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RESEARCH LETTER

Association study of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms and essential hypertension in northern Han Chinese

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Essential hypertension (EH), a complex disease, is an important worldwide public-health challenge and is considered to be caused by the interaction of genetic determinants and environmental factors.[1]

The renin–angiotensin system (RAS) continues to be the focus of substantial research efforts because of its powerful role in both blood pressure (BP) regulation and sodium and volume homoeostasis.[2] Angiotensin-converting enzyme 2 (ACE2), a new member of the RAS, can convert angiotensin I (Ang I) into angiotensin 1–9, which can be further converted to the vasodilator angiotensin 1–7 (Ang 1–7) by ACE or other peptidases. ACE2 can also directly convert angiotensin II (Ang II), a powerful vasoconstrictor, to Ang 1–7. Thereby, ACE2 may counteract the function of ACE by converting Ang II into Ang 1–7 or by competing with ACE for the same substrate Ang I,[3] implicating that ACE2 may potentially be cardio- and reno-protective for hypertension.

ACE2 maps to the chromosomal location Xp22 in human, and to a quantitative trait locus on the X chromosome in hypertensive rats previously identified as a quantitative locus for hypertension.[3] In three mice models, despite the lack of BP changes in the Ace2 mutant mice, there is an increase in Ang II levels.[3] Thus, ACE2 gene should still be regarded as a candidate gene for hypertension, perhaps with gender-specific effect.

A few association studies of ACE2 variants and hypertension have been reported. Benjafield *et al.*[4] found that no association between four single-nucleotide polymorphisms (SNPs) of ACE2 gene and hypertension in an Anglo-Celtic Australian population. Similarly, Lieb *et al.*[5] reported that five ACE2 SNPs were not associated with systolic or diastolic BP or pulse pressure in German subjects. Recently, Zhong *et al.*[6] showed that the ACE2 A/C polymorphism (rs2285666) is associated with hypertension in female Chinese patients with metabolic syndrome.

Considering the functional importance of ACE2 gene in cardiovascular system, we performed a case–control association study with hypertension. We selected the rs1978124 (intron 1), rs2285666 (intron 3), rs4646142 (intron 7) and rs879922 (intron 11) polymorphisms roughly evenly interspersed in the ACE2 gene, aiming at examining whether these polymorphisms are associated with hypertension among northern Han Chinese.

The samples analysed consisted of 494 cases (241 females/253 males) and 484 controls (233 females/251 males), were selected from the InterASIA study and matched for area, gender and age, using criteria described elsewhere.[7] The local bioethical committee approved the study protocol and each participant signed the written consent.

We genotyped the rs1978124 and rs2285666, using the method described by Benjafield *et al.*[4] with a minor modification. Rs4646142 and rs879922 were genotyped by means of the polymerase chain reaction-restriction fragment length polymorphism method (available upon request). The minor allele frequency (MAF) of rs879922 is lower than 0.05, and is therefore not included in later analysis.

The statistical analyses in clinical data were carried out using Stata. Because males are hemizygous, Hardy–Weinberg equilibrium (HWE) was assessed only in females by Fisher's exact test using the programme HWE.[8] Pairwise linkage disequilibrium (LD) coefficients were calculated with estimated haplotype frequencies using the R programme 'LD' within the R package 'genetics' (http://cran.r-project.org/src/contrib/Descriptions/genetics.html). For females, Haplo.stats[9] was used for the estimation of haplotype frequencies and haplotype-based case–control analysis with adjustment for environment covariates. For males, haplotypes were calculated through direct counting.

All SNPs did not deviate from HWE in females. Genotype and allele frequencies for each polymorphism in female subgroup and allele data for male subjects are given in Table 1. None of the polymorphisms showed an association with hypertension status. For females, analysis of variance, adjusted for age, body mass index, cholesterol, triglycerides, creatinine, glucose, high-density lipoprotein cholesterol, revealed no statistical difference between BP level and any of the three SNPs.

Table 1 Results of single-locus analysis and haplotype analysis

Group	n	*Genotype frequencies*				*Allele frequencies*			
		MM	*Mm*	*mm*	P-*value*	*M*	*m*	χ^2	P-*value*
Intron 1 (A→G)		AA	AG	GG		A	G		
Female									
NT	233	63 (0.27)	115 (0.49)	55 (0.24)	0.31	241 (0.52)	225 (0.48)	0.13	0.71
EH	241	60 (0.25)	135 (0.56)	46 (0.19)		255 (0.53)	227 (0.47)		
Male									
NT	247	—*	—*	—*		126 (0.51)	121 (0.49)	0.28	0.60
EH	253	—*	—*	—*		135 (0.53)	118 (0.47)		
Intron 3 (G→A)		AA	GA	GG		A	G		
Female									
NT	232	62 (0.27)	115 (0.50)	55 (0.24)	0.64	239 (0.52)	225 (0.48)	0.10	0.75
EH	237	61 (0.26)	127 (0.54)	49 (0.21)		249 (0.53)	225 (0.47)		
Male									
NT	247	—*	—*	—*		128 (0.52)	119 (0.48)	0.003	0.96
EH	252	—*	—*	—*		130 (0.52)	122 (0.48)		
Intron 7 (G→C)		GG	GC	CC		G	C		
Female									
NT	233	64 (0.28)	115 (0.49)	54 (0.23)	0.60	243 (0.52)	223 (0.48)	0.25	0.62
EH	239	65 (0.27)	127 (0.53)	47 (0.20)		257 (0.54)	221 (0.46)		
Male									
NT	250	—*	—*	—*		131 (0.52)	119 (0.48)	0.002	0.96
EH	251	—*	—*	—*		131 (0.52)	120 (0.48)		

Haplotype[a]	*Females*[b]				*Males*[c]			
	Total (474)	*NT (233)*	*EH (241)*	P-*value*	*Total (504)*	*NT (251)*	*EH (253)*	P-*value*
A-A-G	0.499	0.493	0.506	0.699	0.498	0.498	0.498	0.859
A-G-G	0.007	0.002	0.011	0.148	0.008	0.004	0.012	0.624
A-G-C	0.017	0.022	0.013	0.380	0.012	0.008	0.016	0.686
G-A-G	0.020	0.024	0.016	0.250	0.014	0.016	0.012	0.724
G-G-C	0.449‘	0.457	0.441	0.723	0.464	0.474	0.454	0.858

Abbreviations: EH, essential hypertension; *M*, major allele, *m*, minor allele; NT, normotensive.
*Because males are hemizygous for ACE2 gene, genotype data are not presented.
[a]Haplotypes in the order of rs1978124, rs2285666 and rs4646142, respectively.
[b]Estimated using haplo.stats.
[c]Calculated through direct counting.

The MAF for females and males in different studies is different. For rs2285666 and rs4646142, the allele frequencies in our study are similar to those in other studies of Chinese population both with respect to females and males.[6,10] For rs1978124 and rs2285666, the MAF in Chinese Population is much higher than that in Australian and German populations.[4–6,10] For rs879922, the MAF in Australians is as high as 0.42 in females and 0.31 in males, and up to 0.36 in females and 0.33 in males in Germans, but it was lower than 0.05 in our study. The MAF differences may be owing to founder effect or genetic drift. Furthermore, the SNPs studied have similar MAFs in our study and in Chiu *et al.*'s study[10] and in the study of Lieb *et al.*[5] (except rs2285666), but not in the study by Benjafield *et al.*[4] (with relatively small sample), suggesting Chinese and Caucasian populations roughly have similar MAFs, respectively.

The three SNPs in our study were found to be in strong LD with each other with pairwise LD coefficients (D′) approximating to 1 (D′ = 0.98–1, $P < 0.001$), showing that they were all part of the same LD block. Benjafield *et al.*[4] demonstrated that rs1978124, rs2285666, rs879922 and rs714205 (intron 16) were in LD (D′ = 0.54–1, $P = 0.05$-0.001). Recently, Lieb *et al.*[5] showed that rs4646156 (intron 8), rs879922, rs4240157 (intron 14) and rs233575 (intron 16), but not rs2285666, were in LD. Taken together, the strong LD and the similar MAFs of the studied SNPs suggest that the ACE2 region has both a low mutation rate and a low recombination rate, implying that this genomic region is very stable.

Both simulations and empirical studies have demonstrated that haplotype analyses may be more powerful than single-locus analyses.[11] From the haplotype analysis in our study, it was observed that the haplotype frequencies in females approximately equalled those in males, and that the most

common haplotypes of the rs1978124, rs2285666 and rs4646142 polymorphisms among the northern Han Chinese were A-A-G (49.9% in females versus 49.8% in males) and G-G-C (44.9% in females versus 46.4% in males) (Table 1), which suggests that these two haplotypes are predominant in the northern Chinese Han population. Consistent with the result of Benjafield *et al.*,[4] the haplotype analysis could not provide evidence to support an association between haplotype and hypertension status and BP.

The negative result in our study may be owing to several reasons. First, polymorphisms selected in our study do not cover the gene fully and extensively; however, as indicated by the strong LD between variants in different studies, this might not be a serious problem. Second, EH is a complex disease that is thought to result from interplay between an individual's genetics background and various environmental factors.[1] Thus, only when a sufficient number of control mechanisms are altered in concert would high BP ensue.[3] Third, ACE2 has complex pleiotropic roles in physiology and various pathophysiological states (such as hypertension, heart failure, atherosclerosis, kidney disease, diabetes and severe acute respiratory syndrome, etc.).[2] According to the hypothesis by Otto,[12] genes with pleiotropic effects have low rates of evolution, that is, the ACE2 region is stable and conserved, indicating that the genetic effect is small. On the other aspect, the ACE2 gene intertwines and functions in concert with many other genes, suggesting epistatic effects may exist.[1] Thus, this study is not powerful enough to find the underlying predisposing variants.

This study did not provide evidence to support an association between polymorphisms in ACE2 gene and hypertension in the northern Chinese Han population. However, our study partly revealed the limitation of the traditional method in finding susceptible genetic locus for complex disease. Further investigation is warranted to reveal the relationship between ACE2 gene and hypertension.

What is known on this topic

- Data have shown that ACE2 located on the X chromosome may counteract the function of ACE and has pleiotropic effects: ACE2 may act as protective protein, preventing the development of hypertension; on the other hand, ACE2 involves other various pathophysiological states (such as heart failure, atherosclerosis, kidney disease, diabetes and severe acute respiratory syndrome, etc.).
- Benjafield *et al.*[4] found that there was no association between four ACE2 gene polymorphisms and hypertension in an Australian population of white Anglo-Celtic origin, and that the four SNPs studied were in LD. Similarly, Lieb *et al.*[5] reported that five ACE2 SNPs were not associated with systolic or diastolic BP or pulse pressure in German subjects.
- Recently, Zhong *et al.*[6] showed that the ACE2 A/C polymorphism (rs2285666) is associated with hypertension in female Chinese patients with metabolic syndrome.

What this study adds

- There is an allele frequency difference in different population with different origin and ethnic background.
- It was found that rs1978124, rs2285666 and rs4646142 were in strong LD, and that two haplotypes (A-A-G and G-G-C) were predominant in Chinese northern Han population.
- Despite a relatively larger sample, neither single-locus analysis nor haplotype analysis provided statistical evidence to support a role of ACE2 gene in genetic predisposition to hypertension in the present study.

Abbreviations: ACE, angiotensin-converting enzyme; BP, blood pressure; SNP, single-nucleotide polymorphism.

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