Author's response to reviews

Title: Angiotensin converting enzyme DD genotype is associated with severity and sudden cardiac death of acute coronary syndrome in Taiwan - a hospital based emergency room study

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Author's response to reviews: see over
Editorial comments:

We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional language editing service. For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz (www.edanzediting.com/bmc1). BioMed Central has negotiated a 10% discount to the fee charged to BioMed Central authors by Edanz.

A: This draft had been edited by Edanz, and the certification note was attached.
10/1/2012

To whom it may concern,

Re: "Angiotensin converting enzyme DD genotype is associated with acute coronary syndrome severity and sudden cardiac death in Taiwan: a case-control emergency room study"

By Yu Cheng-Ping (Department of Emergency Medicine, Tri-Service General Hospital, National Defense Medical Center, No.325, Sec.2, Chenggong Rd., Neihu District, Taipei City 114, Taiwan(R.O.C))

This is to confirm that this text has been edited by Dr Mary Cant (1080 PhD Pharmacology, University of Edinburgh, UK), an editor working for Edanz Group Ltd.

Sincerely yours,

Kerry Greer
Chief Editor
Edanz Group Ltd.
Reviewer #1:

My opinion this manuscript; Authors investigated the association between angiotensin converting enzyme DD genotype polymorphism and severity and sudden cardiac death of acute coronary syndrome in Taiwan - a hospital based emergency room study.

It is original and interesting study. If the editor appropriate, it can be acceptable for published the Journal.

A: Thanks for your opinion.

Reviewer #2:

Q(1). Introduction is relatively relevant.

A(1): Thanks for your opinion.

Q(2). The discussion is very good.

A(2): Thanks for your opinion.

Q(3). Materials & methods, Mistyping of ID heterozygote as D homozygotes may occur. Thus, each sample which had the DD genotype must submitte to PCR amplification using the forward: 5’- TCG GAC CAC AGC GCC CGC CAC TAC-3’ and the reverse 5’-TCG CCA GCC CTC CCA TGC CCA TAA-3’ primers with
identical PCR conditions except for an annealing temperature of 67°C.

A(3): We compared the DD genotype (490bp) and II genotype (190bp) with designated primer set (primer set 1). Besides, all DD genotype samples were further confirmed by using a pair of primers that produce a 335bp product for I allele (with 62°C annealing temperature, Lee and Tsai, 2002) and the suggested primer set (with 67°C annealing temperature, Namazi S et al., 2010). These two primer sets are similar in sequences especially in 3’-termini, therefore the PCR productivity was also similar in both primer sets. These is no mistyping of ID heterozygote as D homozygotes in this study, and a representative agarose gel electrophoresis of each ACE genotype was showed as followed.

<table>
<thead>
<tr>
<th>Primer set 1</th>
<th>F- 5’-CTGGAGACCACCTCCCATCTTTTCT-3’</th>
<th>I allele: 490bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Tiret et al., 1992)</td>
<td>R-5’-GATGTGGCCATCACATTCGTCAGAT-3’</td>
<td>D allele: 190bp</td>
</tr>
<tr>
<td>Primer set 2</td>
<td>F- 5’-TGGGACCACAGCGCCCCGACTAC-3’</td>
<td>I allele: 335bp</td>
</tr>
<tr>
<td>(Lee and Tsai, 2002)</td>
<td>R- 5’-TCGCCAGCCCTCCCATGCCCATTA-3’</td>
<td>D allele: no product</td>
</tr>
<tr>
<td>Primer set 3</td>
<td>F-5’-TCGGACCACAGCGCCCCGACTAC-3’</td>
<td>I allele: 335bp</td>
</tr>
<tr>
<td>(Namazi S et al., 2010)</td>
<td>R-5’-TCGCCAGCCCTCCCATGCCCATTA-3’</td>
<td>D allele: no product</td>
</tr>
</tbody>
</table>


Q(4). How is the statistical power of the analysis?

A(4): The statistical power of those analyses were evaluated by using the G*Power ver 3.1. In Table 1, the total sample size is 306 (ACS, n=111, and NCS, n=195), when set \( \alpha \) error equal to 0.001 and under the odd ratio 2.6, that gave a high power (1-\( \beta \) error probability) equal to 0.96. Besides, in Table 2 and Table 3, the total sample size is 111 (ACS only), when set \( \alpha \) error equal to 0.001 and under the odd ratio 3, that gave a moderate power (1-\( \beta \) error probability) equal to 0.79 (otherwise, if set \( \alpha \) error as 0.01 and the odd ratio as 3, that gave a high power equal to 0.94). At last, in Table 4, the
total sample size is also 111, when set \( \alpha \) error equal to 0.001 and under the odd ratio 6, that gave a high power (1-\( \beta \) error probability) equal to 0.93.

**Q(5).** The language of text is well but it needs a little bit correction.

**A(5):** This article had been edited by Edanz (the attachment is the certification note).

Reviewer #3:

**Major Revisions**

**Q(1).** In the end of Introduction, the authors have explained about their results, that is not standard

**A(1):** The introduction had been revised and also edited by Edanz.

**Q(2).** In my opinion the authors must matched the patients and controls for age. It is obvious that in the older individual the risk of most disease will increase. For this reason by logistic regression analysis, only age but no other factors is the independent risk factor for ASC.

**A(2):** Age-matched case-control study is a better experimental design for most association studies. However, most elder visitors of emergency-room were also CAD carriers, and therefore be excluded from this study to avoid any other confounding
risk factor (non-coronary subjects recruited criteria were described as methods). To avoid the difference between diagnostic age would misleading the risk of ACE genotypes for ACS incidence, we used the multivariate logistic regression to adjust possible interactions between those significantly different risk factors in univariate analysis, including diagnostic age, systolic blood pressure, diastolic blood pressure, and diabetes mellitus. As Table 1 in text, ACE genotypes and diagnostic age were kept their significance after adjustment, and it implicated that systolic blood pressure, diastolic blood pressure, and diabetes mellitus maybe highly interacted; however, ACE genotypes and diagnostic age were act as independent risk factors. In other words, the risk of ACE genotypes for ACS incidence may not be modified by the difference of diagnostic age between ACS and NCS groups in this study.

Q(3). In my opinion the criteria for ASC is not adequate.

A(3): We had replaced this part as definition of a recent review (Trost et al. Crit Care Med 2011). As line 13 of page 8 to line 2 of page 9, it was replaced as followed.

Fifty four ACS patients had experienced an ST-segment elevation myocardial infarctionSTEMI, with has cardiac chest pain, serologic evidence of myonecrosis, and persistent (> 20 mins) ST-segment elevation.; tThe other 57 ACS patients had experiencedeither an unstable angina pectoris (UAP) andor non-ST-segment elevation
myocardial infarction (NSTEMI), that was clinically documented as: (1) The patient with unstable anginaUAP has when cardiac chest pain that was new or worsening with and without serologic evidence of myonecrosis (i.e., no elevation of serum troponin or creatine kinase MB isoenzyme concentration), or dynamic electrocardiographic (ECG) changes (i.e., ST depression and/or T wave inversion); (2) NSTEMI is diagnosed when the patient with had cardiac chest pain has and serologic evidence of myocardial necrosis in the absence of ST-segment elevation on the ECG.

Q(4). I offer authors to check the primer sequences by NCBI Blast

A(4): Literature reviews of primer sets used for ACE I/D genotyping revealed the primer set of Tiret et al., 1992 (the sense primer: 5’-CTGGAGACCACCTCCCATCCTTTCT-3’, and the antisense primer: 5’-GATGTGCGCCATCAGATCAGAT-3’) was the most employed primer set for studies of ACE I/D genotyping. After checking the primer sequences of Tiret et al. by NCBI Blast, these is one gap in forward primer and one mismatch in reverse primer compared to reference genomic DNA sequence of human (assceion no. reflNG_011648.1). In addition of the unexpected polymorphisms of different ethnic groups, these positions were adjacent 5’-terminus of each primer and thus may not abolish the PCR results of each genotype.
Minor Revisions

**Q(5).** The article has some grammatical errors for example

1- In abstract section line 2 replace polymorphisms with polymorphism

2- In methods section page2, line17 replace protocols with protocol

3- In Results section line6, delete respectively.

**A(5):** These grammatical mistakes had been corrected.