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Association of *ACE2* Genetic Variants With Blood Pressure, Left Ventricular Mass, and Cardiac Function in Caucasians With Type 2 Diabetes

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BACKGROUND
Cardiovascular disease is common in diabetes, and is associated with activation of the renin–angiotensin system (RAS). Angiotensin-converting enzyme (ACE)2 is a recently described member of the RAS, and this study investigated whether *ACE2* polymorphisms are associated with hypertension, left ventricular (LV) mass, and cardiac function in type 2 diabetes.

METHODS
Variants in *ACE2* (rs1978124, rs2074192, rs4240157, rs4646156, rs4646188) were examined in 503 Caucasian subjects with type 2 diabetes. As *ACE2* is located on the X chromosome, analyses were performed separately for men and women. Hypertension was defined by a history of hypertension, and/or antihypertensive medications or blood pressure (BP) >130/80 mm Hg. LV mass and systolic function (ejection fraction) were assessed by transthoracic echocardiography.

RESULTS
In men, hypertension was more prevalent with the *ACE2* rs2074192 C allele ($P = 0.023$), rs4240157 G allele ($P = 0.016$) and rs4646188 T allele ($P = 0.006$). In men, the rs1978124 A allele was associated with a significantly lower ejection fraction compared to the G allele (62.3 ± 13.3 vs. 67.2 ± 10.9 %, $P = 0.002$). This association remained significant after covariate adjustment for age, body mass index, hypertension, antihypertensive treatment, and BP. In women, the prevalence of hypertension was higher ($P = 0.009$) with the rs4240157 G allele, and the rs1978124 A allele was associated with significantly higher LV mass ($P = 0.008$).

CONCLUSIONS
In Caucasians with type 2 diabetes, genetic variation in *ACE2* is associated with hypertension and reduced systolic function in men, and hypertension and increased LV mass in women.

Keywords: *angiotensin-converting enzyme 2; blood pressure; cardiac function; cardiac hypertrophy; hypertension; renin–angiotensin system; type 2 diabetes*

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Type 2 diabetes mellitus is a coronary heart disease risk equivalent[1] associated with a significant cardiovascular disease burden,[2,3] which includes a two to threefold increase in cardiac death[4] and an increased risk of heart failure.[5] Diabetes also directly contributes to the development of hypertension and left ventricular (LV) hypertrophy (LVH).[6]

Activation of the renin–angiotensin system (RAS) plays an important role in the development and progression of cardiovascular complications in diabetes, and blockade of the RAS by angiotensin-converting enzyme (ACE) inhibitors and angiotensin (Ang) II receptor blockers have been shown to reduce blood pressure (BP) and the cardiac and renal complications of diabetes.[7] The enzyme ACE converts Ang I to the vasoconstrictor peptide Ang II which is the main effector of the RAS. ACE2 is a recently discovered homologue of ACE,[8,9] with one active enzymatic site and is responsible for degrading Ang II to the vasodilator Ang 1–7. ACE2 expression is mainly limited to the heart and kidney, and ACE2 is thought to act in a counter-regulatory manner to ACE to limit the detrimental effects of Ang II.[10] The *ACE2* gene maps to chromosome Xp22 and contains 18 exons spanning ~40 kb of genomic DNA and encoding a protein of 805 amino acids.[9]

A number of experimental studies suggest that ACE2 may protect against increased BP and cardiac dysfunction. In three strains of genetically hypertensive rats, the *ACE2* gene maps to a quantitative trait locus on the X chromosome that was previously identified as a quantitative locus for BP.[11] Higher BP has been associated with reduced renal ACE2 mRNA and protein levels in experimental models[11,12] suggesting that ACE2 may

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have a protective effect. Certainly, in ACE2 knockout mice,[11] which lack ACE2, BP is increased, and cardiac systolic function is severely impaired. Interestingly the abnormalities are more severe in male than female mice, and the phenotype more pronounced in older animals. Other studies have shown that deletion of ACE2 accelerates cardiac hypertrophy and shortens the time taken for mice to go from compensatory hypertrophy to cardiac failure.[13]

Currently, few studies have investigated whether common single-nucleotide polymorphisms (SNPs) in the human *ACE2* locus correlate to changes in BP or cardiac function. The results available differ according to the ethnicity of the population, as well as the phenotype of the population studied. The allele frequencies of *ACE2* SNPs are significantly different between subjects of Asian and European ancestry, and the SNP alleles associated with BP in Chinese populations are not common or represented in European populations.[14–16] Thus in Chinese women, there are significant associations with *ACE2* SNP rs228566 and essential hypertension[14] and diastolic BP[17] and *ACE2* (rs1978124, rs4646142) with myocardial infarction.[18] However, in Australian Caucasians with essential hypertension, no association between four *ACE2* SNPs (rs1978124, rs2285666, rs879922, rs714205) and hypertension was found in either men or women.[15] Similarly in a cohort of Austrian subjects randomly recruited from the general population as part of the MONICA study,[19] no association with BP levels was reported in men or women. However, there were significant associations with four *ACE2* gene SNPs (rs4646156, rs879922, rs4240157 and rs233575) with higher LV mass and LVH in men, but not in women. In a study of 729 men recruited after admission for an acute coronary syndrome event, the A allele of the *ACE2* rs1978124 was significantly associated with male survival in a New Zealand Caucasian population.[20] There appear to be similarities in the association of *ACE2* rs1978124 in Chinese and Caucasian populations with regard to coronary heart disease and myocardial infarction.[18,20]

In summary, the limited reported studies to date have given conflicting results which may be partially explained by the cohort under investigation. Moreover, although cardiovascular disease is common in diabetes,[21,22] there have been no previous reports of associations of *ACE2* polymorphisms and cardiovascular disease in diabetes. Thus, the aim of this study was to assess whether genetic variations in *ACE2* are associated with hypertension, LVH and cardiac dysfunction in a cohort of subjects with type 2 diabetes.

METHODS

Study sample. Subjects with type 2 diabetes attending for a transthoracic echocardiograpam as part of a routine complications surveillance program at Austin Health, Melbourne, Australia were prospectively recruited as previously described.[22] As our primary referral base (80%) is from general practitioners, with only 20% referred from within the hospital, the cohort is representative of subjects with type 2 diabetes seen in the wider community. Ethical approval was obtained from the Human Research Ethics Committee at Austin Health. Subjects of non-European ancestry were excluded, as were those with moderate/severe valvular dysfunction.

Medical history and clinical measurements. Each subject completed a questionnaire at the time of the echocardiogram, crosschecked by medical record review. Information on diabetes duration, history of hypertension, antihypertensive drug therapy (ACE inhibitors, Ang receptor blockers, diuretics, calcium-channel blockers, β-blockers) and ethnic background was obtained. Height and weight were measured as previously described.[22] BP was measured manually using a mercury sphygmomanometer at the time of the echocardiogram in a supine position, after the subject was rested quietly for 5 min. Hypertension was defined as present if participants were on antihypertensive medication, had a history of hypertension and/or had evidence of hypertension (clinic BP >130/80 mm Hg).[23] Glycosylated hemoglobin (HbA_{1c}) was measured at Austin Health, and whole blood collected for DNA extraction.

Echocardiography. Transthoracic echocardiography was performed as previously described using a commercially available ultrasound system (Vivid 7, 3.5 MHz transducer).[22,24,25] Standard parasternal and apical views were used to assess LV mass and LV systolic function. M-mode echocardiography was used to measure cardiac dimensions and wall thickness. Body surface area was calculated using the Mosteller formula[26] and LV mass was indexed to body surface area.[24] LVH was defined as LV mass index >115 g/m^2 in men and >95 g/m^2 in women.[24] LV ejection fraction (LVEF) was calculated as previously described.[22]

Genotyping. Genomic DNA was extracted using a Nucleon BACC2 kit DNA (GE Healthcare, Sydney, Australia). Analysis of the linkage disequilibrium (D′ and r^2) and haplotype structure of the *ACE2* gene was performed using the HapMap phase II project data from CEPH Caucasian trios and the Haploview software (version 4.2).[27,28] The haplotype tagging SNPs that defined the underlying haplotype structures were identified using pairwise tagging with r^2 thresholds of 0.8 and minor allele frequency thresholds of 0.05. Five tagging SNPs captured all the common variation across the *ACE2* gene. These SNPs were rs1978124 G/A, rs2074192 C/T, rs4240157 A/G, rs4646156 A/T, rs4646188 T/C. The tagging SNPs were genotyped using the Sequenom MassARRAY system (Sequenom, San Diego, CA) at the Australian Genome Research Facility (AGRF, Brisbane, Australia). Of the genotyped samples, 10% were duplicates and there were at least four negative controls per 96 well plate. Genotyping accuracy was determined by the genotype concordance between duplicate samples and was 100% for each of the SNPs.

Statistical analyses. As the *ACE2* gene is located on the X chromosome, males and females were analyzed separately. As males are hemizygous for ACE2, Hardy–Weinberg equilibrium was assessed only in females. All analysis was performed using

SPSS version 18 (SPSS, Chicago, IL). All continuous variables studied were normally distributed (except for diabetes duration) and presented as means ± s.d. Diabetes duration is presented as the median and the interquartile range (25th–75th quartile). The relationship between each SNP with continuous variables (systolic and diastolic BP, LV mass, LVEF) were examined by linear regression using the additive, dominant and recessive genetic models (men are hemizygous therefore only the dominant model was examined). By linear regression analysis the rs1978124 SNP associations were significant with the dominant genetic model (genotypes GG vs. AA) with LVEF in men, and the recessive model (genotypes GG + GA vs. AA) with LV mass in women. The significant SNP associations were examined further using multiple linear regression analysis to test for associations between SNP rs1978124 genotypes and continuous variables (LV mass, LVEF) after adjusting for confounding variables (age, body mass index (BMI), hypertension, use of antihypertensive medication, systolic and diastolic BP). Normality was assessed by evaluating quantile–quantile plots for continuous variables and all quantile–quantile plots were normal. Differences in the prevalence of hypertension and LVH with SNPs were assessed by chi square analysis using the dominant genetic model. Significant SNP associations with hypertension were further assessed using a logistic regression model with hypertension as a dependent variable and genotype, age, BMI, and antihypertensive medication use as independent variables. Logistic regression analysis is presented as odds ratio (95% confidence intervals), *P* values. Two-tailed *P* values <0.05 were considered significant; however, allowance for multiple testing was done by the interpretation of the significant results for testing the five *ACE2* SNPs (a global significance level of 0.05 means a single test significance level of 0.01).

Table 1 | Characteristics of study participants, data shown in the whole cohort and stratified by gender

	Whole cohort	Men	Women
n	503	279	224
Age (years)	61.9 ± 14.2	61.0 ± 15.0	63.1 ± 13.0
BMI (kg/m^2)	30.7 ± 6.2	29.8 ± 5.0	31.9 ± 7.2
Diabetes duration (years)[a]	11 (5.0, 18.0)	10 (5.0, 17.0)	11 (5.1, 19.8)
HbA_{1c} (%)	7.7 ± 1.2	7.7 ± 1.3	7.8 ± 1.1
Blood pressure			
Systolic BP (mm Hg)	137 ± 19	135 ± 18	139 ± 20
Diastolic BP (mm Hg)	75 ± 10	76 ± 10	75 ± 10
Hypertension, % (*n*)	80 (404)	79 (216)	84 (188)
Antihypertensive medication use, % (*n*)	68 (339)	66 (182)	70 (157)
Cardiac structure			
LV mass index (g/m^2)	99.4 ± 27.5	102.8 ± 27.8	95.2 ± 26.7
LVH, % (*n*)	32 (139)	24 (60)	40 (79)
Systolic function			
LV ejection fraction (%)	67.7 ± 11.9	65.5 ± 12.5	70.4 ± 10.6

Data is expressed as mean ± s.d.
BMI, body mass index; BP, blood pressure; HbA_{1c}, glycosylated hemoglobin; LV, left ventricle; LV mass index, indexed to body surface area; LVH, left ventricular hypertrophy (defined as LV mass index >115 g/m^2 men and >95 g/m^2 in women).
[a]Median (25th, 75th quartiles) or % (numbers).

RESULTS

Characteristics of the study participants with type 2 diabetes

As shown in Table 1, there were 503 subjects (279 men, 224 women) aged 61.9 ± 14.2 years (mean ± s.d.), with a BMI of 30.7 ± 6.2 kg/m^2 and median (25th, 75th interquartile range) diabetes duration of 11 (5, 18) years. Mean HbA_{1c} was 7.7 ± 1.2% and similar in men and women. Hypertension was present in 80% of the cohort. The LV mass index was 99.4 g/m^2, and LVEF was 67.7 ± 11.9%. Women were older and had higher systolic BPs, increased prevalence of hypertension and antihypertensive medication use compared to men. In keeping with this, LVH was present in 40% of females and 24% of men. Men had a lower LVEF (65%) compared to women (70%), although in both genders, systolic function was in the normal range.

ACE2 SNPs and genotype frequencies

All genotyped SNPs were in Hardy–Weinberg equilibrium. Five tagging SNPs (rs1978124, rs2074192, rs4240157, rs4646156, and rs4646188) captured all the common variation (minor allele frequencies >5%) in the *ACE2* region from HapMapII (captured 20 SNPs with an r^2 >0.8 and mean r^2 of 0.981). Information on SNP locations, allele and genotype frequencies are shown in Table 2.

Table 2 | Descriptive information on *ACE2* SNPs and genotype frequencies

SNPs[a]	Chromosome X position[b]	Gene region	Major/minor alleles	MAF	Genotype frequencies, % (*n*)				
					Men		Women		
rs1978124	15618063	Intron 1	G/A	0.49	GG 48.5 (133)	AA 51.5 (141)	GG 24.6 (55)	GA 48.2 (108)	AA 27.2 (61)
rs4646188	15601343	Intron 7	T/C	0.10	TT 85.4 (229)	CC 14.6 (39)	TT 83.4 (176)	TC 13.7 (29)	CC 2.8 (6)
rs4646156	15597043	Intron 8	A/T	0.38	AA 62.2 (173)	TT 37.8 (105)	AA 40.8 (91)	AT 42.2 (94)	TT 17.0 (38)
rs4240157	15586964	Intron 14	A/G	0.37	AA 60.9 (170)	GG 39.1 (109)	AA 41.5 (93)	AG 42.4 (95)	GG 16.1 (36)
rs2074192	15582790	Intron 16	C/T	0.44	CC 57.1 (149)	TT 42.9 (112)	CC 34.0 (70)	CT 44.7 (92)	TT 21.4 (44)

ACE2, angiotensin-converting enzyme 2; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.
[a]dbSNP rs identification numbers (http://www.ncbi.nlm.nih.gov/SNP/; last accessed May 2011). [b]Chromosome X SNP positions using the genomic contig NT_167197.1 (http://www.ncbi.nlm.nih.gov/nuccore/NT_167197.1; last accessed July 2011).

Table 3 | Association of *ACE2* SNPs with blood pressure, hypertension, LVH, and cardiac function in men and women

SNPs	Genotypes	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Hypertension, % (*n*)	LV mass (g/m^2)	LVH, % (*n*)	LV ejection fraction (%)
rs1978124							
Men	GG	135 ± 19	76 ± 11	76.7 (102)	98.1 ± 23.7	19 (20)	67.2 ± 10.9
	AA	136 ± 17	76 ± 9	80.1 (113)	104.6 ± 29.4	25 (29)	62.3 ± 13.3
	P value	0.535	0.887	0.487	0.07	0.185	0.002
Women	GG	137 ± 22	75 ± 10.7	80.0 (44)	92.1 ± 23.3[a]	60 (27)	70.0 ± 9.5
	GA + AA	139 ± 19	74 ± 10.4	85.2 (144)	103.5 ± 32.1[a]	58 (84)	71.7 ± 9.1
	P value	0.346	0.671	0.361	0.008	0.548	0.269
rs4646188							
Men	TT	136 ± 20	76 ± 11	81.2 (186)	102.1 ± 27.7	25 (49)	65.9 ± 12.7
	CC	132 ± 17	76 ± 9	61.5 (24)	99.4 ± 23.0	18 (6)	68.8 ± 10.2
	P value	0.225	0.204	0.006	0.586	0.253	0.219
Women	TT	138 ± 20	74 ± 10	83.0 (146)	95.8 ± 28.5	43 (68)	71.5 ± 9.5
	TC + CC	146 ± 19	78 ± 11	88.6 (31)	90.5 ± 17.4	39 (11)	69.6 ± 8.0
	P value	0.048	0.054	0.409	0.331	0.428	0.316
rs4646156							
Men	AA	135 ± 20	76 ± 11	75.7 (131)	99.7 ± 25.4	25 (36)	67.5 ± 12.1
	TT	138 ± 15	77 ± 9	84.8 (89)	105.2 ± 29.0	24 (22)	64.8 ± 12.5
	P value	0.17	0.233	0.072	0.121	0.505	0.105
Women	AA	136 ± 21	74 ± 10	78.0 (71)	90.8 ± 22.9	36 (29)	70.9 ± 9.0
	AT + TT	142 ± 18	74 ± 11	87.3 (116)	98.4 ± 27.9	48 (54)	71.8 ± 9.4
	P value	0.062	0.953	0.049	0.045	0.064	0.519
rs4240157							
Men	AA	134 ± 20	75 ± 10	74.1 (126)	100.5 ± 25.9	25 (36)	65.3 ± 12.4
	GG	138 ± 15	77 ± 9	86.2 (94)	103.9 ± 28.4	23 (22)	64.1 ± 12.4
	P value	0.126	0.32	0.016	0.339	0.428	0.477
Women	AA	136 ± 21	74 ± 10	76.3 (71)	91.0 ± 23.4	35 (28)	71.0 ± 8.7
	AG + GG	142 ± 19	74 ± 10	89.3 (117)	97.9 ± 27.7	48 (55)	71.7 ± 9.6
	P value	0.047	0.899	0.009	0.07	0.051	0.53
rs2074192							
Men	CC	138 ± 17	77 ± 10	83.9 (125)	102.4 ± 28.0	25 (32)	65.2 ± 12.0
	TT	133 ± 20	75 ± 10	72.3 (81)	101.0 ± 25.5	22 (22)	64.5 ± 12.9
	P value	0.061	0.254	0.023	0.684	0.372	0.687
Women	CC	137 ± 20	74 ± 10	81.4 (57)	98.2 ± 28.6	48 (30)	69.0 ± 12.0
	CT + TT	139 ± 19	74 ± 10	85.3 (116)	94.3 ± 25.4	41 (49)	71.0 ± 9.8
	P value	0.631	0.872	0.474	0.351	0.249	0.239

Data expressed as mean ± s.d. Differences in proportions of subjects with hypertension and LVH were compared by χ^2 analysis.
ACE2, angiotensin-converting enzyme 2; BP, blood pressure; LV, left ventricular mass (indexed to body surface area); LVH, left ventricular hypertrophy (defined as LV mass >115 g/m^2 men and >95 g/m^2 in women); SNP, single-nucleotide polymorphism.
[a]Data and *P* values are for the recessive model for SNP rs1978124, GG + GA vs. AA.

Association of ACE2 SNPs and BP

The association of *ACE2* SNPs with BP and hypertension is shown in **Table 3**. By linear regression analysis, *ACE2* SNPs rs2074192, rs4240157, and rs4646188 were significantly associated with the prevalence of hypertension, and rs4240157 and rs4646188 were significantly associated with systolic BP in the dominant model. In men, there were no associations with *ACE2* variants with systolic or diastolic BP. However, the prevalence of hypertension was significantly higher with the rs2074192 C allele ($P = 0.023$), rs4240157 G allele ($P = 0.016$) and rs464188 T allele ($P = 0.006$). In women, there was an association with two *ACE2* SNPs (rs4240157, rs4646188) and systolic BP, although these associations were of borderline significance. Similar to men, women with the *ACE2* rs4240157 G allele were more likely to have hypertension ($P = 0.009$). After accounting for multiple testing (single test *P* value of 0.01), the association of SNPs rs4646188 in men and rs4240157 in women with the prevalence of hypertension remained significant. The rs4646188 T allele

Table 4 | Association of *ACE2* SNP rs1978124 with cardiac function in men and LV mass in women

		SNP rs1978124 genotypes	Adjusted mean ± s.e.	Adjusted *P* value[a]
Men	LV ejection fraction (%)	GG	67.5 ± 1.2	0.007
		AA	62.3 ± 1.1	
Women	LV mass (g/m^2)	GG ± GA	92.6 ± 2.1[b]	0.040
		AA	104.4 ± 3.8[b]	

Data expressed as mean ± s.e.
ACE2, angiotensin-converting enzyme 2; LV, left ventricular; SNP, single-nucleotide polymorphism.
[a]*P* value for association of LV mass and LV ejection fraction with SNP rs1978124 adjusted for covariates age, body mass index, hypertension, use of antihypertensive medication, and systolic and diastolic blood pressure. [b]Data are for the recessive model for SNP rs1978124, GG + GA vs. AA.

was associated with a higher odds ratio for hypertension in men, independently of age, BMI and antihypertensive medication use (odds ratio 4.8 (95% confidence intervals, 1.2–19.7), $P = 0.031$). The rs4240157 G allele was not independently associated with hypertension after controlling for age, BMI and antihypertensive medication use (odds ratio 1.6 (0.6–4.7), $P = 0.384$).

Relationship between ACE2 SNPs and cardiac hypertrophy

The association of *ACE2* SNPs with LV mass index, LVH, and systolic function is shown in Table 3. In men, there was a trend towards increased LV mass index with the rs1978124 A allele but the association did not reach statistical significance ($P = 0.07$). However in women the rs1978124 A allele was significantly associated with an increased LV mass index in the recessive model ($P = 0.008$). This association remained significant after covariant adjustment for age, BMI, hypertension, antihypertensive medications, and systolic and diastolic BP ($P = 0.040$, Table 4). There was a trend towards increased LV mass index and LVH with the dominant model in women with SNPs rs4240157 and rs4646156. However this did not reach significance after correcting for multiple testing.

Relationship between ACE2 SNPs and systolic function

In men, *ACE2* rs1978124 was associated with systolic function. Men with the A allele of rs1978124 had a significantly lower systolic function (LVEF in GG vs. AA, 67.2% vs. 62.3%, $P = 0.002$). After adjustment for age, BMI, hypertension, antihypertensive medications, and systolic and diastolic BP, the A allele remained significantly associated with a lower LVEF ($P = 0.007$, Table 4). In women, none of the SNPs studied were associated with systolic function.

DISCUSSION

This is the first study to investigate variation in the *ACE2* gene with BP, LV mass, LVH, and cardiac function in a Caucasian population with type 2 diabetes. We report that the *ACE2* SNPs rs464188 and rs4240157 were associated with hypertension in men and women, and that the rs1978124 A allele was associated with a lower ejection fraction in men and with increased LV mass in women.

ACE2, BP and hypertension

To date, association studies with BP, hypertension and *ACE2* have produced inconsistent results, which may reflect the ethnic background of the subjects, the underlying cardiovascular phenotype and the *ACE2* SNPs investigated. A strength of our study was the use of a population in which 80% of subjects had hypertension, thus enriching our cohort to study gene associations. Our results are at variance with a study in a nondiabetic Caucasian population which reported no association with four *ACE2* SNPs (rs1978124, rs2285666, rs879922, and rs714205) and hypertension prevalence.[15] However, our results are in agreement with a study in Austrian Caucasians which reported no associations with *ACE2* and actual systolic and diastolic BP.[19] Although some of the *ACE2* SNPs that we studied were different to the previous Caucasian studies, we performed a comprehensive analysis of the *ACE2* gene. The five genotyped *ACE2* SNPs in this study captured 100% of common alleles across the *ACE2* gene. Three of the initially selected SNPs were unsuitable for the genotyping assay and therefore two alternative haplotype tagging *ACE2* SNPs that were in strong linkage disequilibrium ($D' = 1.0$) with the initial SNPs were selected and genotyped.

By contrast, in a study of 1494 Han Chinese subjects,[29] the *ACE2* rs1978124 SNP was a risk factor for stage 2 hypertension in males, and in the GenSalt study in subjects of Chinese ancestry, *ACE2* SNP rs4646174 was associated with systolic and diastolic BP responses to potassium supplementation in men, but not in women.[30] Zhong *et al.* showed that the *ACE2* rs2285666 GG genotype was associated with higher diastolic BP in female Chinese subjects with the metabolic syndrome,[17] but a different group showed no association of *ACE2* rs2285666 with essential hypertension in men or women from the northern Han Chinese population.[31]

ACE2 and LVH

We observed a trend towards increased LV mass index with *ACE2* rs1978124 in men and a significant association with LV mass index in women. In the Austrian study,[19] there were significant associations with the minor alleles of four *ACE2* SNPs (rs4646156, rs879922, rs4240157, and rs233575) and LV mass after adjusting for age, BMI, antihypertensive medications, and systolic BP, but this association was not seen in women. The same four SNPs and a haplotype consisting of the minor alleles of these SNPs were significantly associated with LVH in men.[19] In our study, the *ACE2* rs1978124 SNP was significantly associated with LV mass index in women, but this SNP was not genotyped in the Austrian study.[19]

ACE2 and systolic function

In women, none of the SNPs studied were associated with systolic function, whereas in men, the rs1978124 A allele was associated with a significantly lower ejection fraction, although still within the normal range between the genotype groups. It is important to note that heart failure is a common complication of diabetes[32,33] and can occur with both reduced and normal (or preserved) ejection fraction.[34] Interestingly,

the same SNP (which is also known as A1075G) is associated with increased mortality in men with acute coronary syndrome,[20] with increased systolic BP in Han Chinese men,[29] and with myocardial infarction in Chinese women.[18] The MONICA Augsburg study[19] found no association with the SNPs they studied and systolic function in men or women, but as mentioned earlier, the *ACE2* rs1978124 was not genotyped in this cohort.

Previous studies that have investigated *ACE2* SNPs have also reported inconsistencies in their association with cardiovascular and metabolic risk factors in men and women. The *ACE2* gene is located on the X chromosome and it is well known that the development of cardiovascular diseases display gender-specific characteristics. Gender and sex hormones are known to affect components of the RAS[35] and the lack of *ACE2* gene associations in women may reflect the effects of gender. In addition, *ACE2* is located on Xp22 where many genes are known to escape X-inactivation and there is a variable degree of expression that can contribute to sexually dimorphic traits.[36]

The underlying functional mechanism by which *ACE2* rs1978124 affects systolic function in men are unclear and requires further investigation, but our finding is supported by experimental evidence in ACE2 knockout mice,[11] which have severe impairment of cardiac systolic function. Interestingly as in our study, the abnormalities are more severe in male than female mice, although with time, cardiac dysfunction develops in both male and female animals. Others have reported that genetic deletion of ACE2 accelerates cardiac hypertrophy and shortens the time taken to develop heart failure.[13] The extent of linkage disequilibrium with rs1978124 and SNPs upstream of intron 1 particularly in the promoter region need further examination, as it may indicate that the functional variant lies in this region of the gene. We are currently following-up the carriers of the A allele of *ACE2* rs1978124 to assess whether they too go on to develop heart failure.

A limitation of this study, as well as all other gene association studies, is a lack of data on plasma ACE2 activity levels. We have previously shown that cardiac ACE2 protein expression is upregulated in the explanted human ischemic failing heart,[37] and others have shown that plasma ACE2 levels correlate with the severity of heart failure in man,[38] and are increased in prehypertensive subjects.[39] It is not yet clear if the increase/decrease in circulating ACE2 is the cause or the consequence of heart failure or high BP. Given the opposing roles that ACE and ACE2 play in the metabolism of Ang II, the relative balance of ACE and ACE2 at both the tissue level, and in the circulation will play a role in the relative balance of the constrictor versus the dilator arm of the RAS.

Most recently, there have been two studies that have investigated *ACE2* and nephropathy in type 1 diabetes in Caucasian populations from Finland (n = 823)[16] and the British Isles (n = 1,467).[40] Neither study found any association with the *ACE2* SNPs investigated and diabetic nephropathy, and in the Finnish study, no association with BP was reported.

In conclusion, this is the first study to investigate genetic variation in *ACE2* with BP, LV mass, LVH and systolic function in a Caucasian population with type 2 diabetes. We have found evidence of associations between *ACE2* and hypertension in men. The *ACE2* rs1978124 variant was associated with LV mass in women and with systolic function in men. Further investigation into the association between *ACE2* SNPs rs464188 and rs4240157 with hypertension, and rs1978124 with systolic function in a population without diabetes is warranted. Our results do require replication in other cohorts of patients with type 2 diabetes in whom cardiac function has also been assessed.

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