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Association between angiotensin-converting enzyme insertion/deletion gene polymorphism and atrial fibrillation: a meta-analysis

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Aims Recent observations have raised concerns regarding the activation of the renin–angiotensin system and the development of atrial fibrillation (AF). Some initial studies indicated an association between an angiotensin-converting enzyme insertion/deletion (*ACE* I/D) polymorphism and AF, however, the results have been inconsistent. Our aim was to perform a meta-analysis of relevant studies to assess the validity of this association.

Methods and results PubMed, Cochrane clinical trials database, and EMBASE were searched through July 2009, and a manual search was also performed. Of the 68 initially identified studies, 18 case–control studies with 7577 patients were finally analysed. No statistically significant associations were found between the *ACE* I/D polymorphism and AF risk in the genetic additive model and dominant model, whereas a significant association was observed in the recessive model. A significant heterogeneity between individual studies was evident in all three models. Subgroup analyses showed a strong association between the *ACE* I/D polymorphism and hypertensive AF without significant heterogeneity.

Conclusion Our meta-analysis suggests that there is insufficient evidence to demonstrate an association between *ACE* I/D polymorphism and AF risk. However, there seems to be a significant association between *ACE* I/D gene polymorphic variation and AF in patients with hypertension. Additional studies are warranted to further explore this association in ethnically diverse populations and varied cardiovascular substrates.

Keywords Angiotensin-converting enzyme • Gene polymorphism • Atrial fibrillation • Arrhythmias • Meta-analysis

Introduction

Atrial fibrillation (AF) is a rapidly evolving epidemic representing a multifactorial, dynamic disorder with serious health consequences.[1,2] Despite contemporary achievements in the pharmacological and non-pharmacological treatment of AF, its management remains a difficult task. Recently, the role of the renin–angiotensin system (RAS) in AF has been intensively investigated.[3–5]

The human angiotensin-converting enzyme (*ACE*) gene is located in chromosome 17q23.3. In the intron 16 of this gene, a polymorphism consisting of an insertion (I) or a deletion (*D*) of a 287 bp *Alu* repeat sequence has been identified, which results in three genotypes: homozygous D/D, I/I, and heterozygous I/D.[6] Previous reports of an insertion/deletion (I/D) polymorphism within the *ACE* gene have been associated with cardiovascular disease, including essential hypertension, myocardial infarction, dilated cardiomyopathy, and left ventricular hypertrophy.[7–9] It seems that the *ACE* I/D polymorphism accounts for approximately half of the observed variance in ACE levels.[6] Individuals who are homozygous for the D allele have the highest levels of the enzyme, those who are homozygous for the I allele have the lowest, and heterozygous individuals have an intermediate level.

The *ACE* I/D polymorphism represents a candidate gene for association studies in AF.[10,11] However, previous reports have

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shown inconsistent and even contradictory results.[12–19] We therefore conducted a comprehensive meta-analysis of all available data regarding the association between the *ACE* I/D gene polymorphism and AF risk. We sought to examine the strength of genetic association, to determine the between-study heterogeneity, and to explore the reasons for heterogeneity through predefined sensitivity and subgroup analyses.

Methods

Meta-analyses of observational studies present particular challenges because of inherent biases and differences in study designs. Consequently, we performed this analysis according to the guidelines of the Meta-analysis of Observational Studies in Epidemiology Group (MOOSE).[20]

Inclusion criteria

We restricted our meta-analysis to case–control or cohort studies which fulfilled the following inclusion criteria: (i) examined the association between the *ACE* I/D gene polymorphism and AF; (ii) the generally accepted criteria for AF, usually based on patient's history, serial electrocardiography (ECG), and/or ambulatory ECG monitoring; (iii) cases of AF and control subjects without AF were examined; (iv) genotype or allele frequencies were reported in both cases and controls, and (v) validated genotyping methods were used. We included both published and unpublished studies without language restriction. In studies with overlapping patients or controls, the most recent study with the greatest number of subjects was included in the meta-analysis. Attempts were made to the contact the corresponding authors if there were incomplete published data.

Search strategies

We carefully searched PubMed (January 1966 to July 2009), EMBASE (January 1980 to July 2009), the Cochrane Controlled Trials Register (Cochrane Library Issue 2, 2009), and Chinese National Knowledge Infrastructure (CNKI) (January 1980 to July 2009) databases to identify relevant studies. We used the following keywords: 'ACE', 'angiotensin-converting enzyme', 'polymorphism', and 'atrial fibrillation'. Titles and abstracts as well as the reference lists of all the identified reports were examined by two independent investigators (T.L. and G.X.) in order to include potentially relevant studies. The two investigators agreed on the inclusion/exclusion status in 94% of the reviewed studies. Discrepancies were resolved by discussion or consensus with a third reviewer (G.L.). Additionally, a manual search was conducted using review articles on this topic, bibliographies of original papers, and abstracts of the scientific sessions of the American College of Cardiology, the American Heart Association, the European Society of Cardiology, the European Heart Rhythm Association, and the Heart Rhythm Society during the past 5 years.

Data extraction

In order to control for potential bias and improve the reliability, two independent investigators (T.L. and G.X.) performed data extraction using a standard data extraction form to determine eligibility for inclusion and to obtain data via a standardized protocol. Discrepancies were resolved by consensus with a third reviewer (G.L.). The extracted data elements of this review included: (i) publication details: first author's family name, publication year; (ii) study design; (iii) characteristics of the studied population: country of origin, ethnic information, sample size, mean age, gender distribution, definition, characteristics and numbers of cases and controls, matching; (iv) genotyping method used; and (v) genotype distribution and frequencies of the alleles among cases and controls for the *ACE* I/D polymorphism. For the studies that had more than one control group, we selected the most matched controls for the case group. In the study from Wang *et al.*[15] which reported two different matching case and control groups, we extracted related data separately for different subgroup analyses (*Table 1*).

Statistical analysis

The strength of association between the *ACE* I/D gene polymorphism and AF was estimated using odds ratios (OR), with the corresponding 95% confidence intervals (95% CI). For the *ACE* I/D polymorphism, we first estimated the risk of the variant genotype D/D compared with the wild-type I/I homozygote (additive model) and then evaluated the risks of D/D vs. (I/D + I/I) (recessive model) and (D/D + I/D) vs. I/I (dominant model). Heterogeneity was examined using the standard χ^2 test. Since this test has poor power in the case of few analysed studies, we considered the presence of significant heterogeneity at the 10% level of significance and values of I^2 exceeding 56% as an indicator of significant heterogeneity.[21] If the χ^2 test for heterogeneity was significant, a pooled effect was calculated with a random-effects model which was used to take into account the within-study and between-study variance, otherwise, with a fixed-effects model. Pearson's χ^2 test was used to determine whether the observed frequencies of genotypes in control group conformed to the Hardy–Weinberg equilibrium (HWE).[22] Studies with controls that violated HWE ($P < 0.05$) were subjected to a sensitivity analysis. Sensitivity analyses were also performed by excluding four studies with a small number of cases ($n < 50$) or those two were presented as preliminary abstracts to check the consistency of the overall effect estimate. Subgroup analyses were performed for populations of differing ethnicity, different AF co-morbidities (i.e. lone AF, AF with hypertension, AF with heart failure), and different sources of controls (hospital-based or population-based) to investigate the possible origin of heterogeneity. Egger's regression asymmetry tests were performed to examine publication bias.[23]

All analyses were performed using SPSS 11.5 Statistical Package Program for Windows (SPSS Inc., Chicago, IL, USA) and Review Manager version 4.2 (Revman, The Cochrane Collaboration, Oxford, UK). All the tests were two-sided and the significant *P*-value <0.05.

Results

Eligible studies

Sixty-eight records were identified by the primary literature search. However, after screening the titles and abstracts, 41 studies were excluded secondarily as they were review articles or irrelevant to the current analysis. We retrieved the 27 remaining relevant manuscripts for detailed review. Among these, five studies were excluded due to duplicate publications, two additionally had incomplete data on genotyping frequency and we did not receive any response from the corresponding authors after requesting extra information,[24,25] and an additional two were excluded due to the absence of a control group.[26,27] Consequently, the remaining 18 case–control studies which examined the association between the *ACE* I/D gene polymorphism and AF were finally included (*Figure 1*).[12–19,28–37] The main characteristics of these studies are shown in *Table 1*. The total number of subjects enrolled in the 18 studies was 7577, comprising 2188 patients and

Table 1 Characteristics of studies included in the meta-analysis

Author (ref. no.)	Year	Country	Ethnicity	Cases	Controls	Case/control (*n*)	Case/control, mean age (years)	Case/control, men (%)
Yamashita *et al.*[12]	1997	Japan	East Asian	Lone AF	Healthy volunteers	77/83	65/64	71.4/NA
Ogimoto *et al.*[13]	2002	Japan	East Asian	HCM patients with AF	HCM patients without AF	26/112	66/62	85/73
Gensini *et al.*[14]	2003	Italy	Caucasian	Documented AF lasting >24 h (lone AF 20%)	Healthy volunteers	148/210	67/65	62.2/59.0
Wang *et al.*[15] (1)	2003	China	East Asian	Lone AF	Healthy subjects	53/50	43/41	71.7/64.0
Wang *et al.*[15] (2)	2003	China	East Asian	Hypertensive patients with AF	Hypertensive patients without AF	32/38	45/41	65.6/52.6
Tsai *et al.*[16]	2004	China (Taiwan)	East Asian	Non-familial structural AF	Matched controls with structural heart disease	250/250	68/66	58/58
Asselbergs *et al.*[17]	2006	Netherlands	Caucasian	AF subjects within a general population	Age- and gender-matched controls	97/97	60/59	56.7/56.7
Bedi *et al.*[18]	2006	USA	Caucasian	Non-familial AF with CHF	CHF patients without AF	51/289	63/56	87/70
Fatini *et al.*[19]	2007	Italy	Caucasian	Non-valvular AF (lone AF 20%)	Healthy subjects	510/520	71/70	61.2/62.7
Chen *et al.*[28]	2007	China	East Asian	Persistent AF hospitalized for EC	Hospitalized patients without AF	40/36	59/57	77.5/72.2
Chang *et al.*[29]	2007	China	East Asian	Hospitalized non-familial AF	Hospitalized matched controls without AF	189/194	56/56	63.5/61.9
Tziakas *et al.*[30]	2007	Greece	Caucasian	Hypertensive patients with AF	Matched hypertensive patients without AF	158/174	71/71	45.6/50.6
Tsai *et al.*[31]	2008	China (Taiwan)	East Asian	Non-familial documented AF	Patients without documented or history of AF	227/1009	68/62	52.9/50.6
Wei *et al.*[32]	2008	China	East Asian	Documented structural AF	Patients without AF (63.5%) and healthy subjects	55/63	70/64	56.4/55.6
Darrieux *et al.*[33] (abstract)	2008	Brazil	South American	Lone AF	Individuals from general population without AF and structural heart disease	64/1576	44/45	75.0/45.4
Huang *et al.*[34]	2009	China	East Asian	AF patients with hypertensive disease	Hypertensive disease patients without AF	97/529	73/71	67.0/62.6
Yang *et al.*[35]	2009	China	East Asian	Non-familial AF	Hospitalized patients without AF	20/30	77/77	80.0/83.3
Dana *et al.*[36] (abstract)	2009	Romania	Caucasian	Heart failure patients with permanent AF	Heart failure patients without AF	25/11	71 (total)	55.5 (total)
Watanabe *et al.*[37]	2009	USA	Caucasian	Paroxysmal lone AF	Healthy subjects	69/118	38/27	72/39

AF, atrial fibrillation; HCM, hypertrophy cardiomyopathy; CHF, congestive heart failure; EC, electrical cardioversion.

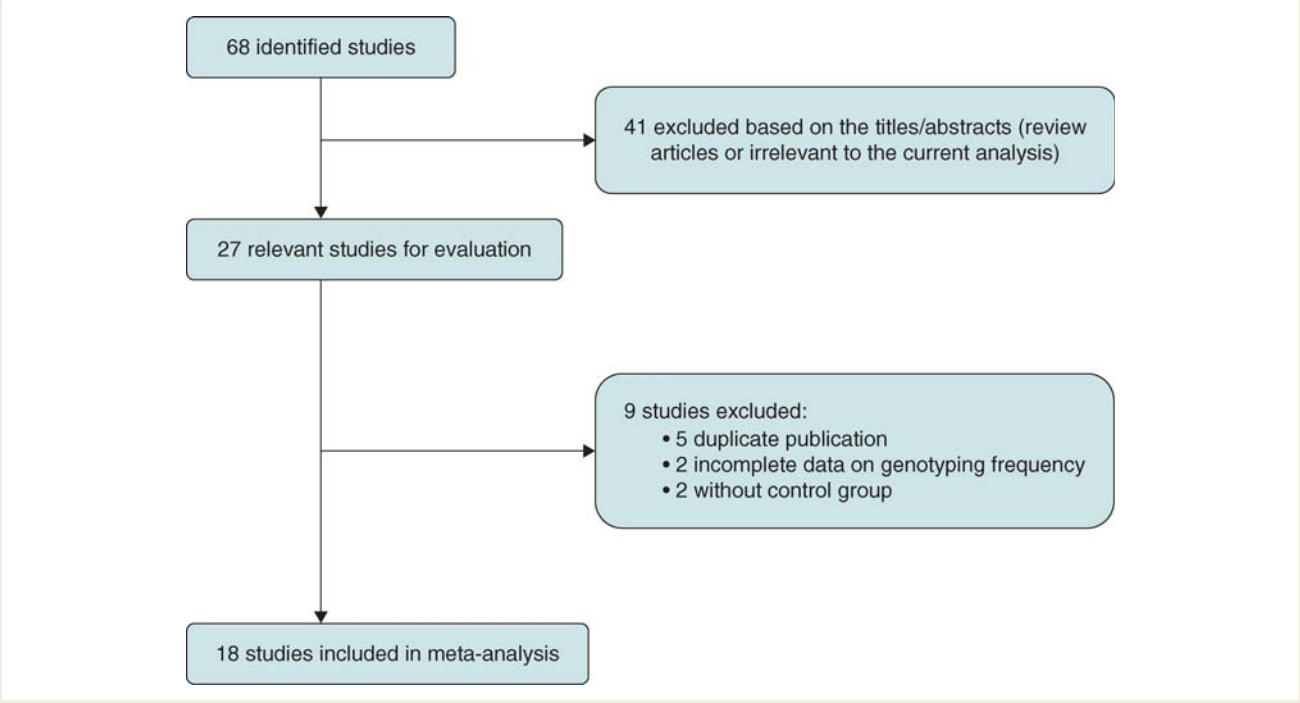

Figure 1 Flow diagram of the trial selection process.

Table 2 Distribution of genotype and allele frequencies in individual studies

Author (ref. no.)	Cases				Controls				HWE in control, *P*-value
	D/D	I/D	I/I	D allele	D/D	I/D	I/I	D allele	
Yamashita *et al.*[12]	11	37	29	0.38	13	40	30	0.40	0.96
Ogimoto *et al.*[13]	3	7	16	0.25	18	57	37	0.42	0.61
Gensini *et al.*[14]	68	62	18	0.67	50	95	65	0.46	0.19
Wang *et al.*[15] (1)	11	26	16	0.45	8	24	18	0.41	1.00
Wang *et al.*[15] (2)	5	16	11	0.41	7	18	13	0.42	0.86
Tsai *et al.*[16]	54	98	98	0.41	61	105	84	0.45	0.02
Asselbergs *et al.*[17]	25	42	30	0.47	26	49	22	0.52	0.91
Bedi *et al.*[18]	23	16	12	0.67	85	153	51	0.56	0.21
Fatini *et al.*[19]	222	218	70	0.65	143	251	126	0.52	0.44
Chen *et al.*[28]	7	20	13	0.43	6	17	13	0.40	0.91
Chang *et al.*[29]	50	52	87	0.40	30	44	120	0.27	<0.01
Tziakas *et al.*[30]	62	70	26	0.61	38	88	48	0.47	0.84
Tsai *et al.*[31]	50	88	89	0.41	216	496	297	0.46	0.74
Wei *et al.*[32]	18	23	14	0.59	12	28	23	0.52	0.51
Darrieux *et al.*[33]	17	31	16	0.51	460	862	254	0.57	<0.01
Huang *et al.*[34]	20	40	37	0.41	43	222	264	0.29	0.70
Yang *et al.*[35]	7	6	7	0.50	14	8	8	0.60	0.01
Dana *et al.*[36]	12	5	8	0.58	5	3	3	0.59	0.15
Watanabe *et al.*[37]	17	38	14	0.25	41	53	24	0.34	0.37

D, deletion; I, insertion; HWE, Hardy–Weinberg equilibrium.

5389 controls. The average age ranged from 38 to 77 years in the case groups and from 27 to 77 years in the control groups. The prevalence of male gender varied from 45.6 to 87% in the case groups and from 39 to 83.3% in the control groups. Ten studies[12,13,15,16,28,29,31,32,34,35] examined East Asian populations, seven examined Caucasian subjects,[14,17–19,30,36,37] and the

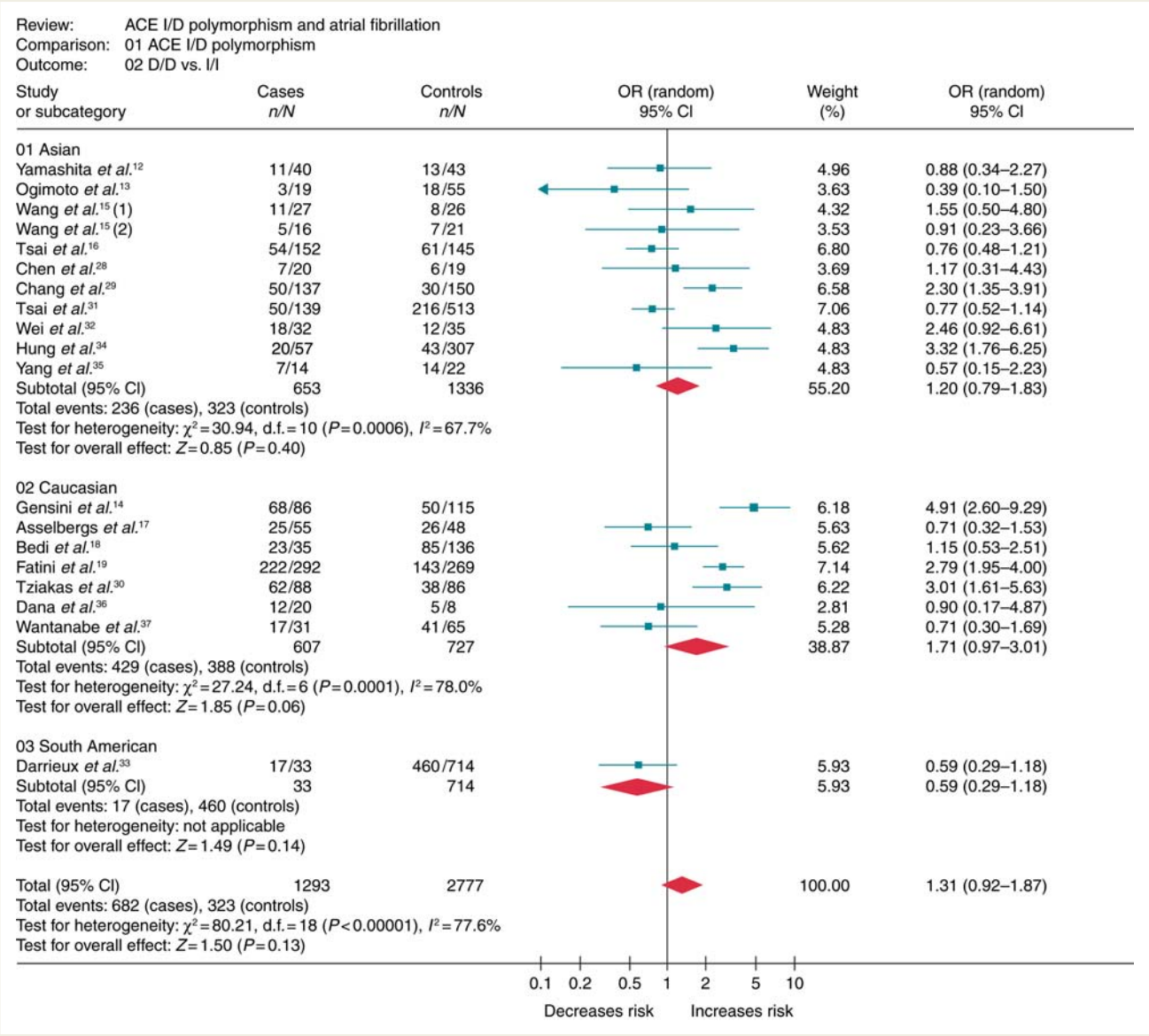

Figure 2 Forest plots of OR with 95% CI for the association between the *ACE* I/D gene polymorphism and AF risk [additive genetic model: D/D vs. I/I].

remaining one enrolled South American subjects.[33] Of the 18 studies, 16 were published as full manuscripts and 2 in abstract form.[33,36] The HWE test that was performed in the control group of each study revealed that four of them[16,29,33,35] violated the HWE (*Table 2*). The distribution of the genotype in these studies is listed in *Table 2*.

Results of meta-analysis

Among the 18 studies analysed, the association between the D/D genotype compared with I/I genotype and AF risk showed no significance utilizing the random-effects model (OR = 1.31, 95% CI: 0.92–1.87, $Z = 1.50$, $P = 0.13$) (*Figure 2*). Additionally, no significant association was demonstrated in the dominant model (OR = 1.09, 95% CI: 0.82–1.43, $Z = 0.58$, $P = 0.56$) (*Figure 3*). However, this association was significant in the recessive model (OR = 1.36, 95% CI: 1.07–1.74, $Z = 2.49$, $P = 0.01$) (*Figure 4*). Significant heterogeneity was present in all three models [additive model ($P < 0.0001$, $I^2 = 77.6\%$), recessive model ($P < 0.0001$, $I^2 = 65.8\%$), and dominant model ($P < 0.0001$, $I^2 = 76.2\%$)].

Sensitivity and subgroup analyses

We subsequently performed sensitivity and subgroup analyses to investigate the origin of this heterogeneity. After removing four studies[13,28,35,36] which included a small number of cases ($n < 50$) and two studies[33,36] which were presented as abstracts, no significant difference from main analysis was noted. Moreover, exclusion of studies[16,29,33,35] having controls that were not in HWE did not alter the results of the main meta-analysis.

Subgroup analysis stratified by ethnicity of populations (*Table 3*) showed significant association between the *ACE* I/D polymorphism

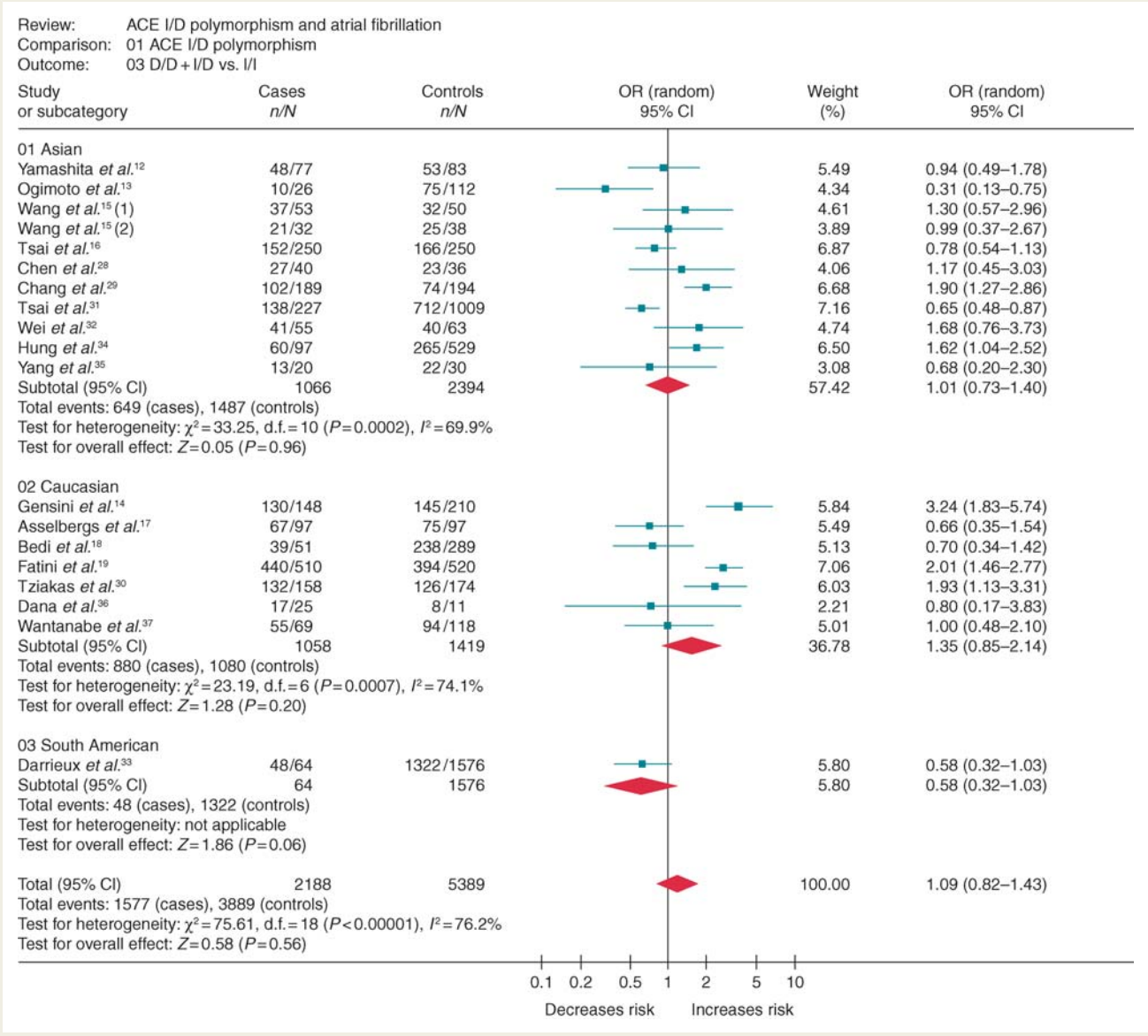

Figure 3 Forest plots of OR with 95% CI for the association between the *ACE* I/D gene polymorphism and AF risk [dominant genetic model: (D/D + I/D) vs. I/I].

and AF in the recessive model (random-effects OR = 1.62, 95% CI: 1.13–2.34) in the Caucasian population[14,17–19,30,36,37] (*Figure 4*). No significant statistical association was observed in the additive and dominant models (*Figures 2* and *3*). For the Eastern Asian subjects,[12,13,15,16,28,29,31,32,34,35] no significant association was evident in all three models (*Figures 2–4*). A significant heterogeneity was noticed among the stratified studies by ethnicity in the three different models (*Table 3*).

In a subgroup analysis according to AF co-morbidities, there was a significant association between the *ACE* I/D gene polymorphism and AF risk in patients with hypertension[15,30,34] (*Table 3*), evident in all three genetic models (fixed-effects OR = 2.76, 95% CI: 1.81–4.22 for additive model; fixed-effects OR = 2.27, 95% CI: 1.59–3.25 for recessive model; fixed-effects OR = 1.64, 95% CI: 1.19–2.26 for dominant model) without significant heterogeneity between the studies. In patients with heart failure,[18,36] a significant association was demonstrated in the recessive model for D allele without heterogeneity (fixed-effects OR = 1.79, 95% CI: 1.03–3.14). In lone AF patients[12,15,33,37] or other composite groups of AF, the random-effects pooled OR were not significant in the three gene models and also had significant heterogeneity.

Finally, the subgroup analysis classified by different sources of controls showed that in studies with hospital-based controls,[13,16,18,29–32,34–36] the relationship between the *ACE* I/D polymorphic variant and AF was evident only under the recessive model (random-effects OR = 1.43, 95% CI: 1.05–1.94) which was similar to the main analysis. In the studies that enrolled

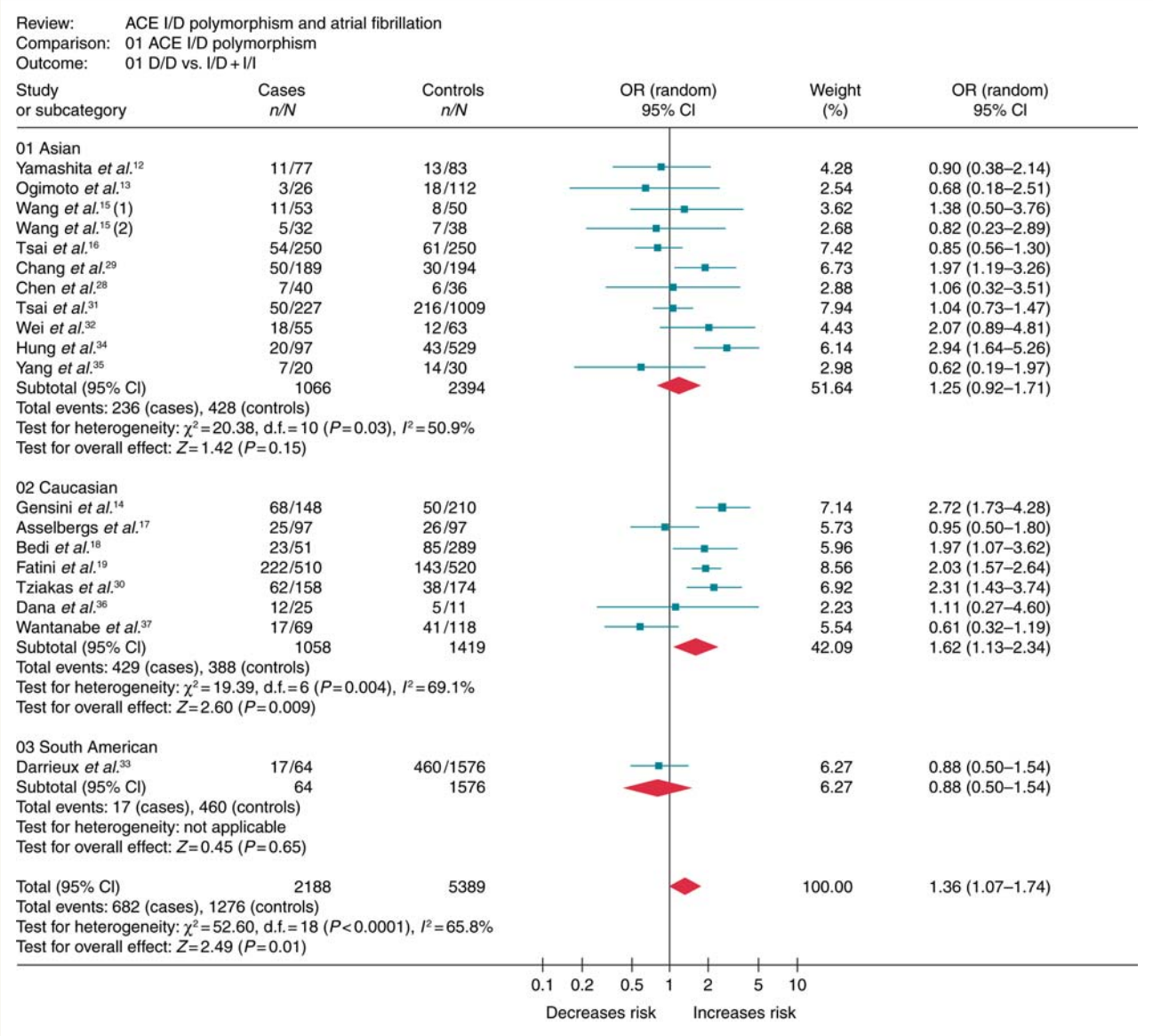

Figure 4 Forest plots of OR with 95% CI for the association between the *ACE* I/D gene polymorphism and AF risk [recessive genetic model: D/D vs. (I/D + I/I)].

population-based controls,[12,14,15,17,19,33,37] the *ACE* I/D polymorphism was not associated with AF. There was also significant heterogeneity noted in both the analyses (*Table 3*).

Egger's regression asymmetry tests showed no evidence of publication bias in all three genetic models (additive: $P = 0.34$; recessive: $P = 0.16$; and dominant: $P = 0.61$).

Discussion

This comprehensive meta-analysis indicates that there is insufficient evidence to draw clear conclusions on the potential association between the *ACE* I/D polymorphism and AF risk. Moreover, a significant heterogeneity was evident across studies. On the other hand, the subgroup analysis demonstrated that there was a significant association between *ACE* I/D gene polymorphic variation and AF risk in patients with hypertension (in all three genetic models). Of note, no heterogeneity was observed across these studies.

Recent evidence indicates that activation of the RAS plays an important role in the development and perpetuation of AF.[3–5] *ACE* expression is elevated in atrial biopsies of patients with AF[38] and angiotensin II (Ang II) concentrations are increased in the rapid ventricular pacing-induced congestive heart failure model of AF.[39] We have previously demonstrated that ACE-inhibitors (ACEIs), angiotensin-receptor blockers (ARBs), and angiotensin-(1–7) prevent atrial electrical and structural remodelling and reduce AF vulnerability in chronic rapid atrial pacing in canines.[40,41] Recent clinical evidence also suggests that RAS inhibition with ACEIs and/or ARBs may suppress AF in patients with systolic left ventricular dysfunction and left ventricular hypertrophy.[42] Angiotensin II is a

Table 3 Odds ratios and heterogeneity tests for the *ACE* I/D gene polymorphism and risk of atrial fibrillation in different subgroup populations

	n	Additive model, D/D vs. I/I [OR (95% CI); Ph]	Dominant model, (D/D + I/D) vs. I/I [OR (95% CI); Ph]	Recessive model, D/D vs. (I/D + I/I) [OR (95% CI); Ph]
Total	18	1.31 (0.92–1.87); <0.0001	1.09 (0.82–1.43); <0.0001	1.36 (1.07–1.74)*; <0.0001
Ethnicity				
East Asian	10	1.20 (0.79–1.83); 0.0006	1.01 (0.73–1.40); 0.0002	1.25 (0.92–1.72); 0.03
Caucasian	7	1.71 (0.97–3.01); 0.0001	1.35 (0.85–2.14); 0.0007	1.62 (1.13–2.34)*; 0.004
South American	1	0.59 (0.29–1.18); NA	0.58 (0.32–1.03); NA	0.88 (0.50–1.54); NA
AF groups[a]				
Lone AF	4	0.78 (0.50–1.20); 0.54	0.85 (0.61–1.20); 0.39	0.84 (0.59–1.21); 0.59
AF with hypertension	3	2.76 (1.81–4.22)*; 0.24	1.64 (1.19–2.26)*; 0.51	2.27 (1.59–3.25)*; 0.20
AF with heart failure	2	1.10 (0.54–0.23); 0.80	0.71 (0.37–1.37); 0.88	1.79 (1.03–3.14)*; 0.46
Other AF[b]	10	1.33 (0.84–2.24); <0.0001	1.10 (0.72–1.69); <0.0001	1.36 (0.99–1.89); 0.0003
Source of controls[a]				
Hospital	12	1.31 (0.86–1.99); <0.0001	1.02(0.73–1.43); <0.0001	1.43(1.05–1.94)*; 0.005
Population	7	1.32 (0.68–2.56); <0.0001	1.19(0.73–1.95); <0.0001	1.25(0.81–1.94); 0.0003

ACE, angiotensin-converting enzyme; D, deletion; I, insertion; *n*, number of studies involved; OR, odds ratio; CI, confidence interval; Ph, *P*-value of the χ^2 test for heterogeneity between the studies; NA, not available; AF, atrial fibrillation.
[a]In the study from Wang *et al*. which reported two different matching case and control groups, we extracted related data separately. Therefore, there are 19 data sets in the subgroup analysis of AF groups and source of controls.
[b]Other AF includes data from studies with mixed AF groups or those in which co-morbidities were not clearly defined.
**P*-value calculated by *Q*-test for OR was <0.05.

potent promoter of interstitial fibrosis triggering the mitogen-activated protein kinase pathway[39] which is responsible for the proliferation of fibroblasts and hypertrophy of cardiomyocytes.[5] Theoretically, the DD genetic variant may increase ACE activity and consequently promote Ang II production and atrial fibrosis, causing atrial structural remodelling and promoting development of AF. However, studies that have examined the association between the *ACE* I/D polymorphism and AF showed inconsistent, even contradictory results. Our present meta-analysis demonstrated no conclusive association between the *ACE* I/D polymorphism and the risk of AF, possibly due to the significant heterogeneity between different individual studies.

When between-study variation cannot be explained by chance, exploration of the reasons for heterogeneity rather than derivation of a single summary estimate emerges as the main goal of a meta-analysis. The heterogeneity test in our analysis showed that there were significant differences between individual studies. Subsequently, sensitivity and subgroup analyses were performed in order to investigate the underlying causes. Our sensitivity analysis showed that even after excluding studies with a small number of cases ($n < 50$), studies presented as abstracts, or having controls violating the HWE, the results of the main meta-analysis do not change.

Alternatively, the subgroup analysis showed some interesting results. First, although the ethnic background does not seem to have obvious influence on the heterogeneity of our analysis, white subjects have a significant association between the *ACE* I/D polymorphism and AF under the recessive model, whereas no significant association was found in all the three genetic models for Eastern Asian populations. In addition, our analysis indicated that there was a strong relationship between the *ACE* I/D polymorphism and hypertensive AF without significant heterogeneity in all the three genetic models. Finally, we demonstrated that differing sources of controls were not the cause of heterogeneity in our meta-analysis. Such possible sources of heterogeneity in our analysis may include differences in the underlying cardiovascular substrates, different sample selection, ethnic differences, or to varied methods of genotyping.

It is prudent to acknowledge that several potential limitations are apparent. We did not receive a response from the corresponding authors of two studies and therefore we did not acquire the data on genotyping frequency necessary for analysis. These articles were excluded from our meta-analysis. Only published studies were included in the meta-analysis and thus we cannot exclude the possibility of publication bias, although the results of statistical tests showed that publication bias is unlikely. Additionally, our analysis is based on observational studies and unadjusted estimates and may be subject to the potential biases of such studies. The interactions between gene–gene and gene–environment could not be included in our meta-analysis due to a lack of relative data. Finally, we have to acknowledge that meta-analysis represents a retrospective research tool that is subject to the methodological deficiencies of the included studies. Therefore, our results should be interpreted cautiously.

Conclusion

In conclusion, our comprehensive meta-analysis indicates that there is insufficient evidence to demonstrate a conclusive association between the *ACE* I/D polymorphism and the risk of AF,

whereas significant heterogeneity was evident across the individual studies. The subgroup analysis indicated that there is a significant association between *ACE* I/D gene polymorphic variation and AF risk in patients with hypertension. Further larger, well-designed studies are needed to elucidate this potential association, examining subjects with different ethnic backgrounds and diverse cardiovascular substrates.

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References

1. Kannel WB, Benjamin EJ. Current perceptions of the epidemiology of atrial fibrillation. *Cardiol Clin* 2009;**27**:13–24.
2. Zhang S. Atrial fibrillation in mainland China: epidemiology and current management. *Heart* 2009;**95**:1052–5.
3. Ehrlich JR, Hohnloser SH, Nattel S. Role of angiotensin system and effects of its inhibition in atrial fibrillation: clinical and experimental evidence. *Eur Heart J* 2006;**27**:512–8.
4. Iravanian S, Dudley SC. The renin–angiotensin–aldosterone system (RAAS) and cardiac arrhythmias. *Heart Rhythm* 2008;**5**:S12–7.
5. Novo G, Guttilla D, Fazio G, Cooper D, Novo S. The role of the renin–angiotensin system in atrial fibrillation and the therapeutic effects of ACE-Is and ARBS. *Br J Clin Pharmacol* 2008;**66**:345–51.
6. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;**86**:1343–6.
7. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Witteman JC. ACE polymorphisms. *Circ Res* 2006;**98**:1123–33.
8. Zintzaras E, Raman G, Kitsios G, Lau J. Angiotensin-converting enzyme insertion/deletion gene polymorphic variant as a marker of coronary artery disease: a meta-analysis. *Arch Intern Med* 2008;**168**:1077–89.
9. Rudnicki M, Mayer G. Significance of genetic polymorphisms of the renin–angiotensin–aldosterone system in cardiovascular and renal disease. *Pharmacogenomics* 2009;**10**:463–76.
10. Andalib A, Brugada R, Nattel S. Atrial fibrillation: evidence for genetically determined disease. *Curr Opin Cardiol* 2008;**23**:176–83.
11. van den Berg MP, Van Gelder IC. New insight into the association among atrial fibrillation, electrophysiological remodeling, and the ACE insertion/deletion polymorphism—toward a more patient-tailored therapy? *Heart Rhythm* 2009;**6**:1333–4.
12. Yamashita T, Hayami N, Ajiki K, Oikawa N, Sezaki K, Inoue M *et al.* Is ACE gene polymorphism associated with lone atrial fibrillation? *Jpn Heart J* 1997;**38**:637–41.
13. Ogimoto A, Hamada M, Nakura J, Miki T, Hiwada K. Relation between angiotensin-converting enzyme II genotype and atrial fibrillation in Japanese patients with hypertrophic cardiomyopathy. *J Hum Genet* 2002;**47**:184–9.
14. Gensini F, Padeletti L, Fatini C, Sticchi E, Gensini GF, Michelucci A. Angiotensin-converting enzyme and endothelial nitric oxide synthase polymorphisms in patients with atrial fibrillation. *Pacing Clin Electrophysiol* 2003;**26**:295–8.
15. Wang S, Zhang P, Chen F, Zhang Y, Du L, Zhao J *et al.* Correlation between ACE-I gene polymorphism and atrial fibrillation (In Chinese). *Chin J Pract Intern Med* 2003;**23**:537–8.
16. Tsai CT, Lai LP, Lin JL, Chiang FT, Hwang JJ, Ritchie MD *et al.* Renin–angiotensin system gene polymorphisms and atrial fibrillation. *Circulation* 2004;**109**:1640–6.
17. Asselbergs FW, Moore JH, van den Berg MP, Rimm EB, de Boer RA, Dullaart RP *et al.* A role for CETP TaqIB polymorphism in determining susceptibility to atrial fibrillation: a nested case–control study. *BMC Med Genet* 2006;**7**:39.
18. Bedi M, McNamara D, London B, Schwartzman D. Genetic susceptibility to atrial fibrillation in patients with congestive heart failure. *Heart Rhythm* 2006;**3**:808–12.
19. Fatini C, Sticchi E, Gensini F, Gori AM, Marcucci R, Lenti M *et al.* Lone and secondary nonvalvular atrial fibrillation: role of a genetic susceptibility. *Int J Cardiol* 2007;**120**:59–65.
20. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D *et al.* Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000;**283**:2008–12.
21. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;**21**:1539–58.
22. Schaid DJ, Jacobsen SJ. Biased tests of association: comparisons of allele frequencies when departing from Hardy–Weinberg proportions. *Am J Epidemiol* 1999;**149**:706–11.
23. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315**:629–34.
24. Ravn LS, Benn M, Nordestgaard BG, Sethi AA, Agerholm-Larsen B, Jensen GB *et al.* Angiotensinogen and ACE gene polymorphisms and risk of atrial fibrillation in the general population. *Pharmacogenet Genomics* 2008;**18**:525–33.
25. Funada A, Ino H, Fujino N, Hayashi K, Uchiyama K, Masuta E *et al.* Impact of renin–angiotensin system polymorphisms on atrial fibrillation associated with heart failure in hypertrophic cardiomyopathy. *CVD Prev Control* 2009;**4**(Suppl. 1):S66.
26. Darbar D, Motsinger AA, Ritchie MD, Gainer JV, Roden DM. Polymorphism modulates symptomatic response to antiarrhythmic drug therapy in patients with lone atrial fibrillation. *Heart Rhythm* 2007;**4**:743–9.
27. Wei W, Zhang H, Wei L, Wang D. Correlation between angiotensin converting II enzyme gene polymorphisms and myocardial fibrosis in patients with atrial fibrillation (In Chinese). *Int J Cardiovasc Dis* 2008;**35**:390–2.
28. Chen X, Ling F, Shen F. The study of the association between the insertion/deletion (I/D) polymorphism of angiotensin-converting enzyme (ACE) gene and atrial fibrillation (In Chinese). *Zhejiang Med J* 2007;**29**:908–10.
29. Chang H, Yang S, Han W. Associations of the polymorphisms in renin–angiotensin system with nonfamilial structural atrial fibrillation (In Chinese). *Hei Long Jiang Med J* 2007;**31**:731–3.
30. Tziakas DN, Chalikias GK, Stakos DA, Papazoglou D, Papanas N, Papatheodorou K *et al.* Effect of angiotensin-converting enzyme insertion/deletion genotype on collagen type I synthesis and degradation in patients with atrial fibrillation and arterial hypertension. *Expert Opin Pharmacother* 2007;**8**:2225–34.
31. Tsai CT, Hwang JJ, Chiang FT, Wang YC, Tseng CD, Tseng YZ *et al.* Renin–angiotensin system gene polymorphisms and atrial fibrillation: a regression approach for the detection of gene-gene interactions in a large hospitalized population. *Cardiology* 2008;**111**:1–7.
32. Wei L, Wei W, Lu G, Yang X, Zhang Y, Zhang X *et al.* Study of association of polymorphism in angiotensin-converting enzyme insertion/deletion gene and β2-adrenergic receptor 16 locus genes with atrial fibrillation (In Chinese). *Med J Natl Defend Forces Southwest China* 2008;**18**:495–8.
33. Darrieux F, Pereira A, Pisani C, Grativol P, Silva R, Macatrão M *et al.* Association between angiotensin converting enzyme and angiotensinogen gene polymorphisms and lone atrial fibrillation. *Heart Rhythm* 2008;**5**(Suppl):S377.
34. Huang M, Gai X, Yang X, Hou J, Lan X, Zheng W *et al.* Functional polymorphisms in ACE and CYP11B2 genes and atrial fibrillation in patients with hypertensive heart disease. *Clin Chem Lab Med* 2009;**47**:32–7.
35. Yang C, Liang X, Huang L, Xu X, Zhai L. Associations between angiotensin-converting enzyme (ACE), angiotensinogen (AGT) and nonfamilial atrial fibrillation. *Guangdong Med J (In Chinese)* 2009;**30**:86–7.
36. Dana P, Dumitru Z, Lucia P, Adela S, Adela P. Gene polymorphism of angiotensin-converting enzyme and angiotensin II type 1 receptor in heart failure patients with atrial fibrillation. *Europace* 2009;**11**(Suppl. 1):i45,No. 191.
37. Watanabe H, Kaiser DW, Makino S, MacRae CA, Ellinor PT, Wasserman BS *et al.* ACE I/D polymorphism associated with abnormal atrial and atrioventricular conduction in lone atrial fibrillation and structural heart disease: implications for electrical remodeling. *Heart Rhythm* 2009;**6**:1327–32.
38. Goette A, Staack T, Rocken C, Arndt M, Geller JC, Huth C *et al.* Increased expression of extrACEllular signal-regulated kinase and angiotensin-converting enzyme in human atria during atrial fibrillation. *J Am Coll Cardiol* 2000;**35**:1669–77.
39. Li D, Shinagawa K, Pang L, Leung TK, Cardin S, Wang Z *et al.* Effects of angiotensin-converting enzyme inhibition on the development of the atrial fibrillation substrate in dogs with ventricular tachypacing-induced congestive heart failure. *Circulation* 2001;**104**:2608–14.
40. Liu E, Xu Z, Li J, Yang S, Yang W, Li G. Enalapril, irbesartan, and angiotensin-(1–7) prevent atrial tachycardia-induced ionic remodeling. *Int J Cardiol* 2011;**146**:364–70.
41. Liu E, Yang S, Xu Z, Li J, Yang W, Li G. Angiotensin-(1–7) prevents atrial fibrosis and atrial fibrillation in long-term atrial tachycardia dogs. *Regul Pept* 2010;**162**:73–8.
42. Healey JS, Baranchuk A, Crystal E, Morillo CA, Garfinkle M, Yusuf S *et al.* Prevention of atrial fibrillation with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: a meta-analysis. *J Am Coll Cardiol* 2005;**45**:1832–9.