Author’s response to reviews

Title: ACE gene insertion/deletion polymorphism has a moderate influence on the acute development of left ventricular dysfunction in patients with ST elevation myocardial infarction treated with primary PCI

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Author’s response to reviews: see over
Dear sir,

Thank you for the comments, I believe the article has been improved. I am sending the article corrected according to the suggestions.

The title: ACE gene insertion/deletion polymorphism has a mild influence on the acute development of left ventricular dysfunction in patients with ST elevation myocardial infarction treated with primary PCI
Author(s): Parenica et al.

Reviewer: Philip Binkley
A major flaw of this investigation is the assumption that the Insertion/Deletion(I/D) genotypes of the ACE gene constitute the functional polymorphisms governing ACE activity and outcomes. More recent data published in 2009 by Johnson and colleagues in Clinical Pharmacology and Therapeutics describe two promoter polymorphisms that appear to be the true functional gene variants demonstrated by allelic expression imbalance studies. A multitude of previous studies examining I/D genotypes have had such variable results in associating cardiovascular outcomes that there has emerged genuine doubt regarding the functional significance of the I/D polymorphism. The identification of promoter polymorphisms that are associated with genuine expression imbalances and have been shown to have impact on outcomes in hypertensive populations strongly suggests that these gene variants are truly functional and therefore most likely associated with outcomes. Therefore, the reliance of this study on the D/I genotypes which have become suspect in terms of functional significance greatly weakens the findings of this paper.

The association between I/D polymorphism and the level of ACE activity was demonstrated several times, even in the cardiac tissue. Also our results support this association, below we quote new results in comparison with the previous version of the article:
The citation from the results:
"In comparison with the II genotype group, the DD genotype group had a higher level of ACE activity upon admission, while ID genotype group had an intermediate level of ACE activity (medians of ACE activities of DD vs ID vs II genotype groups were 36.7 U/L vs 31.4 U/L vs 24.1 U/L, p < 0.001)."

"Using multivariate linear regression, only age, previous treatment with ACE inhibitors or AT1-antagonists and I/D polymorphism were detected as variables with a statistically significant influence on ACE activity (25% of variability of ACE activity was explained by a given model; 4.9% of the total variability of ACE activity could be explained by ACE gene I/D polymorphism in this model)."

We found very important data published in 2009 by Johnson, we have revised our results and discussion:

The citation from the results:
"We found a significantly higher level of ACE activity for patients with moderate LV dysfunction (EF 40-54%; n= 301; described by median and 5–95th percentile (30.9 U/L (10.2–65.5)) in comparison both with patients with preserved LV function (EF ≥55%; n = 185; 28.5 U/L (8.2-60.4)) and in patients with severe LV dysfunction (EF ≤40%; n = 70; level of ACE activity 27.8 U/L (5.4-62.6)) (p = 0.028)."

The citation from the discussion:
"A completely new look Johnson [18] brought at 2009, he detected three promotor single-nucleotide polymorphisms (SNPs) of the ACE gene connected with reduced ACE mRNA expression in cardiac tissue. These identified SNPs (rs7213516 and rs4290) were tested in a large cohort of 1032 hypertonics and they were associated with adverse cardiovascular outcomes, largely attributable to nonfatal MI in African Americans. The high allele frequency was found in African Americans (16%), but low in Hispanics (4%) and very low in Caucasians (<1%). We do not suppose these SNPs play a relevant role in our study population according to low allele frequency in Caucasians only 1 or 2 subjects could be expected. But very important is an idea that very low level of ACE activity could be harmfull. So far the higher levels of ACE activity associated with DD/ID genotypes of ACE were connected with
worse prognosis [25], but existing results were not consistent [24,25]. For the first time our results suggest a non-linear type of a dependence of ACE activity and adverse cardiac outcomes. As we showed, higher levels of ACE activity were associated with moderate LV dysfunction (EF 40-54%), but lower levels of ACE activity were found in patients with preserved LV function (EF ≥ 55%) as well as in patients with severe LV dysfunction (EF < 40%). The results of Johnson study [18] and the Valsartan Heart Failure (Val-HeFT) trial [26] raise the possibility, that excessive neurohormonal inhibition may contribute to adverse outcomes in heart failure treatment.

Although the authors find statistically significant differences in genotypic groups, these differences are small and it is difficult to believe they are clinically relevant. The differences in end-systolic volume and ejection fractions are of margin clinical significance, even if statistically significant. Similarly, it Table 2, many of the associations are weak although there is a statistically significant linear relationship. For example, although there is a significant relationship between the EDV normalized for body surface area and ACE activity, only 9% of the variation of ACE activity is explained by the EDV.

We are agree the differences between genotype groups in ejection fractions and in end-systolic volume are of borderline clinical significance. We discuss it in the discussion:

"We consider the difference of EF between the both genotype groups on the border of clinical significance, the absolute value of the EF difference was only 1.5% after adjustments for other variables. This borderline influence of ACE polymorphism may explain inconsistent results of previous smaller studies on the development of left ventricular dysfunction, especially when ACE inhibitors were administered."

The authors state that the genotypic groups remain independent predictors even when adjusting for other variables. However, this is not the same as testing for confounding. Two variables may remain as independent predictors in a statistical model, yet one may still confound the other. This is usually reflected in large changes in the coefficients of one of the variables when the confounding variable is added. To
this point, a larger proportion of patients having the D polymorphism were diabetic. It is of course known that diabetic patients have worse outcomes and therefore, without more information regarding the modeling process, the claim that the genotypes independently predict outcomes is difficult to support. To this point, better descriptions of the modeling process, what variables were tested as covariates, and the results of different models would strengthen the manuscript.

*During the process of modelling the all parameters in table 2 were tested as univariate predictors for left ventricular dysfunction. The parameters with \( p < 0.1 \) in univariate regression were then tested for redundancy (controlled by expert opinion on clusters of redundant variables) to produce final set for multivariate analysis. Multivariate model was based on this set of parameters and backward stepwise algorithm. Although the interaction of genotypic groups with predictors included in the model exists, it is also included in the adjusted model; the testing of relationship of genotypic groups and adjusted left ventricular dysfunction should be also adjusted for these interactions of genotypic groups and other parameters with influence on left ventricular dysfunction.*

It is stated that logistic regression modeling was used in some cases. Yet it is unclear as to what was the outcome variable.

*Logistic regression was adopted for the odds ratio estimation of the DD/ID genotype group for EF <45% before hospital discharge (only in text, not in tables). Odds ratio estimation of the DD/ID genotype group for EF <45% only for subgroup of non-diabetics patients was added:*

"The DD/ID group had a significantly higher risk of EF <45% before hospital discharge (OR (95% CI) 2.04 (1.28; 3.25) (p = 0.003)), a similar result was found for DD/ID subgroup of patients without diabetes mellitus (OR (95% CI) 2.17 (1.25; 3.80) (p = 0.006)."

There are other methodological concerns. The authors note that Teicholz method of ejection fraction determination was used in some subjects. This is a much less reliable measure and appears to have been mixed with two dimensional estimates of ejection fraction. If one group had a greater proportion of ejection fractions determined by the Teicholz methods, a difference in ejection fractions could have resulted on this basis alone.
The citation from the article:
"Left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), and left ventricular ejection fraction (LVEF) were estimated using the bi-planar Simpson's rule from apical two- and four-chamber views (93.8% and 94.6 % of all measurements in DD/ID genotype group, resp. in II genotype group) or by the Teicholz formula. Echocardiography was assessed by two operators using Vivid 7 or Vivid i (GE Vingmed Ultrasound)."

The authors also report determining ventricular dP/dT based on measures from a fluid filled catheter system. Unless such a system is appropriately damped for such readings, the measures are unreliable.

We agree and we excluded the results of dP/dt/P and LVEDP from the article.

It is unclear why the proportion of male participants is so great in both genotypic groups. Women are afflicted by coronary artery events to the same, or in some groups, greater extent as men. The great majority of men enrolled in the study may reflect a study bias that can alter findings.

Only older age of women hospitalized with acute coronary syndrom can explain the disproportion between men and women, as we excluded patients older than 75 years. We found the same relationship between groups of DD/ID and II for both men and women (men – DD/ID group – 71%, women - DD/ID group - 69,5%). According to the multivariate model (Table 3) we did not find the relationship between sex and ejection fraction. We believe the higher proportion of men enrolled in the study does not alter the results.

The discussion is entirely too long. Much of the discussion reviews past literature rather than describing how the authors feel their findings add to this literature or explain gaps in the understanding the impact of genetic variations on dinase outcomes.

We agree and we have redesigned and shortened the discussion.
Reviewer: Jan Murin

I would like in the conclusion the statement “whether in a clinical practice there is a need (1) for a general genetic analysis (ACE polymorphism) in patient after acute myocardial infarction and (2) if not for “general” then for some special patients group(s) like “diabetics, renal insufficiency patients or so”.

The citation from the article:

"…. we do not believe the stratification of patients according to I/D polymorphism and ACE activity is useful in the daily clinical practice."

With our best regards,

On behalf the authors Jiri Parenica