PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	The association of ACE gene D polymorphism with left ventricular hypertrophy in patients with diastolic heart failure: A case control study
AUTHORS	Bahramali, Ehsan; Rajabi, Mona; Jamshidi, Javad; Mousavi, Seyyed Mohammad; Zarghami, Mehrdad; Manafi, Alireza; Firouzabadi, Negar

VERSION 1 - REVIEW

REVIEWER	Mehdi Dianatpour Shiraz university of Medical Sciences- Shiraz-IRAN
REVIEW RETURNED	09-Nov-2015

GENERAL COMMENTS	Please explain briefly about the method of genotyping. how you confirmed your genotyping for example sequencing of some PCR products.

REVIEWER	Meral URHAN KÜÇÜK Mustafa Kemal University Turkey
REVIEW RETURNED	09-Nov-2015

GENERAL COMMENTS	This study is original, well desgined and interesting. The tested parameters are suitable and enough.The manuscript is suitable for publication in the journal.

REVIEWER	Justin Keogh Bond University, Australia
REVIEW RETURNED	27-Nov-2015

GENERAL COMMENTS	General comments The authors are to be applauded for conducting this study which seeks to determine some of the genetic predispositions to diastolic heart failure in patients with left ventricular hypertrophy. I am impressed with many aspects of the paper, but still feel the authors need to address a number of my comments as well. The specific comments are provided below. Specific comments Page four, lines 6 to 18: perhaps a little bit more detail here about the condition's actual prevalence, morbidity and mortality risk would be useful. Page four, line 30: "key players here" should be written as "key players". Page five, line 3 – 20: I would also appreciate little bit more detail

	here regarding the role of the ACE gene in other healthy outcomes for middle-aged and older adults. For example, how may the ACE gene influence the level of body composition, physical function/disability and all physical activity in older adults? There has been some relatively new studies in this area which be an idea to cite in a quick summary paragraph here or at least please describe in the discussion. Keogh, J. W. L., Palmer, B. R., Taylor, D., & Kilding, A. E. (2015). ACE and UCP2 gene polymorphisms and their association with baseline and exercise-related changes in the functional performance of older adults. PeerJ, 3, e980. doi: 10.7717/peerj.980 Seripa, D., Paroni, G., Matera, M. G., Gravina, C., Scarcelli, C., Corritore, M., . . . Pilotto, A. (2011). Angiotensin-converting enzyme (ACE) genotypes and disability in hospitalized older patients. Age, 33(3), 409-419. doi: 10.1007/s11357-010-9192-2 Page six and seven: how valid is the eyeballing technique for determining LVEF? Page nine, line 32: I would suggest removing or at least rephrasing "our results though not in large sample size" as it doesn't read overly well. Page nine, line 51: "that also patients" should just read "that patients". Page nine – 11: while the D allele of the ACE gene appears associated with increased risk of LVH, it may also be associated with other protective or positive outcomes associated with the ageing process. Please refer back to the recent papers I suggested on the ACE gene and older adults which may allow you to provide a somewhat more rounded summary of the potential benefits and disadvantages of the ACE gene to the health of older adults. Table 1: please provide abbreviations for the variables underneath this table. Could you also provide some details for the duration of hypertension and diagnosis of HFpEF for the two groups? If there was a significant between group differences in the duration of these variables, how could such differences impact on your interpretation of the genetic differences?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:
We used a simple PCR method for genotyping as is described in the methods section of the manuscript. It has been widely used in the past 20 years with plenty of articles published with the same method. Method included an internal control mechanism with another pair of primers to recheck for the possible genotyping mistakes.

Reviewer 2:
We appreciate her comments.

Reviewer 3:
-Details about the prevalence and mortality risk of HFpEF are added to further clarity the burden of the disease, In the introduction section we added the following statements: "At 5 years, the cumulative mortality rate is reported to be 65% for patients with HFpEF with an adjusted hazard ratio of 1.48 when compared with persons with no HF and a normal LVEF. Besides being associated with a high incidence of systemic hypertension (70-88%), HFpEF is highly prevalent in older and obese patients and in females."
-All the English writing suggestions were applied in pages 4,5 and 9.
-We read the suggested articles carefully and found their findings relevant to our study. So the

following statements were added to the introduction section page 5: "Furthermore, ACE gene I/D polymorphism has been related to baseline muscular strength and power in older adults, indicating its role in overall physical performance and functional capacity which is seriously limited in patients with HFpEF"; and we enriched the final part of our discussion with these inferences as well: "Besides there are reports that older adult carriers of D allele of ACE gene have greater physical performance level in a 6 minute walk test compared to those with II genotype. This is consistent with our findings and implicates the muscular hypertrophic role of D allele in augmenting muscular mass in adults alongside inducing cardiac hypertrophy. It may have implications in clinical assessment of HFpEF and affect the severity of symptoms; an issue that needs to be addressed in future studies".
-Abbreviations for the variables underneath table-1 were added. Regarding the duration of hypertension and diagnosis of HFpEF and the impact they make on our interpretations, we discussed with detail in the manuscript with the following statements on page 10: "Furthermore, as this was a case control study, determination of the exact duration of hypertension in the patients was not feasible. Although it was their first documentation of HFpEF and symptom presentation, these patients likely has hypertension undiagnosed or untreated over a longer time period. This problem needs to be addressed in a cohort study of healthy individuals without hypertension with frequent and long enough follow ups to detect the development of hypertension and consequent HFpEF.

VERSION 2 – REVIEW

REVIEWER	Justin Keogh Bond University, Australia
REVIEW RETURNED	12-Jan-2016

GENERAL COMMENTS	the authors have now addressed all my comments on the previous version of the manuscript