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ORIGINAL ARTICLE

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Association of angiotensin-converting enzyme 2 (ACE2) gene polymorphisms with parameters of left ventricular hypertrophy in men

Results of the MONICA Augsburg echocardiographic substudy

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Abstract Angiotensin-converting enzyme (ACE) activity is considered to be of major importance for the conversion of angiotensin (Ang) I to Ang II. Recently, a second ACE, named ACE2, has been identified. Experimental data provide evidence that ACE2 might be involved in modulating cardiac structure and function. In the present explorative study, we assessed whether polymorphisms in the ACE2 gene are related to echocardiographically determined parameters of left ventricular mass, structure or function in the general population. Five intronic single nucleotide polymorphisms (SNPs) were genotyped using the 5′-exonuclease activity (TaqMan) assay in the echocardiographic substudy of the third MONICA Augsburg survey. As ACE2 is located on the X chromosome, women and men were analysed separately. Four SNPs showed high pairwise linkage disequilibrium (rs4646156, rs879922, rs4240157 and rs233575). The minor alleles of these four SNPs were associated with higher left ventricular mass index (LVMI) and higher septal wall thickness (SWT) in men. Likewise, male carriers of a common haplotype

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(frequency 29.9%) consisting of the minor alleles of these four SNPs displayed higher values for LVMI and SWT than non-carriers (LVMI: TGGC 98.8±1.52 vs non-TGGC 94.8±0.99 g/m^2, p=0.027; SWT: TGGC 11.5±0.14 vs non-TGGC 11.1±0.09 mm, p=0.019). Furthermore, this haplotype was associated with an increased odds ratio (OR) for left ventricular hypertrophy (OR 3.10, p=0.006). In women, similar but less pronounced and consistent trends were observed. No association was observed between any of these SNPs and parameters of left ventricular systolic or diastolic function nor with blood pressure levels. This study provides evidence that genetic variants in the ACE2 gene may be associated with left ventricular mass, SWT and left ventricular hypertrophy in hemizygous men.

Keywords Hypertrophy · ACE2 · Genetics · Polymorphism

Introduction

The angiotensin-converting enzyme (ACE) is a key enzyme of the renin-angiotensin system (RAS) producing angiotensin (Ang) II by removing the C-terminal dipeptide from Ang I [1]. Ang II is considered to be the main effector of the system. For example, isolated rat hearts display a hypertrophic response to Ang II which is associated with increased protein uptake [2]. Moreover, elevated Ang I to Ang II conversion rates were found in rat hearts with adaptive left ventricular hypertrophy (LVH) [3]. In clinical and experimental studies of hearts exposed to pressure overload, ACE inhibition ameliorates cardiac hypertrophy [4–8]. More evidence for involvement of RAS in modulation of cardiac structure is provided by genetic studies. In particular, genetic variants within the RAS were found to be associated with electrocardiographically or echocardiographically determined LVH [9–12].

Recently, a homologue of ACE, termed ACE2, has been discovered [13, 14]. It is highly expressed in heart, kidney and testes [13, 14]. The ACE2 gene maps to the X chromosome and contains 18 exons, encoding an 805 amino acids protein. This protein has been shown to catalyse the hydrolysis of several biological peptides [13–15]. Of the peptides of RAS, Ang II is hydrolysed into Ang 1–7 with high catalytic efficiency, whereas Ang I is hydrolysed only partially by ACE2 [15]. Thus, ACE2 may act as a counter-regulatory system to ACE by degrading Ang II and producing the vasodilator Ang 1–7 [16–18]. Furthermore, the ACE2 gene has been mapped to a quantitative trait locus (QTL) on the X chromosome in three different rat models of hypertension, suggesting ACE2 as a candidate gene for hypertension in rats [19]. In mice, targeted disruption of ACE2 gene resulted in severely reduced cardiac contractility and increased circulating Ang II levels, suggesting ACE2 may also modulate cardiac function [19]. In addition, ACE2 mRNA was found to be up-regulated in patients with dilated or ischaemic cardiomyopathy and in rats after myocardial infarction [20, 21]. Taken together, these data suggest that ACE2 may be an important factor in the modulation of cardiac structure or function [18].

Given these experimental data, we assessed in the present epidemiological study whether single nucleotide polymorphisms (SNPs) in ACE2 gene are associated with blood pressure or with parameters of left ventricular mass, structure or function.

Materials and methods

Study population

The subjects of this study participated in the echocardiographic substudy (total n=1,674) of the third MONICA (Monitoring of trends and determinants in Cardiovascular disease) Augsburg survey 1994/1995 [22, 23]. The third survey represents a gender- and age-stratified random sample of all German residents of the Augsburg area and consists of individuals 25 to 74 years of age, with about 300 subjects for each 10-year increment. The population was studied by physical examination, blood testing and a standardized interview, including medical history, physical activity, medication and personal habits. Resting blood pressure was taken according to MONICA guidelines using the random zero method and standard mercury

Table 1 Baseline characteristics of the study population stratified by gender

	Men	Women
n (%)	536 (48.9)	561 (51.1)
Age (year)	50.3±13.7	50.0±13.5
BMI (kg/m^2)	26.9±3.2	26.4±4.5
SBP (mmHg)	136±19	130±21
DBP (mmHg)	83±12	79±11
Diabetes (%)	4.7	2.9
LVMI (g/m^2)	96.0±22.6	82.0±19.9
Septal wall (mm)	11.2±2.2	10.0±2.0
Posterior wall (mm)	9.2±1.4	8.3±1.4
LVEDD (mm)	50.1±4.4	45.9±4.2
n	534	561
EF (%)	58.58±7.6	60.02±7.6
FS (%)	35.12±5.8	36.18±5.9
n	485	514
E/A ratio	1.21±0.436	1.27±0.539
n	361	411
IVRT (ms)	82.0±18.3	77.4±17.5

Data are mean±standard deviation (age, BMI, SBP, DBP, LVMI, septal wall, posterior wall, LVEDD, E/A ratio and IVRT) or per cent (diabetes, EF and FS)

BMI Body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *LVMI* left ventricular mass index, *LVEDD* left ventricular end-diastolic diameter, *EF* ejection fraction, *FS* fractional shortening, *E/A ratio* early/late diastolic filling velocities, *IVRT* isovolumetric relaxation time

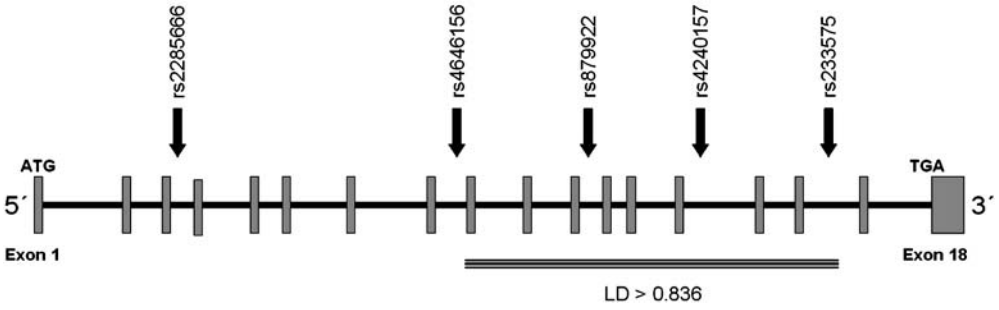

Fig. 1 Genomic organization of the human ACE2 gene and localization of the studied SNPs (according to http://snpper.chip.org/). ACE2 gene contains 18 exons. Each exon is represented by a *box*; exon length and intron length are not on the scale. The *arrows* mark the positions of the SNPs. The LD block of four of these SNPs, namely, rs4646156, rs879922, rs4240157 and rs233575, is represented by a *black line*

sphygmomanometers after subjects had been in a sitting position. Body weight in kilogram and height in metres were determined with subjects wearing light clothing. Written informed consent was obtained from all subjects, and a local ethical committee approved the study protocol.

Echocardiographic measurements

A two-dimensionally guided M-mode echocardiogram recorded on a strip-chart paper at 50 mm/s was performed on each subject by one of two expert sonographers using a single recorder (Sonos 1500, Hewlett-Packard Inc.). Only tracings that demonstrated optimal visualisation of left ventricular interfaces were used, a requirement that resulted in exclusion of 15% of potential subjects. The echocardiographers were blinded for clinical and biochemical data.

Left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), septal wall thickness (SWT) and posterior wall thickness (PWT) were measured as recommended by the American Society of Echocardiography [24]. Left ventricular mass was calculated using the formula $0.8\times1.04[(\mathrm{LVEDD+SWT+PWT})^3-(\mathrm{LVEDD})^3]+0.6$ as described by Devereux et al. [25]. Left ventricular mass was indexed to body surface area (LVMI). LVH was defined as LVMI >134 g/m^2 in men and LVMI >110 g/m^2 in women [24, 26].

Left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) were determined with the Teichholz equations [27]. The ejection fraction (EF) was calculated as EF=(LVEDV−LVESV)/LVEDV.

Doppler echocardiograms were recorded at 100 mm/s using pulsed wave Doppler with the sample volume at the tips of the mitral valve in the apical four chamber view. Early (E) and late (A) diastolic filling velocities and ratios of the early and late diastolic filling velocities (E/A ratio) were determined. Isovolumetric relaxation time (IVRT) was determined as the interval between the end of the aortic outflow and the start of the mitral inflow signal.

Genotyping

Five SNPs, spanning almost the whole ACE2 gene (rs2285666 in intron 3, rs4646156 in intron 8, rs879922 in intron 11, rs4240157 in intron 14 and rs233575 in intron 16), were chosen for the present study. Information for SNPs (rs number, polymorphic site and localization within the gene) was taken from SNPper, a web-based application designed to facilitate the retrieval and use of human SNPs (http://snpper.chip.org/), and from the SNP Database of NCBI (http://www.ncbi.nih.gov/). SNPs were genotyped using 5′-exonuclease activity (TaqMan) assay on a HT7900 (Applied Biosystems, Darmstadt, Germany). SNP assays were ordered from Applied Biosystems either as Custom TaqMan SNP genotyping assays (rs4646156 and rs4240157) or TaqMan SNP genotyping assays (rs2285666, rs879922 and rs233575). Probes were labelled with the fluorophores, FAM or VIC. Genotyping was done on 384-well plates prepared with the GENESIS Freedom pipetting robot from Tecan (Crailsheim, Germany). The Universal PCR Master Mix from Applied Biosystems was used in a 5-μl total

Table 2 Genotype frequencies of ACE2 SNPs in men and women

	Men		Women		
	1	3	1	2	3
Rs2285666, *n* (%)	414 (77.2)	122 (22.8)	367 (65.4)	176 (31.4)	18 (3.2)
Rs4646156, *n* (%)	350 (65.3)	186 (34.7)	222 (39.6)	264 (47.1)	75 (13.4)
Rs879922, *n* (%)	358 (66.8)	178 (33.2)	231 (41.2)	262 (46.7)	68 (12.1)
Rs4240157, *n* (%)	358 (66.8)	178 (33.2)	229 (40.8)	263 (46.9)	69 (12.3)
Rs233575, *n* (%)	364 (67.9)	172 (32.1)	241 (43.0)	255 (45.5)	65 (11.6)

1=(homozygous) common allele, 2=heterozygous, 3=(homozygous) minor allele

Table 3a Association of different SNPs within the ACE2 gene with echocardiographic parameters of LV mass, systolic and diastolic function in men

	Rs2285666			Rs4646156		
	C	T	*p* values[a]	A	T	*p* values[a]
n	414	122		350	186	
LVMI (g/m^2)	96.2±0.95	95.2±1.76	0.61	94.9±1.03	98.2±1.41	0.057
Septal wall (mm)	11.2±0.09	11.4±0.16	0.23	11.1±0.09	11.5±0.13	0.033
Posterior wall (mm)	9.2±0.06	9.2±0.11	0.64	9.2±0.06	9.2±0.09	0.55
LVEDD (mm)	50.2±0.21	49.6±0.39	0.15	50.0±0.23	50.2±0.32	0.70
n	412	122		349	185	
EF (%)	58.7±0.37	58.2±0.68	0.49	58.7±0.40	58.4±0.55	0.70
FS (%)	35.2±0.28	34.7±0.52	0.33	35.2±0.31	35.0±0.42	0.71
n	369	116		324	161	
E/A ratio	1.22±0.017	1.18±0.031	0.27	1.19±0.019	1.25±0.026	0.064
n	274	87		239	122	
IVRT (ms)	81.2±1.02	84.4±1.82	0.13	82.8±1.10	80.4±1.54	0.21
	Rs879922			Rs4240157		
	C	G	*p* values[a]	A	G	*p* values[a]
n	358	178		358	178	
LVMI (g/m^2)	94.8±1.02	98.4±1.45	0.040	94.6±1.01	98.8±1.44	0.017
Septal wall (mm)	11.1±0.09	11.5±0.13	0.008	11.1±0.09	11.5±0.13	0.006
Posterior wall (mm)	9.1±0.06	9.3±0.09	0.18	9.1±0.06	9.3±0.09	0.12
LVEDD (mm)	50.1±0.23	50.0±0.33	0.65	50.1±0.23	50.0±0.33	0.73
n	357	177		357	177	
EF (%)	58.7±0.40	58.4±0.56	0.74	58.7±0.40	58.4±0.56	0.71
FS (%)	35.2±0.30	35.0±0.43	0.71	35.2±0.30	35.0±0.43	0.68
n	329	156		329	156	
E/A ratio	1.19±0.018	1.25±0.027	0.065	1.19±0.018	1.25±0.027	0.097
n	242	119		242	119	
IVRT (ms)	82.9±1.09	80.0±1.56	0.13	82.9±1.09	80.1±1.56	0.14
	Rs233575					
	T		C		*p* values[a]	
n	364		172			
LVMI (g/m^2)	94.8±1.01		98.7±1.47		0.029	
Septal wall (mm)	11.1±0.09		11.5±0.13		0.012	
Posterior wall (mm)	9.1±0.06		9.3±0.09		0.14	
LVEDD (mm)	50.1±0.23		50.0±0.33		0.86	
n	363		171			
EF (%)	58.6±0.39		58.5±0.57		0.90	
FS (%)	35.1±0.30		35.1±0.44		0.88	
n	335		150			
E/A ratio	1.20±0.018		1.24±0.027		0.19	
n	248		113			
IVRT (ms)	82.7±1.08		80.5±1.60		0.26	

Values are least square means± SE. LVMI, septal and posterior wall are adjusted for age, BMI, antihypertensive medications and systolic blood pressure. EF and FS are adjusted for BMI. E/A ratio and IVRT are adjusted for age and diastolic blood pressure

LVMI Left ventricular mass index, *LVEDD* left ventricular end-diastolic diameter, *EF* ejection fraction, *FS* fractional shortening, *E/A ratio* early/late diastolic filling velocities, *IVRT* isovolumetric relaxation time

[a]Two-tailed descriptive *p* values from *t* test for independent groups for the frequent vs the rare allele

reaction volume with 10 ng DNA per reaction. Allelic discrimination was measured automatically on the ABI Prism HT7900 (Applied Biosystems) using the Sequence Detection Systems 2.1 software (autocaller confidence level 95%). Ten per cent of all genotypes were repeated in independent PCR reactions to check for consistency and to ensure intra-and inter-plate genotype quality control. No genotyping discrepancies were detected between the repeated samples. The overall mis-genotyping rate of 23% was due to insufficient PCR amplification.

Statistical analysis

To determine whether the genotypes of the different polymorphisms deviated from Hardy–Weinberg equilibrium, actual and predicted genotype counts were compared by a chi-squared goodness-of-fit test with 1 degree of freedom. Men and women were analysed separately, because the ACE2 gene is located on the X chromosome. Least square means for echocardiographic parameters according to genotypes of the different ACE2 SNPs were

Table 3b Association of different SNPs within the ACE2 gene with echocardiographic parameters of LV mass, systolic and diastolic function in women

	Rs2285666					Rs4646156				
	CC	CT	TT	*p* values[a]	*p* values[b]	AA	AT	TT	*p* values[a]	*p* values[b]
n	367	176	18			222	264	75		
LVMI (g/m^2)	83.1±0.80	79.5±1.15	84.6±3.59	0.47	0.022	81.3±1.03	82.1±0.95	83.3±1.78	0.44	0.43
Septal wall (mm)	10.0±0.08	10.0±0.12	10.3±0.36	0.46	0.91	9.9±0.10	10.1±0.09	10.1±0.18	0.49	0.25
Posterior wall (mm)	8.3±0.06	8.3±0.09	8.3±0.27	0.82	0.66	8.2±0.08	8.3±0.07	8.5±0.13	0.16	0.52
LVEDD (mm)	46.2±0.21	45.2±0.30	46.3±0.94	0.68	0.010	46.0±0.27	45.7±0.25	46.1±0.47	0.56	0.65
EF (%)	60.0±0.39	59.9±0.57	62.2±1.78	0.20	0.86	59.7±0.51	60.4±0.46	59.8±0.87	0.77	0.36
FS (%)	36.1±0.31	36.1±0.44	38.1±1.38	0.16	0.84	35.9±0.39	36.5±0.36	36.0±0.67	0.74	0.36
n	335	165	14			206	240	68		
E/A ratio	1.29±0.021	1.26±0.030	1.13±0.103	0.17	0.34	1.26±0.027	1.30±0.025	1.25±0.047	0.53	0.47
n	258	138	15			170	189	52		
IVRT (ms)	77.5±0.96	77.3±1.31	75.7±3.97	0.67	0.82	77.2±1.18	78.0±1.12	76.0±2.13	0.47	0.79
	R879922					Rs4240157				
	CC	CG	GG	*p* values[a]	*p* values[b]	AA	AG	GG	*p* values[a]	*p* values[b]
n	231	262	68			229	263	69		
LVMI (g/m^2)	81.8±1.01	82.2±0.95	81.8±1.86	0.93	0.79	81.1±1.01	82.7±0.94	82.0±1.85	0.98	0.28
Septal wall (mm)	9.9±0.10	10.1±0.09	10.2±0.18	0.38	0.060	9.8±0.10	10.1±0.09	10.2±0.18	0.42	0.022
Posterior wall (mm)	8.2±0.08	8.3±0.07	8.4±0.14	0.34	0.45	8.2±0.08	8.3±0.07	8.4±0.14	0.38	0.43
LVEDD (mm)	46.2±0.26	45.7±0.25	45.7±0.49	0.68	0.13	46.0±0.27	45.8±0.25	45.8±0.48	0.90	0.46
EF (%)	59.7±0.50	60.3±0.47	60.2±0.91	0.81	0.39	59.7±0.50	60.2±0.46	60.3±0.91	0.73	0.46
FS (%)	35.9±0.39	36.4±0.36	36.3±0.71	0.84	0.36	35.9±0.39	36.3±0.36	36.4±0.71	0.74	0.41
n	216	236	62			214	237	63		
E/A ratio	1.24±0.026	1.32±0.025	1.21±0.049	0.16	0.12	1.25±0.026	1.32±0.025	1.19±0.048	0.061	0.20
n	178	188	45			176	191	44		
IVRT (ms)	78.0±1.15	76.7±1.12	78.0±2.29	0.80	0.51	77.5±1.16	77.2±1.11	78.3±2.31	0.68	0.96

	Rs233575				
	TT	TC	CC	*p* values[a]	*p* values[b]
n	241	255	65		
LVMI (g/m^2)	81.2±0.99	82.7±0.96	82.0±1.90	0.99	0.29
Septal wall (mm)	9.9±0.10	10.1±0.10	10.2±0.19	0.32	0.028
Posterior wall (mm)	8.2±0.07	8.3±0.07	8.4±0.14	0.29	0.44
LVEDD (mm)	46.1±0.26	45.8±0.25	45.6±0.50	0.59	0.35
EF (%)	59.7±0.49	60.2±0.47	60.3±0.94	0.72	0.41
FS (%)	35.9±0.38	36.3±0.37	36.4±0.73	0.74	0.40
n	226	229	59		
E/A ratio	1.25±0.025	1.32±0.025	1.20±0.050	0.093	0.28
n	184	185	42		
IVRT (ms)	77.5±1.13	77.1±1.13	78.6±2.37	0.60	0.92

Values are least square means±SE. LVMI, septal and posterior wall are adjusted for age, BMI, antihypertensive medications and systolic blood pressure. LVEDD is adjusted for age, BMI and systolic blood pressure. EF and FS are adjusted for BMI. E/A ratio and IVRT are adjusted for age and diastolic blood pressure

LVMI Left ventricular mass index, *LVEDD* left ventricular end-diastolic diameter, *EF* ejection fraction, *FS* fractional shortening, *E/A ratio* early/late diastolic filling velocities, *IVRT* isovolumetric relaxation time

[a]Two-tailed descriptive *p* values from *t* test for independent groups for the recessive model (homozygous rare vs heterozygous+homozygous frequent allele)

[b]Two-tailed descriptive *p* values from *t* test for independent groups for the dominant model (homozygous rare+heterozygous vs homozygous frequent allele)

Table 4 Association of the different four SNP haplotypes with parameters of LV mass in men (order of the SNPs: rs4646156, rs879922, rs4240157 and rs233575)

	TGGC	ACAT	TCAT	Others	*p* values[a]
n (%)	160 (29.9)	336 (62.7)	20 (3.7)	20 (3.7)	
LVMI (g/m^2)	98.8±1.52	94.7±1.05	93.7±4.30	98.1±4.32	0.027
Septal wall (mm)	11.5±0.14	11.1±0.09	11.0±0.38	11.6±0.39	0.019

Values are least square means±SE adjusted for age, BMI, antihypertensive medications and systolic blood pressure. LVMI, left ventricular mass index

[a]Two-tailed descriptive p-values from t-test for independent groups for the TGGC-haplotype vs all other haplotypes

calculated, adjusting for relevant covariates. LVMI, SWT, PWT, EF and fractional shortening (FS) were adjusted for age, BMI, systolic blood pressure and antihypertensive medications. IVRT and E/A ratio were adjusted for age and diastolic blood pressure. The covariates were kept in the model if $p \leq 0.05$. For men, *p* values of a two-tailed *t* test for independent groups were calculated. For women, *p* values of a two-tailed *t* test for independent groups for a dominant model and for a recessive model were determined.

Thereafter, in men, least square means for LVMI and SWT according to different four SNP haplotypes were calculated (adjusted for age, BMI, systolic blood pressure and antihypertensive medications), and two-tailed *t* tests for independent groups (carrier of the four SNP haplotype consisting of those alleles being associated with higher LVMI and SWT in the separate evaluation of each SNP vs non-carrier of this haplotype) were calculated. For men, haplotypes were determined directly from the genotypes, whereas, for women, haplotype frequencies were estimated with the expectation–maximization algorithm. To assess whether the SNPs or haplotypes were associated with LVH as a binary variable (presence vs absence of LVH), odds ratios (ORs) and 95% confidence intervals (CI) were calculated using a logistic regression model with LVH as dependent variable and genotype or haplotype, age, BMI, systolic blood pressure and antihypertensive medications as independent variables.

To evaluate in women whether LVMI, SWT or LVH were associated with the most frequent haplotypes consisting of those three SNPs that were associated with SWT in the separate analyses of each SNP, score statistics based on a chi-squared distribution with 1 degree of freedom, *p* values and simulated *p* values (number of simulations 10,000) adjusted for age, BMI, antihypertensive medications and systolic blood pressure were calculated [28]. Due to the exploratory character of the present study, all *p* values are considered descriptive.

Results

For the present study, 1,294 individuals were successfully genotyped for five ACE2 SNPs. Six individuals were excluded due to missing covariates. Two-dimensional echocardiograms of optimal quality were obtained in 1,097 of the 1,294 participants. Doppler mitral profiles of sufficient quality were obtained in 999 participants to determine diastolic filling velocities and in 772 participants to determine IVRT.

Baseline characteristics stratified by gender display the expected differences in systolic and diastolic blood pressure, BMI and in the different echocardiographic phenotypes between men and women (Table 1). Figure 1 shows a schematic illustration of the genomic organization of the human ACE2 gene and localization of the studied SNPs. The genotype frequencies of all SNPs (Table 2) did not deviate from those predicted by Hardy–Weinberg equilibrium. Pairwise correlations of neighbouring SNPs suggest that rs4646156, rs879922, rs4240157 and rs233575 were in linkage disequilibrium (LD; data not shown).

Association of ACE2 SNPs with blood pressure

We did not find an association of systolic or diastolic blood pressure or pulse pressure with any of the SNPs analysed.

Table 5 ORs with 95% CI for LVH associated with the minor allele of different ACE2 SNPs in men

		OR (CI) for LVH[a]	*p* values[b]
Rs2285666	Minor vs frequent allele (Ref)	0.69 (0.25–1.94)	0.48
Rs4646156	Minor vs frequent allele (Ref)	2.47 (1.11–5.49)	0.027
Rs879922	Minor vs frequent allele (Ref)	3.23 (1.44–7.24)	0.004
Rs4240157	Minor vs frequent allele (Ref)	3.68 (1.63–8.30)	0.002
Rs233575	Minor vs frequent allele (Ref)	3.17 (1.42–7.10)	0.005
Haplotype[c]	TGGC vs non-TGGC (Ref.)	3.10 (1.38–6.96)	0.006

[a]Adjusted for age, body mass index, systolic blood pressure and antihypertensive medications

[b]Descriptive *p* values from binary regression analysis

[c]Haplotype consisting of the SNPs rs4646156, rs879922, rs4240157 and rs233575

In particular, neither in men nor in women, ACE2 SNPs display association with blood pressure or pulse pressure in a multivariate model corrected for age and BMI as covariates (data not shown).

Association of ACE2 SNPs with echocardiographic parameters of left ventricular mass, structure and function

The association of each SNP with parameters of left ventricular mass, geometry and function in a multivariate model is reported, and descriptive *p* values are given. In men, the minor alleles of four SNPs (rs4646156, rs879922, rs4240157 and rs233575) were consistently associated with higher values for LVMI and SWT (Table 3a) as well as with a higher OR for LVH (Table 5), whereas rs2285666 showed no association with LVMI, SWT or LVH in men (Tables 3a and 5). Therefore, the association of one haplotype (TGGC), consisting of the minor alleles of these four SNPs with LVMI, SWT and LVH, is reported in men. In a multivariate model, male carrier of the TGGC haplotype displayed higher values for LVMI and SWT than non-carrier (Table 4). Furthermore, in a multivariate model, this haplotype was independently associated with a higher OR for LVH (Table 5).

In women, the minor alleles of three SNPs (rs879922, rs4240157 and rs233575) were associated with slightly higher SWT in a dominant model. However, none of these SNPs was associated with LVMI, and no haplotype consisting of these three SNPs was associated with LVMI or SWT in women. Furthermore one SNP (rs2285666) outside the LD block mentioned above was associated with lower LVEDD and lower LVMI (Table 3b). Neither in men nor in women an association with parameters of left ventricular systolic or diastolic function was found (Tables 3a and 3b).

Discussion

In the present study, we assessed systematically the association of five polymorphisms within the ACE2 gene, spanning from intron 3 to intron 16, with echocardiographic parameters of left ventricular mass, structure and function. Four of these SNPs (located in introns 8, 11, 14 and 16) define a haplotype block with high pairwise LD values.

The main finding of our study is that a haplotype (TGGC) consisting of the minor alleles of these four SNPs (rs4646156, rs879922, rs4240157 and rs233575) was associated with increased LVMI and increased SWT as well as with a higher OR for LVH in men. Separate analyses of each SNP revealed that, consistently, the minor alleles of these four SNPs (rs4646156, rs879922, rs4240157 and rs233575) were associated with higher LVMI and SWT as well as with a higher OR for LVH in men (Tables 4 and 5). In women, however, the results are inconclusive. Likewise, the minor alleles of three SNPs (r879922, rs4240157 and rs233575) within the above-mentioned LD block were associated with slightly higher SWT. However, none of these SNPs was associated with LVMI. Furthermore, no haplotype consisting of these SNPs was associated with SWT or LVMI in women. Therefore, the association of these three SNPs with SWT in women may be a chance finding and thus false positive. In addition, one SNP (rs2285666) outside this LD block was associated with lower LVEDD and LVMI in women. As we found no association with LVMI for rs4646156, rs879922, rs4240157 and rs233575 in women, we addressed the question of sufficient power to detect small differences in LVMI between both alleles of these SNPs in women in our sample. We found that the present sample had satisfying power to detect a minimal difference in LVMI of 2.6 g/m^2 between the minor and the frequent allele of the respective SNPs in women ($1-\beta$=0.8, α=0.05). Thus, if the allelic association of LVMI in women would have been comparable to that found in men, it should have been detectable.

The data should be seen in the context of experimental evidence, suggesting that ACE2 modulates cardiac structure and function [18]. In mice, targeted disruption of ACE2 resulted in a severely reduced cardiac contractility and increased Ang II levels [19]. Furthermore, in rats, cardiac mRNA expression of ACE2 was increased after myocardial infarction [21]. Likewise, ACE2 immunoreactivity was found to be significantly increased in cardiac tissue of patients with failing hearts due to ischaemic heart disease [21]. Furthermore, in another study, cardiac ACE2 mRNA was found to be increased in patients with dilated or ischaemic cardiomyopathy [20]. Our data add evidence that the genetic variability in the ACE2 gene might modulate cardiac structure, although no evidence could be obtained that it may affect cardiac systolic or diastolic function.

The functional mechanisms underlying the observed association between SNPs in ACE2 gene and echocardiographically determined LVMI and SWT are not yet clear.

The association of ACE2 SNPs with LVMI, SWT and LVH could be observed in men but was inconsistent in women. This is in line with gender-specific differences in RAS [29]. Numerous studies demonstrated that genetic variants of ACE, angiotensinogen and Ang II receptor genes display more profound effects in men, and several studies were only positive in men [9, 29–34]. It is therefore conceivable that also genetic variants in ACE2 display gender-specific effects.

Experimental data provide evidence that ACE2 might also be involved in the pathophysiology of hypertension. In rats, ACE2 gene mapped within a QTL for hypertension in three different rat models of hypertension [19]. However, in an association study in Australia, no association of polymorphisms in ACE2 gene with hypertension was found [35]. In line with these data, we could not observe an association of any of the five SNPs with diastolic or systolic blood pressure nor with pulse pressure in the present study from the general population (data not shown).

Finally, some limitations of the present study need to be mentioned. First, despite the size of our sample, it is important to reproduce the findings in several independent

populations. Second, functional data to explain the association between the TGGC haplotype and LVMI, SWT and LVH in men will be needed. The first step may therefore be to find the causative genetic variant for this association. Third, the results in women are inconsistent.

In conclusion, the present study provides for the first time, to our knowledge, evidence that ACE2 gene variants might be involved in modulation of left ventricular mass in men. No evidence could be obtained that ACE2 modulates left ventricular systolic or diastolic function nor blood pressure levels in the general population.

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