastolic dysfunction grade compared with 18% and 8% of patients with TC and CC genotypes, respectively (adjusted $P = 0.008$; **Figure S3B**). Changes in diastolic dysfunction grade were not significant by the *AGT* rs5051 (adjusted $P = 0.218$) or *NR3C2* rs2070950 (adjusted $P = 0.123$) genotypes (**Figure S3C,D**).

Change in QOL by genotype

The greatest improvement in QOL with spironolactone therapy was observed with the *AGT* rs699 CC and *CYP11B2* rs1799998 TT and TC genotypes (**Table 4**). Neither the *AGT* rs5051 nor *NR3C2* rs2070950 genotype was associated with change in QOL (**Table 4**).

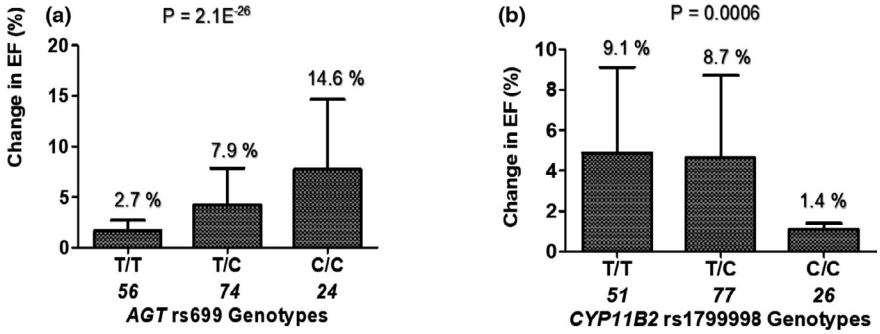

Figure 1 Change in left ventricular ejection fraction (EF) with spironolactone by (**a**) *AGT* rs699 and (**b**) *CYB11B2* rs1799998 genotypes.

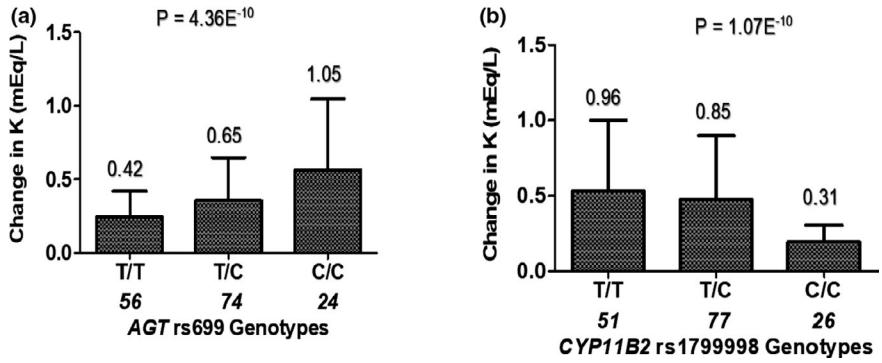

Figure 2 Change in serum potassium (K) with spironolactone by (**a**) *AGT* rs699 and (**b**) *CYB11B2* rs1799998 genotypes.

Change in serum K by genotype

Both the *AGT* rs699 CC and *CYP11B2* rs1799998 TT and TC genotypes were associated with lower baseline serum K level (**Table S1**) and greater increases in serum potassium with spironolactone therapy (**Table 4**, **Figure 2a,b**) compared with other genotypes. There were no significant changes in K observed for the *AGT* rs5051 and *NR3C2* rs2070950 genotypes (**Table 4**). No patient developed hyperkalemia, defined as $K > 5.0$ mEq/L.

Effect of SNP combination

Results from univariate analysis showed that patients carrying both *AGT* rs699 CC and *CYP11B2* rs1799998 TT (9.1%) had the greatest improvement in LVEF (*P* value = 1.4E-31) and reductions in LVESV (*P* value = 1.6E-13) and LVEDV (*P* value = 8.4E-06), as well as the greatest increase in serum K (*P* value = 2.1E-29; **Table S3**). However, the combined effect of these two SNPs was not significant for QOL changes (*P* value = 0.742).

Genetic and clinical predictors of MRA response

Multiple linear regression analyses were performed to identify predictors of variability in changes in LVEF, K, and QOL measures with MRA therapy. The results showed that *AGT* rs699, *CYP11B2* rs1799998, and baseline K were jointly associated with changes in LVEF, K level, and QOL. A model, including baseline K, *AGT* rs699, and *CYP11B2* rs1799998, explained 71%, 63%, and 39% of the variability in ΔLVEF, ΔK, and ΔQOL, respectively, with MRA therapy (**Table 5**). Exclusion of the two patients treated with the 100 mg/day dose did not alter results.

DISCUSSION

In this study, we observed a significant improvement in echocardiographic parameters and QOL with spironolactone therapy among Egyptian patients with HFrEF, consistent with data on spironolactone effects in other populations.[25,26] We also found that the *AGT* rs699, *CYP11B2* rs1799998, and *NR3C2* rs2070950 SNPs, but not *AGT* rs5051, significantly influenced response to spironolactone in this population. Specifically, both the *AGT* rs699 CC genotype and *CYP11B2* rs1799998 T allele were associated with improvement of echocardiographic parameters, QOL, and diastolic dysfunction grade. *NR3C2*

Table 5 Multivariate regression analysis for predictors of variability in clinical response among HFrEF patients in response to 6 months of MRA therapy

	Model 1 Change in EF		Model 2 Change in K		Model 3 Change in MLHFQ	
Predictors	**β (SE)**	***P* value**	**β (SE)**	***P* value**	**β (SE)**	***P* value**
AGT rs699	6.1 (0.46)	2.10E-26	0.3 (0.045)	4.36E-10	–2.93 (0.57)	1E-06
CYB11B2 rs1799998	–1.54 (0.44)	0.0006	–0.3 (0.043)	1.07E-10	1.71 (0.55)	0.002
NR3C2 rs2070950	–	–	–	–	–	–
Baseline K	–2.44 (1.03)	0.0186	–0.41 (0.1)	7E-05	3.07 (1.28)	0.018
Baseline EDV	–	–	–	–	–	–
Baseline ESV	–	–	–	–	–	–
Diabetes	–	–	–	–	–	–
Sex	–	–	–	–	–	–
Hypertension	–	–	–	–	–	–
Baseline diastolic dysfunction grade	–	–	–	–	–	–
Intercept	13.1	0.001	2.3	2.25E-08	–18.6	2.3E-04
R^2	0.71		0.63		0.39	

EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; K, potassium; MLHFQ, Minnesota Living with Heart failure Questionnaire.
AGT rs699, *CYB11B2* rs1799998, and *NR3C2* rs2070950 genotypes were included as predictor with three levels (coded as 0, 1, and 2). Clinical variables with *P* value < 0.20 were tested in the model. Variables were retained in the model if they achieved statistical significance ($P < 0.05$).

rs2070950 GG and GC genotypes were associated with improvement in LVESV and LVEDV, but not other measures.

To date, few HF pharmacogenetic studies have assessed the extent to which variability in response to HF medications is driven by genetic factors,[27] and no study, to our knowledge, has focused on the contribution of genetic and nongenetic factors to variability in response to MRAs in patients with HFrEF. Consistent with our data, Kurland *et al*.[28] showed that, in patients with hypertension and evidence of left ventricular hypertrophy, there was greater improvement in echocardiographic measures with angiotensin receptor blocker therapy in those with the *AGT* rs699 CC genotype; however, no association was observed with β-blockers. Our data are also consistent with a genetic substudy of the African American Heart Failure Trial, which showed that patients with the *CYP11B2* rs1799998 T allele had better response to HF therapy than those with the CC genotype; the beneficial effect on LVEF was more marked in patients receiving spironolactone.[29] Additionally, our study findings were in line with a study by Yu *et al*.,[30] in which individuals with *CYP11B2* TT or CT genotypes had greater BP reduction with ACEI therapy compared with those with the CC genotype. Although, in a previous study by our group, we showed no significant association between *AGT* rs699 genotype and K response to spironolactone, our sample size was small.[23] We believe our current data add to the accumulating evidence of associations between the *AGT* rs699C and *CYP11B2* rs1799998T alleles and improved response to RAAS inhibitors in cardiovascular disease. Similar to our data, Su *et al*.[31] found no association between *AGT* rs5051 and BP reduction in patients with hypertension treated with an ACEI.

There are very limited data with the *NR3C2* genotype and cardiovascular drug response. However, our findings with the rs2070950 variant are consistent with data by Luo *et al*. as well as previous data by our group.[22,23] Luo *et al*.[22] showed a trend toward greater diastolic blood pressure reduction with ACEI therapy in individuals with the rs2070950 GG genotype.[22] In a mixed US population treated with spironolactone for HF, we showed a greater K increase with the *NR3C2* rs2070951G allele,[23] which has been associated with mineralocorticoid receptor expression and circulating levels of plasma renin and aldosterone.[32] The rs2070950 and rs2070951 SNPs are in strong linkage disequilibrium in populations with European ($D' = 0.996$, $R^2 = 0.9881$) and African ancestry ($D' = 1$, $R^2 = 0.8204$) per 1000-Genomes.[33] Together, these data suggest a possible role for the *NR3C2* gene in mediating spironolactone response.

The cleaved product of AGT is angiotensin II, which stimulates aldosterone release.[34]The secretion of aldosterone is mainly regulated at the level of expression of the *CYP11B2* gene, which is the key rate-limiting enzyme in the final steps of aldosterone biosynthesis in the zona glomerulosa cells of human adrenal glands. Moreover, *CYP11B2* expression is primarily regulated by angiotensin II and serum potassium levels.[35] Previous findings showed that the *AGT* rs699C allele is associated with higher plasma AGT levels,[15,16] which, consequently, may lead to greater aldosterone release. The *CYP11B2* rs1799998 T allele is associated with increased *CYP11B2* activity and higher aldosterone secretion.[20,21] Lower K levels at baseline among patients with the *AGT* rs699CC and *CYP11B2* rs1799998TT genotypes supports greater aldosterone levels in these patients as aldosterone mediates potassium excretion. Based on the totality of our data, we postulate that patients with HFrEF with the *AGT* rs699 CC and *CYP11B2* TT genotypes have increased circulating aldosterone at baseline, and, thus, derive the greatest benefit from blockade of the aldosterone receptor with spironolactone.

In addition to their associations with beneficial effects of MRAs, our study revealed that the *AGT* rs699 CC genotype and *CYP11B2* rs1799998 T allele were associated with greater increase in serum K with MRA therapy compared with other genotypes. Interestingly, the increase in K levels and improvements in echocardiographic parameters and QOL with MRA therapy were all in the same direction. That is, the genotypes associated with greater improvements in echocardiographic parameters and QOL were also associated with greater increases in potassium. The cardiac and renal effects of spironolactone are mediated through blockade of aldosterone binding to the NR3C2 in the heart and kidney, respectively.[36] Thus, it would stand that patients deriving greater cardiac benefit might also have greater effects at the renal level. Overall, our data suggest that MRA therapy will be especially beneficial for patients with HFrEF with the *AGT* rs699 CC and *CYP11B2* rs1799998 TT genotypes, but it should be initiated at very low doses with more careful monitoring and up-titration to avoid the development of hyperkalemia. However, it is notable that none of the patients in our study developed hyperkalemia.

Importantly, the same genotypes associated with improvement in left ventricular function were also associated with improvement in QOL, as assessed by the MLHFQ. We believe these consistent associations between the *CYP11B2* and *AGT* rs699 genotypes and multiple measures in our study further support these genotypes as important predictors of spironolactone response.

The comparison of the minor alleles frequencies of the SNPs tested in this study with population frequencies showed differences emphasizing our previous observation that the frequency of genetic polymorphisms in Egyptians do not mirror the frequency from other continental populations, probably given the known historical ancestral admixture of Egyptians.[37,38] Moreover, the higher frequency of the *AGT* rs699 C and *CYP11B2* rs1799998 T alleles in Africans and Asians suggests that MRA therapy may be especially effective in these populations.

Personalized medicine approaches involve the use of patient-specific factors, including genotype data to predict optimal pharmacotherapy regimens for individual patients. To enable personalized approaches to HF therapy, pharmacogenetic research is needed to uncover genetic factors that, along with clinical factors, underlie differences in response to HF therapy.[39] Herein, we identified genetic and clinical factors that explained a large portion of the variability in response to MRAs. Specifically, baseline K and the *AGT* rs699 and *CYB11B2* rs1799998 SNPs explained 71% and 63% of variability in ejection fraction and K post-MRA treatment.

This study had several strengths. This is one of the first reports of genetic associations with interpatient variability in response to MRAs among patients with HFrEF. Additionally, we prospectively assessed several clinical measures to more completely evaluate the response to MRA. We also acknowledge some limitations to our study, including the relatively small sample size and focus on a limited number of SNPs. However, given the paucity of data to date with genetic determinants of response to MRAs, we believe that assessing candidate variants with known functional effects was a logical place to start. Second, there was no replication cohort, and, thus, our findings should be considered hypothesis generating. In addition, we did not measure aldosterone or other neurohormone levels, and, thus, further studies are needed to elucidate the mechanisms underlying the observed genetic associations.

In summary, results from this study suggest that *AGT* rs699, *CYP11B2* rs1799998, and baseline potassium may serve as predictors of response to MRAs therapy in Egyptian patients with HFrEF. Future replication or validation of our results is needed.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

Figure S1. Change in LVESV with spironolactone by (A) *AGT* rs699, (B) *CYP11B2* rs1799998, (C) *AGT* rs5051, and (D) *NR3C2* rs2070950 genotypes.
Figure S2. Change in LVEDV with spironolactone by (A) *AGT* rs699, (B) *CYP11B2* rs1799998, (C) *AGT* rs5051, and (D) *NR3C2* rs2070950 genotypes.
Figure S3. Diastolic dysfunction grade improvement with spironolactone by (A) *AGT* rs699, (B) *CYP11B2* rs1799998, (C) *AGT* rs5051, and (D) *NR3C2* rs2070950 genotypes.
Table S1. Baseline characteristics by genotype.
Table S2. Minor allele frequencies of studied genetic polymorphisms among Egyptian patients compared to reported population frequencies.
Table S3. Change in clinical measures with spironolactone by genotypic combination.

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