

The *ACE2* gene: its potential as a functional candidate for cardiovascular disease

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Abstract

The RAS (renin–angiotensin system) plays an important role in the pathophysiology of CVD (cardiovascular disease), and RAS blockade is an important therapeutic strategy in the management of CVD. A new counterbalancing arm of the RAS is now known to exist in which ACE (angiotensin-converting enzyme) 2 degrades Ang (angiotensin) II, the main effector of the classic RAS, and generates Ang(1–7). Altered ACE2 expression is associated with cardiac and vascular disease in experimental models of CVD, and ACE2 is increased in failing human hearts and atherosclerotic vessels. In man, circulating ACE2 activity increases with coronary heart disease, as well as heart failure, and a large proportion of the variation in plasma ACE2 levels has been attributed to hereditary factors. The *ACE2* gene maps to chromosome Xp22 and this paper reviews the evidence associating *ACE2* gene variation with CVD and considers clues to potential functional *ACE2* variants that may alter gene expression or transcriptional activity. Studies to date have investigated *ACE2* gene associations in hypertension, left ventricular hypertrophy and coronary artery disease, but the results have been inconsistent. The discrepancies may reflect the sample size of the studies, the gender or ethnicity of subjects, the cardiovascular phenotype or the *ACE2* SNP investigated. The frequent observation of apparent sex-dependence might be of special importance, if confirmed. As yet, there are no studies to concurrently assess *ACE2* gene polymorphisms and circulating ACE2 activity. Large-scale carefully conducted clinical studies are urgently needed to clarify more precisely the potential role of ACE2 in the CVD continuum.

Key words: angiotensin-converting enzyme 2 (*ACE2*), coronary artery disease, hypertension, left ventricular hypertrophy, myocardial infarction, renin–angiotensin system (RAS).

INTRODUCTION

The RAS (renin–angiotensin system) plays a major role in the pathophysiology of CVD (cardiovascular disease) [1,2], a leading cause of death and disability worldwide [3]. Within the RAS, the enzyme ACE (angiotensin-converting enzyme) converts Ang (angiotensin) I into the vasoconstrictor AngII, the main effector of the system, which mediates its effects via the AT₁R (angiotensin type 1 receptor). AngII has actions to raise BP (blood pressure) through vasoconstriction and salt and water retention, and contributes to cardiac remodelling, inflammation, thrombosis and plaque rupture. Therapeutic strategies that block RAS activation using ACE inhibitors or AngII receptor blockers are first-line therapies in hypertension and can favourably affect remodelling and reduce morbidity and mortality in post-MI (myocardial in-

fraction) and heart failure patients [4]. Despite their CV (cardiovascular) benefits, RAS blockade slows, but does not prevent, the progression of disease, prompting the search for other therapeutic approaches.

It is now 12 years since *ACE2* was discovered by two independent groups, who cloned it from a human cardiac left ventricle cDNA library prepared from the explanted heart of a heart transplant recipient [5] and a human lymphoma cDNA library [6]. The *ACE2* amino acid sequence shares approximately 40% homology with the NT (N-terminal) catalytic domain of ACE and a hydrophobic region near the C-terminus that probably serves as a membrane anchor. Like ACE, *ACE2* is a type 1 membrane protein with the catalytic domain on the extracellular surface [5,6]. The *ACE2* protein is 805 amino acids in length and is encoded by the gene *ACE2* located on chromosome Xp22 (Figure 1).

Abbreviations used: ACE, angiotensin-converting enzyme; Ang, angiotensin; AT₁R, angiotensin type 1 receptor; BMI, body mass index; BNP, B-type natriuretic; BP, blood pressure; CAD, coronary artery disease; CEPH, Centre d'Étude du Polymorphisme Humain; CTCF, CCCTC-binding factor; CV, cardiovascular; CVD, cardiovascular disease; ENCODE, Encyclopaedia of DNA Elements; HCM, hypertrophic cardiomyopathy; LD, linkage disequilibrium; LV, left ventricular; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; MAF, minor allele frequency; MI, myocardial infarction; miRNA, microRNA; NT, N-terminal; RAS, renin–angiotensin system; SNP, single nucleotide polymorphism; UTR, untranslated region.

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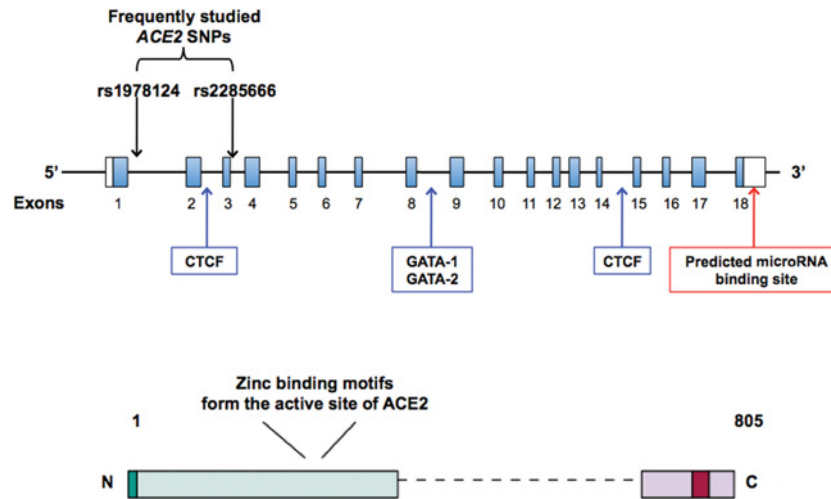


Figure 1 Schematic representation of the *ACE2* gene (not shown to scale)

Exons are shown as blue boxes, the 5' and 3'-UTRs are shown as white boxes and the horizontal black line represents the introns. The locations of the two commonly studied *ACE2* SNPs (rs1978124 and rs2285666) are shown above the gene image. The locations of high signal strength transcriptional factor-binding sites (identified from ENCODE data) are shown by blue arrows below the gene image. The red arrow below the gene image indicates the location of the predicted miRNA-binding site in the *ACE2* 3'-UTR. Bottom panel: *ACE2* domain structure showing the N-terminal ectodomain, the C-terminal cytoplasmic domain, the signal peptide (green) and the transmembrane domain (red) with the residue numbers shown above the protein [80]. Adapted from Trends Pharmacol. Sci., 23, Turner, A.J. and Hooper, N.M., The angiotensin-converting enzyme gene family: genomics and pharmacology, 177–183, © (2002), with permission from Elsevier.

ACE2 has been identified in many tissues, including the heart [5,7,8], blood vessels [9–11], kidney [12–14] and brain [15], as well as the retina [16], liver [17], gastrointestinal tract [18,19] and lungs [20]. *ACE2* acts as a monooxypeptidase and removes a single C-terminal residue from AngI to generate Ang-(1–9) [5], but its major role is to degrade AngII to the heptapeptide Ang-(1–7) [21]. Thus *ACE2* may limit the vasoconstrictor action of AngII through its inactivation, as well as counteracting the actions of AngII through the formation of Ang-(1–7), which is reported to have vasodilatory and anti-fibrotic actions [22] at the Ang-(1–7) or *mas* receptor [23]. There is now substantial experimental evidence that the classical RAS pathway *ACE*/AngII/AT₁R is balanced by the 'new' arm of the RAS, namely the *ACE2*/Ang-(1–7)/*mas* receptor axis [24,25]. A full discussion of the role of *ACE2*, Ang-(1–7) and *mas* receptor in experimental studies can be found in recent reviews [26–29].

The aim of the present review is to discuss the evidence regarding circulating *ACE2* activity in patients with CVD. We also consider the potential for functional variants in and around the *ACE2* gene to alter gene expression or transcriptional activity in a way that might alter *ACE2* activity and predispose to CVD in humans.

ACE2 ACTIVITY AND CARDIOVASCULAR DISEASE

ACE2 is an integral cell membrane protein and an ectoenzyme that undergoes 'shedding' to release the catalytically active ectodomain [30]. The process involves the proteinase ADAM (a dis-

integrin and metalloproteinase) 17, also known as TACE [TNF α (tumour necrosis factor α)-converting enzyme] [30,31], and results in *ACE2* that can be detected in the circulation [32–36]. Most assays for the measurement of *ACE2* activity use *ACE2*-specific quenched fluorescent substrates [30,32,37,38], which can detect activity in tissue or plasma/serum. To date, there have been eight studies to assess circulating *ACE2* activity in humans, and these include normal subjects and those with CVD [35,37,39–44].

The Leeds Family study of healthy subjects and family members comprised 537 individuals [37]. Plasma *ACE2* activity was detectable in only 40 subjects who tended to be older, with greater central adiposity and higher BP, fasting glucose and lipid levels than those without detectable levels [37]. Using a similar quenched fluorescent substrate-based assay to Rice et al. [37], we found that plasma *ACE2* levels were low in normal healthy volunteers [35], but significantly increased in the presence of cardiac disease [36,45]. Small early studies were not always consistent in this regard. For example, compared with normotensive individuals, *ACE2* activity in monocyte-derived macrophages was reported to be increased in pre-hypertensive men, but not in untreated hypertensive subjects [39]. Other studies suggest that serum *ACE2* activity is sex-dependent, with higher levels in males compared with females [44].

In the vasculature, *ACE2* is present in all layers of the vessel wall [10,11]. More direct evidence of a role for *ACE2* in atherosclerotic disease comes from studies that have shown human carotid atherosclerotic lesions to express *ACE2* mRNA in early and advanced lesions [11], with *ACE2* protein present in endothelial cells, smooth muscle cells and macrophages. In patients with CAD (coronary artery disease) undergoing coronary artery bypass surgery, we found that *ACE2* was present in

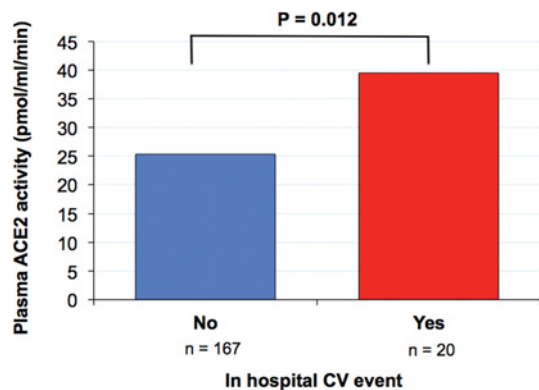


Figure 2 Post-operative median plasma ACE2 activity is increased after emergency orthopaedic surgery in those with a CV event

Adapted from Am. J. Cardiol., 109, Chong, C.P., Lim, W.K., Velkoska, E., van Gaal, W.J., Ryan, J.E., Savige, J. and Burrell, L.M., N-terminal pro-brain natriuretic peptide and angiotensin-converting enzyme-2 levels and their association with postoperative cardiac complications after emergency orthopaedic Surgery, 1365–1373, © (2012), with permission from Elsevier.

both normal as well as atherosclerotic internal mammary and radial arteries [10]. In these studies, ACE2 was present in the neo-intima and the media, and was clearly evident in small newly formed angiogenic vessels as well as the vaso vasorum [10].

Furthermore, in patients with suspected CAD, plasma ACE2 activity is significantly increased in those with angiographically defined CAD compared with those with normal coronary arteries, suggesting that it may be a useful biomarker of disease [45]. In this respect, in a study of high-risk patients undergoing emergency orthopaedic surgery ($n = 187$), we found that post-operative plasma ACE2 activity was significantly increased in those who had an in-hospital CV event ($n = 20$) including MI, heart failure, atrial fibrillation, major arrhythmia or cardiac arrest (Figure 2) [36]. Plasma ACE2 activity was not a significant predictor of CV events in multivariate analysis [36], but further studies in larger patient cohorts are needed to confirm these findings.

Clinical studies of heart failure patients [41] report that higher serum ACE2 activity is correlated with increasing severity of heart failure on the New York Heart Association function classification, lower ejection fraction and increasing BNP (B-type natriuretic) peptide levels. Serum ACE2 activity was increased in heart failure of both ischaemic and non-ischaemic origin, as well as in those with symptomatic heart failure and preserved ejection fraction, presumably as a result of diastolic dysfunction. Other studies of stable chronic systolic heart failure corroborate these correlations between serum ACE2 activity and lower ejection fraction and higher plasma NT-proBNP [42]. In addition, serum ACE2 was an independent predictor of adverse clinical events after adjustment for LVEF (left ventricular ejection fraction) and NT-proBNP levels [42]. Similar results have been seen in non-ischaemic causes of heart failure in which plasma ACE2 activity was higher in heart failure due to Chagas' disease and was predictive of cardiac death and heart transplantation [43]. Interestingly, one early study suggested that spironolactone ther-

apy in heart failure increased ACE2 activity [40]. The effects of other treatments for heart failure have not been reported.

Our findings of increased ACE2 immunoreactivity in explanted ischaemic failing human heart tissue [7] help us to understand the higher plasma ACE2 activity levels in heart failure. Others have shown that the *ACE2* gene is up-regulated in human idiopathic and ischaemic cardiomyopathy [46], and in myocardial biopsies from patients with severe heart failure [8]. Whether the increase in cardiac ACE2 represents an important adaptive mechanism to retard the progression of adverse cardiac remodelling is not clear from these cross-sectional studies. Moreover there are no studies that have simultaneously measured both tissue and circulating ACE2, and therefore it is unclear if the changes in plasma ACE2 seen in patients with heart failure reflect cardiac tissue or blood vessel ACE2 activity.

Although serum ACE2 activity does not appear to differ between subjects with Type 1 diabetes compared with healthy controls [44], the noted associations between ACE2 activity and other cardiovascular risk factors are seen in diabetes, with increased levels being associated with male sex, higher BP and coronary heart disease. Additionally, in men with Type 1 diabetes, microvascular disease reflected by microalbuminuria was associated with increased serum ACE2 activity.

GENETICS OF ACE2

If activity of ACE2 is associated, either as a cause or an effect of CVD, it is important to understand intrinsic factors that might influence ACE2 activity under a given set of circumstances. In this regard genetic variation is worth considering. Evidence of genetic influences comes from the Leeds Family Study in which it was estimated in 89 pedigrees that up to 67% of the variability in circulating ACE2 levels could be explained by heritable factors [37]. This conclusion was based on the observation that among the 7% of healthy subjects with detectable ACE2 activity, such activity was detectable in at least one other family member among half of the subjects [37]. Indeed, eight families were remarkable for having two or more members with detectable ACE2 activity. Formal tests of heritability of ACE2 activity using more sensitive assays have not been published.

Genetic variation in and around the gene encoding ACE2 is a strong candidate for differences in ACE2 activity. Such variation is most often detected using SNPs (single nucleotide polymorphisms). Despite the biological proximity of the phenotype of ACE2 activity to genetic variation of the *ACE2* gene, there are presently no association studies of *ACE2* gene polymorphisms and circulating levels of ACE2 activity. As such, functional variants of the *ACE2* gene that might alter gene expression or protein composition in tissues or cells of CV relevance are yet to be identified.

ACE2 GENE POLYMORPHISMS

The NCBI (National Center for Biotechnology Information) database dbSNP (build 136, last accessed 21/05/2012,

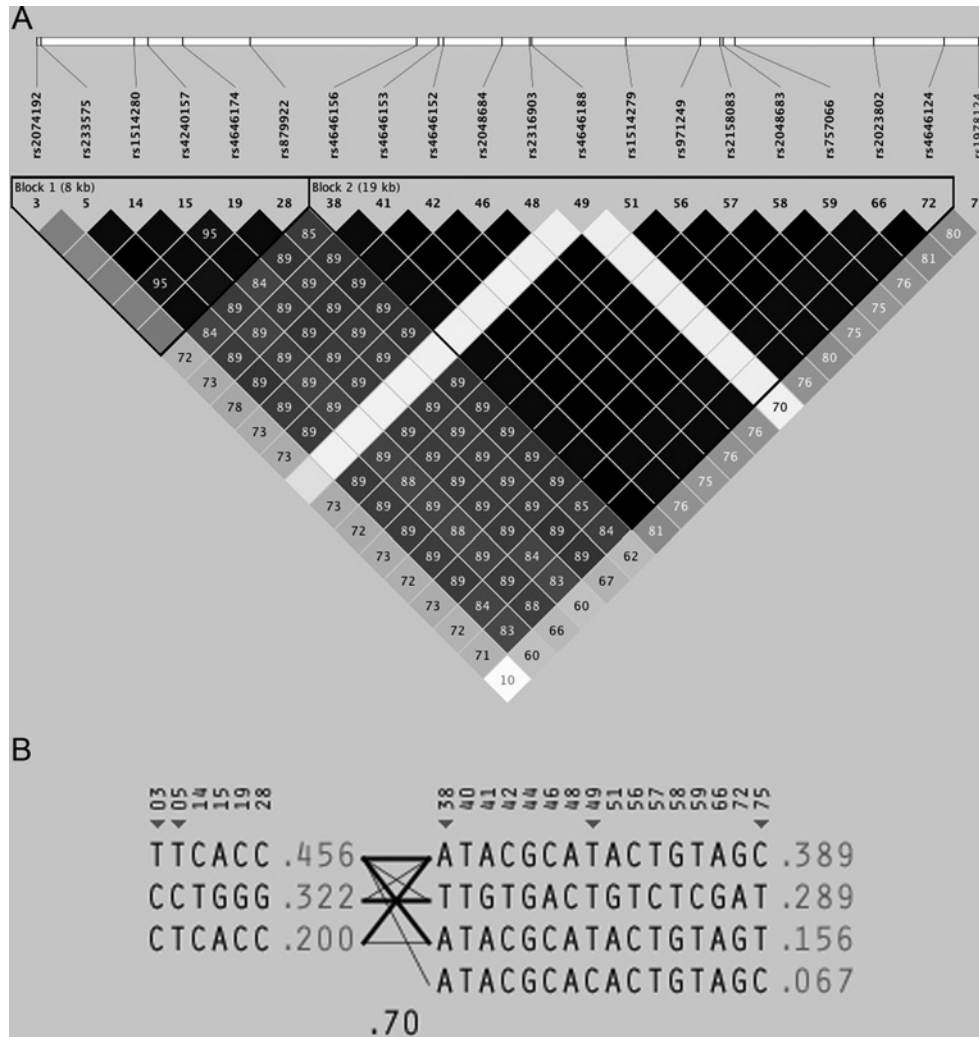


Figure 3 *ACE2* gene analysis performed using the Haploview software with the HapMap Phase II CEPH data (<http://www.hapmap.org>)

(A) Pairwise correlation r^2 plot of common *ACE2* SNPs. Black squares indicate $r^2 = 1$, grey shades indicate $r^2 < 1$ and white indicates an r^2 value of zero. (B) Common haplotypes formed by the common SNPs (minor allele frequency $> 5\%$); haplotype frequencies are shown. The arrowheads below the SNP numbers indicate the tagging SNPs; these correspond in order (left to right) to SNPs rs2074192, rs233575, rs4646156, rs464188 and rs1978124.

<http://www.ncbi.nlm.nih.gov/snp/>) has described 479 *ACE2* SNPs in the gene region of which 38 are located within the coding regions. Of these, 31 are missense polymorphisms and nine have been validated and have MAFs (minor allele frequencies) of $< 1\%$. Examination of the LD (linkage disequilibrium) in the *ACE2* gene using the CEPH (Centre d'Étude du Polymorphisme Humain) population (Utah residents with ancestry from northern and western Europe) HapMap Phase II data suggests that there is very good correlation (r^2) between most of the SNPs across the gene (Figure 3) [47]. Five *ACE2* SNPs capture almost all of the common variation (MAF $> 5\%$) in the *ACE2* gene [captured = 87% (20/23 SNPs)] with $r^2 > 0.8$ and mean $r^2 = 0.969$; Figure 3). Examination of the extent of LD (r^2) in the *ACE2* gene region suggests that this extends approximately 28 kb from the 5' end and 20 kb from the 3' end of the gene.

Functional variants located within transcriptional factor-binding sites may disrupt gene transcription. An analysis

of the ENCODE (Encyclopaedia of DNA Elements) data (<http://genome.ucsc.edu/ENCODE/>) suggests that there are several high signal strength transcriptional factor-binding sites in the *ACE2* gene region that might merit further investigation (Figure 1). These include binding sites for CTCF (CCCTC-binding factor), which is important in a number of regulatory functions, including transcriptional activation/repression and X chromosome inactivation [48]; GATA-binding protein 1 involved in gene regulation during erythropoiesis [49] and GATA-binding protein 2, a transcriptional activator that regulates endothelin-1 gene expression, which is thought to be involved in hypertension [50].

The location of the *ACE2* gene on the X chromosome is particularly interesting, and the development of CVD is known to display gender-specific characteristics, with men more likely to have a coronary event than women [51]. Normally, one female X chromosome is randomly inactivated to maintain equal gene expression between sexes, a process known as X-inactivation

[52]. The *ACE2* gene location on Xp22 encompasses an area where genes are known to escape X-inactivation [53], which may contribute to phenotypic differences between sexes and tissue-specific differences in X-inactivation [54].

Non-coding RNA such as miRNA (microRNA) has the ability to control gene expression in a developmental-stage- and tissue-specific manner. miRNAs bind to recognition sites, and polymorphisms within such sites might alter gene expression. Currently, there are no known experimentally verified miRNA targets in the human *ACE2* gene. However, there is a predicted miRNA target located in a conserved *ACE2* 3'-UTR (untranslated region) (<http://www.targetscan.org>) (Figure 1). The sequence is an exact match to positions 2–8 of three mature miRNAs (*hsa-miR-200b*, *hsa-miR-200c* and *hsa-miR-429*).

Two *ACE2* SNPs, rs2285666 and rs1978124, have been among those most frequently used in association studies (Figure 1). The rs2285666 SNP is located within a splice site and may be involved in the disruption of translation efficiency of the mRNA (<http://fastsnp.ibms.sinica.edu.tw>), although this has yet to be investigated. The rs1978124 SNP has no known function and does not have any predicted effects on gene splicing or transcription-binding sites. The rs1978124 SNP is in strong LD ($r^2 = 1$) with three SNPs located upstream (rs6629110, rs2158082 and rs2106806), but these also have no predicted functional effects. Indels (insertions/deletions) have not been identified in the *ACE2* gene region, although one frameshift variant has been sequenced in the 1000 Genomes Project (<http://www.1000genomes.org>), located in exon 10 and within a predicted weak enhancer element.

Suffice it to say that there is sufficient evidence of *ACE2* polymorphisms to pursue further studies of the association with *ACE2* activity in males and females, at various ages and in particular tissues in parallel with laboratory-based analyses of the control of *ACE2* gene expression.

ACE2 GENE ASSOCIATION STUDIES

A PubMed search with the search terms 'ACE2' and ('polymorphism' or 'variant' or 'variation' or 'genotype' or 'allele') and limited to publications in the English language, human studies and non-reviews since the discovery of *ACE2* (from 2000 until 21/05/2012) revealed 41 publications. Of these 21 have examined *ACE2* gene associations with BP, hypertension, LVH (left ventricular hypertrophy) or CAD. These studies are reviewed below and summarized in Table 1.

ACE2 GENE AND BP

A total of 14 studies have examined the relationship of *ACE2* SNPs with BP or hypertension (Table 1). Eight individual studies were conducted in the Chinese [55–62], one in Koreans [63] and three in Caucasians [64–66]. The intronic *ACE2* SNP rs2285666 has been the most consistently studied SNP in 11 of the 14 studies summarized in Table 1.

Three Chinese studies reported significant associations in women with the rs2285666 SNP with either hypertension or systolic or diastolic BP [56,57,62]. In two of these studies, higher pressure was associated with the A allele [56,57], but in the third study it was the G allele [62], where it was also associated with the metabolic syndrome. One analysis of orthostatic BP responses in 3630 Chinese Han subjects found no association with the rs2285666 SNP [59].

Two meta-analyses of the rs2285666 SNP have been reported [67,68]. One, with a combined total of 2528 case subjects in the Chinese Han population with hypertension and 2024 ethnicity-matched normotensive controls, reported no association with hypertension [67]. The other, a more recent augmented meta-analysis of hypertensive subjects ($n = 7251$) reported a significant association between the rs2285666 AA genotype with hypertension in women [68]. However, this meta-analysis mixed ethnicities (Chinese Han, Chinese Dongxiang and Anglo-Celtic) and reported significant heterogeneity in males, suggesting that the A allele was associated with hypertension only in Chinese Han subjects. In Korean subjects, two *ACE2* SNPs (rs1514282 and rs1514283) were found to be significantly associated with diastolic pressure, but not with hypertension, and separate sex analyses were not reported [63].

The evidence in Caucasian studies is also mixed, with negative association in two studies of hypertension [64] and BP variation [66]. However, in our recent study of 503 Australian Caucasians with Type 2 diabetes, the prevalence of hypertension was significantly higher in both men and women in association with the G allele of the *ACE2* SNP rs4240157 [65].

ACE2 GENE AND BP TREATMENT

A small number of dietary and pharmacological interventions have been analysed in relation to *ACE2* polymorphisms. The GenSalt study in Chinese Han subjects examined responses to variation in sodium and potassium intake [60]. Associations were reported for a diversity of *ACE2* SNPs, with the reduction in BP in response to potassium while on a high salt intake in men only [60] and to variations in sodium intake alone in men and women combined [61].

One study of the BP response to RAS blockade in Chinese Han subjects [58] suggested that the diastolic pressure response to ACE inhibition was 3.3 mmHg greater in women with the rs2106809 T allele after adjustments for age, pre-treatment BP, BMI (body mass index) and fasting blood glucose. There are no studies of *ACE2* gene associations with BP treatment responses in Caucasian populations.

ACE2 GENE AND CARDIAC STRUCTURE AND FUNCTION

In experimental studies, it was initially reported that *ACE2*-knockout mice had severe impairment of cardiac systolic function [69]. The failure of other investigators to duplicate these results

Table 1 ACE2 gene associations with CVD

IVS, interventricular septal thickness; MAP, mean arterial blood pressure; PW, posterior wall thickness.

Reference	ACE2 SNP	Population	Gender	Study design	Sample size (case:control)	Phenotype	Findings significant?	Summary of significant findings
Yi et al. [56]	rs2285666	Chinese populations of (i) Han ethnicity and (ii) Dongxiang ethnicity	Both	Case-control	(i) 198:131 and (ii) 120:102	Hypertension	Yes	Women: the AA genotype frequency greater in the hypertensive Dongxiang population
Niu et al. [57]	rs1978124 and rs2285666	Chinese population of Han ethnicity	Both	Case-control	808:686	Hypertension	Yes	Men: the rs1978124 G allele was associated with an increased risk of hypertension Women: increased systolic BP with the rs1978124 G allele and rs2285666 A allele
Lu et al. [68]	rs2285666	Mixed ethnicity of Chinese Han, Dongxiang, Li and Australian Anglo-Celtic	Both	Meta-analysis (nine case-control and one cross-sectional study)	7251:3800	Hypertension	Yes	Men: the A allele was associated with an increased risk of hypertension in the Chinese Han group only Women: the AA genotype was associated with an increased risk of hypertension across all ethnicities
Fan et al. [58]	rs2106809, rs2285666, rs4646155 and rs879922	Chinese population of Han ethnicity	Both	Two studies: (i) case-control and (ii) clinical trial randomized to four treatment groups	(i) 973:969 and (ii) 3408	Hypertension	Yes	Women: (i) the rs2106809 T allele was associated with a 1.6-fold increase in the risk of hypertension; (ii) the adjusted diastolic BP response to captopril was decreased in rs2106809 T allele carriers
Song et al. [63]	rs1514282 and rs1514283	South Korean	Both	Cohort study	7551	BP	Yes	Analysis was not split by gender; both SNPs were associated with increased diastolic BP in the dominant genetic model, adjusted for age, gender and BMI

Table 1 Continued

Reference	ACE2 SNP	Population	Gender	Study design	Sample size (case:control)	Phenotype	Findings significant?	Summary of significant findings
Zhao et al. [60]	rs4646174, rs879922 and rs4646140	Chinese population of Han ethnicity	Both	Intervention study in probands and their families (parents, siblings, spouses and offspring)	1906	BP	Yes	Men: all three SNPs were associated with decreased systolic BP, diastolic BP and MAP in response to potassium supplementation
Zhao et al. [61]	rs1514283, rs1514282, rs2074192, rs714205, rs4646176 and rs2285666	Chinese population of Han ethnicity	Both	Intervention study in probands and their families (parents, siblings, spouses and offspring)	1906	BP	Yes	Family-based analysis, analysis not split by gender: three SNPs were associated with the systolic BP response to a low-sodium diet; three SNPs were associated with the MAP response to low- and high-sodium intervention
Benjafield et al. [64]	rs1978124, rs2285666, rs879922 and rs714205	Australian white Anglo-Celtic origin	Both	Case-control	152:193	Hypertension	No	—
Huang et al. [55]	rs1978124 and rs2285666	Chinese population of Han ethnicity	Both	Case-control	494:484	Hypertension	No	—
Zhou et al. [67]	rs2285666	Chinese population of Han ethnicity	Both	Meta-analysis (five case-control studies)	2528:2024	Hypertension	No	—
Fan et al. [59]	rs2106809 and rs2285666	Chinese population of Han ethnicity	Both	Case-control	3630:826	Hypertension	No	—
Patel et al. [65]	rs1978124, rs2074192, rs4240157, rs4646156 and rs4646188	Australian Caucasians	Both	Cohort study	503	Type 2 diabetes	Yes	Men: prevalence of hypertension was increased with SNPs rs2074192, rs4240157 and rs464188; decreased LVEF with rs1978124 A allele Women: increased systolic BP with SNPs rs4240157 and rs4646188; increased hypertension with rs4240157; and increased LV mass with rs1978124

Table 1 Continued

Reference	ACE2 SNP	Population	Gender	Study design	Sample size (case:control)	Phenotype	Findings significant?	Summary of significant findings
Lieb et al. [66]	rs2285666, rs4646156, rs879922, rs4240157 and rs233575	German Caucasian	Both	Cross-sectional	1674	Healthy subjects in the MONICA Augsburg echocardiographic substudy	Yes	Men: increased septal wall thickness and LV mass with rs879922, rs4240157 and rs233575; the rs4646156 SNP was associated with increased septal wall thickness; haplotype of minor alleles of these SNPs were associated with LVH ($P = 0.006$)
Wang et al. [73]	rs2106809 and rs6632677	Chinese population (unknown ethnicity)	Both	Case control	261:609	HCM	Yes	Men: the rs2106809 T and rs6632677 C alleles were associated with an increased risk of HCM and increased IVS
van der Merwe et al. [74]	rs1978124, rs2285666, rs879922 and rs4646179	South African (unknown ethnicity)	Both	Pedigrees of 22 HCM families	227	HCM	Yes	Analysis was not split by gender; the rs879922 G allele was associated with increased LV mass, maximum IVS and PW
Kelly et al. [75]	rs2074192 and rs4646156	>66% from Australia (unknown ethnicity)	Both	Cohort study	79	Cardiac structure in Friedreich's ataxia	Yes	Men: trend towards increased LVM in rs4646156 A allele ($P = 0.046$) carriers, but not significant after correction for multiple comparisons
Yang et al. [77]	rs1978124, rs2285666 and rs4646142	Chinese population of Han ethnicity	Both	Case control	508:905	Myocardial infarction	Yes	Men: haplotypes of the three ACE2 SNPs risk alleles (GGC) were associated with an increased risk of MI only in male subjects not consuming alcohol Women: the rs1978124 and rs4646142 SNPs were associated with an increased risk of MI

Table 1 Continued

Reference	ACE2 SNP	Population	Gender	Study design	Sample size (case:control)	Phenotype	Findings significant?	Summary of significant findings
Palmer et al. [76]	rs1978124	New Zealand mixed ethnicity (majority European ancestry and minor Maori, Asian, African and Pacific Islander ancestry)	Men	Prospective cohort study	1042	Acute coronary syndromes	Yes	Men: the rs1978124 A allele was associated with an increased risk of mortality
Vangjeli et al. [78]	rs2285666 and rs971249	Northern European	Both	Prospective case-control follow-up	1959:2278	CV events	Yes	Women: the rs2285666 A allele was associated with a decrease in fatal CV events (hazard ratio of 0.3; $P = 0.04$)
Sotoodehnia et al. [79]	Four SNPs (details not given in the paper)	European-American	Both	Case control	211:730	Sudden cardiac arrest	No	—

in the ACE2-knockout mice may relate to differences in the genetic background of the mice [70]. In aortic constriction models, genetic deletion of ACE2 results in cardiac remodelling [71] and accelerates cardiac hypertrophy and shortens the time taken to develop heart failure [72]. The association of ACE2 SNPs with LVH has been investigated in five studies to date. In studies of Mendelian causes of cardiac enlargement, including HCM (hypertrophic cardiomyopathy) [73,74] and Friedreich's ataxia [75], it has been reported that ACE2 gene polymorphisms might modulate the underlying Mendelian tendency to hypertrophy, particularly in men.

In a large cross-sectional study of German subjects participating in the MONICA Augsburg echocardiographic substudy [66], four ACE2 SNPs (rs4646156, rs879922, rs4240157 and rs233575) were significantly associated with LVH in men, but not in women. In our recent study of cardiac structure and function in 503 Caucasians with Type 2 diabetes, we found in women that the ACE2 rs1978124 A allele was significantly associated with an increased LV (left ventricular) mass independent of age, BMI, hypertension, anti-hypertensive medications and systolic and diastolic BP [65]. In men, we observed a trend towards increased LV mass with the rs1978124 A allele; however, the difference was not statistically significant ($P = 0.07$). The MONICA Augsburg echocardiographic substudy [66] did not examine the rs1978124 SNP with LVH, and further studies are required to clarify the relationship between ACE2 associations with cardiac hypertrophy and the influence of gender and ethnicity.

There have been two studies of ACE2 polymorphisms and LV systolic function. The first study by Lieb et al. [66], reported no significant associations with LVEF or fractional shortening in men or women. In our recent study, the ACE2 rs1978124 A allele was associated with a significantly lower LVEF in men, and the association was independent of age, BMI, hypertension, anti-hypertensive medications and systolic and diastolic BP [65].

ACE2 GENE AND CORONARY ARTERY DISEASE

There have been three studies investigating ACE2 SNPs with CAD [76–78] and one study has investigated ACE2 SNPs with the risk of sudden cardiac arrest [79]. A case control study in the Chinese Han population examined the association of ACE2 SNPs in 811 subjects with CAD of whom 508 also had MI [77]. There were significant associations with ACE2 SNPs (rs1978124 and rs4646142) with MI in men and women [77]. In Caucasian men ($n = 729$) with an acute coronary syndrome, the ACE2 rs1978124 SNP (A allele) was significantly associated with reduced survival in those who did not receive beta-blocker treatment [76].

A recent European study of 1959 cases of CVD [78] suggested that the rs2285666 A allele was associated with a decreased risk of fatal CV events of MI and stroke, but only in women (hazard ratio of 0.3, $P = 0.04$). However this marginal difference was no longer significant after accounting for multiple testing. One study has examined four ACE2 SNPs in European-Americans with the risk of sudden cardiac arrest in 211 cases and 730

age- and gender-matched controls [79]; however, none of the *ACE2* SNPs was associated with a risk of sudden cardiac arrest in men or women.

SUMMARY OF *ACE2* GENETIC STUDIES IN CVD

Many of the studies performed to date and described above have shown gender-specific associations, but this is not routinely addressed in all study designs. In terms of markers, the studies have either genotyped haplotype tagging *ACE2* SNPs or have selected specific SNPs based on previous *ACE2* genetic study associations with CVD. Overall, the results of studies have been inconsistent, which may reflect the sample size, the ethnicity of the population studied [57,64,68], the underlying CV phenotype [66], the *ACE2* SNP investigated [65,66] or the gender of subjects [65,66]. Even within the largest group studied to date, the Chinese Han population, there are conflicting findings of *ACE2* gene associations with BP, hypertension and MI. Some of the SNPs studied in the Chinese populations either occur at a low frequency or are absent in the Caucasian population. There are also differences in the LD patterns between the Chinese and Caucasian populations.

In general terms, association studies based on candidate genes should be large (typically 1000 or more subjects) to ensure sufficient power and employ comprehensive gene-wide mapping with reliable and validated polymorphisms. Steps should also be taken to avoid potential sources of bias, such as population stratification. This has not always been the case in the *ACE2* gene association studies to date. The sample sizes in the case-control studies have been small, with the majority having used cases ranging from 120 to 508 subjects, which limits their power to detect subgroup association, such as sex-dependent associations.

FUTURE DIRECTIONS

Despite the growing evidence linking *ACE2* with CV risk factors and disease, the genetic evidence implicating the *ACE2* gene is unsatisfactory. The goal is to pinpoint *ACE2* gene variation that leads to changes in circulating *ACE2* activity or *ACE2* gene expression or transcriptional activity in tissues or cells of CV relevance. Such studies demand a comprehensive catalogue of candidate variants in and around the *ACE2* gene. All of the *ACE2* genetic studies reviewed in the present paper have investigated intronic *ACE2* SNPs only.

Care needs to be taken in selecting informative phenotypes and the most pressing concern is an attempt to identify functional variants that might influence circulating and tissue *ACE2* levels. However, in view of the recurrent reports to date of apparent sex-dependent associations between the *ACE2* polymorphisms and CV phenotypes, it is important that studies are designed with sufficient power to allow separate analyses of males and females. Large-scale carefully conducted clinical studies in patients with CVD are urgently needed to more precisely clarify the potential role of *ACE2* as a biomarker of disease and provide clues to mechanisms that might offer novel treatment targets.

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