Author's response to reviews

Title: Validation study of candidate single nucleotide polymorphisms associated with left ventricular hypertrophy in the Korean population

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Author's response to reviews: see over
1) The authors claim, that their manuscript reports results from a replication study in which a small number of SNPs previously associated with left ventricular mass index (the HyperGen Study, Arnett DK et al., BMC Med Genet. 2009) are evaluated in Asian population. However, The HyperGen Study was performed on subjects with hypertension and in the presented manuscript the general population (age > 44) was studied, where the prevalence of hypertension was 44.3%. The separate analysis in the subgroup of individuals with hypertension should be performed.

Response) We thanks the reviewer for the insightful comment. In response to the reviewer’s comment, we performed subgroup analysis in non-hypertensive subjects (n=911) and hypertensive subjects (n=726) in supplementary Table 1 and 2. The results showed that the polymorphism of rs4129218 on chromosome 12 had consistently protective effect on LVH defined as LVM/BSA (adjusted OR=0.701; 95% CI, 0.504–0.975; \( P=0.035 \), Supplementary Table 1B) and LVM/height\(^{2.7} \) (adjusted OR=0.672; 95% CI, 0.481–0.941; \( P=0.021 \), Supplementary Table 2B) in hypertensive subjects.

We added the results as follows;

In subgroup analysis, these findings were consistently observed in hypertensive subjects (Supplementary Table 1B and 2B, \( P=0.035 \) and 0.021, respectively). Another SNP, rs6450415 on chromosome 5 also revealed an association with LVH defined as LVM/BSA (adjusted OR=1.463; 95% CI, 1.148–1.865; \( P=0.002 \)) and LVM/height\(^{2.7} \) (adjusted OR=1.320; 95% CI, 1.041–1.673; \( P=0.022 \)). However, no significant associations were noted in quantitative analysis (\( p=0.107 \) and 0.178, respectively). In subgroup analysis, an association between rs6450415 and LVH was remained in only non-hypertensive subjects (Supplementary Table 1A and 2A, \( P=0.002 \) and 0.013, respectively) but, not in hypertensive subjects (Supplementary Table 1B and 2B, \( P=0.174 \) and 0.567, respectively) (Page 7; line 20-Page 8; 4).
2) No clear evaluation of the significance of the findings is indicated. In the Statistical Analysis the authors state, that p-values <0.0056 were designated as significant based on the correction for multiple comparisons, but it is not clear if the correction was applied to the results presented in Table 4 and 5.

Response) We thanks the reviewer for the insightful comment. In present study, we selected 9 SNPs for genotyping. Therefore, multiple linear and logistic regressions were performed with a p value < 0.05/9 (0.0056) to control the type 1 error. However, Table 4 and 5 shows that the association was not statistically significant using cut-off of P value as 0.0056. Therefore, we concluded that the minor allele of rs4129218 did not have significant, but borderline association with lower LVM in this study. Arnett DK et al. also showed that rs4129218 on chromosome 12 showed an association with log-transformed LVMI (P=0.007) and LVH (P=0.038) in Caucasian subjects (BMC medical genetics 2009, 10:43).

3) The authors performed multiple linear and logistic regressions using an additive genetic model and data are presented in Table 4 and 5, but the data presented in Figure 1 were obtained from a dominant genetic model, and there is no information how the best genetic model was chosen.

Response) We thanks the reviewer for the insightful comment. Arnett DK et al. (BMC medical genetics 2009, 10:43) chose to use a dominant model because the number of minor allele homozygotes in sample tended to be small (<20%). Therefore, we reanalyzed data with dominant model (Table 4 and 5) using PLINK 1.07 software to maintain the consistency of the method as previous study (BMC medical genetics 2009, 10:43). In our study, minor allele frequencies of two SNP (rs409045 and rs6450415) were < 20%.

We added the results as follows;

Multiple linear and logistic regressions with a dominant model were performed using PLINK 1.07 software (http://pngu.mgh.harvard.edu/purcell/plink) (Page 6; line 19-21).

Minor essential revisions:
The last sentence in the Abstract and the second sentence in the Statistical Analysis are unclear.

Response) In response to reviewer’s comment, we clarified the sentences

This study suggests that rs4129218 on chromosome 12 showed consistent tendency of possibly related loci for LVH independent of ethnic background. (Page 2; line 18-19).

The examined phenotypes comprised the continuous variables of LVM/BSA and LVM/height^{27}, and the categorical phenotype of LVH, which was defined as aforementioned cut-off value (Journal of the American Society of Echocardiography: official publication of the American Society of Echocardiography 2005, 18(12):1440-1463.) (Page 6; line 16-18).

Discretionary Changes: none

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Response) We made use of a copyediting service.

Statistical review: No, the manuscript does not need to be seen by a statistician.
Reviewer #2

Reviewer: DANIELA CACCAMO

Reviewer's report:

Minor essential revisions

A large body of literature in recent years showed that cardiac disease is associated with VDR polymorphisms also in Asian populations (see Levin et al. JAMA 2012; Alobeidy et al. PloS One 2013). It has also been reported that VDR polymorphisms are associated with left ventricular hypertrophy, although occurring as comorbidity of other pathologies (Testa et al. J Bone Min Res; El-Shehaby et al. Scand J Clin Lab Invest. 2013; Santoro et al. 2014). Notably, the VDR gene is located on chromosome 12 as well as the rs4129218 reported by the authors. In view of these observations it could be worthy to consider if a linkage could exist between these genetic loci on chromosome 12 and comment on this, adding a sentence on the need of future investigations in this direction.

Response) We thanks the reviewer for the insightful comment. In response to reviewer’s comment, we added this in discussion as follows;

Recently, several studies demonstrated that vitamin D receptor (VDR) gene polymorphism is associated with LVM and predicts LVH progression in end-stage renal disease patients [31, 32]. Notably, the VDR gene is located on chromosome 12. In view of these observations it could be worthy to consider if a linkage could exist between these genetic loci on chromosome 12. However, further investigation may be needed to verify this possibility (Page 9; line 14-19).
Reviewer #3

Reviewer: XUWEI HOU

Reviewer's report:

Major Concerns:

Some antihypertensive medication, like ACEI, ARB and even b-blocker, has established effect on the LVH. In this study, a great portion of participants were under antihypertensive medication (483, 29.5%). The effect of antihypertensive medication on LVH should be taken in account. How many were under ACEI, ARB and b-blocker? Also the antihypertensive period should be compared since long term treatment will regress LVH.

Response) We thanks the reviewer for the insightful comment. Present study used community-based data. Therefore, we did not investigate the class of antihypertensive medication. And, the period of antihypertensive was not available. To adjust the effect of antihypertensive medication on LVH, we only used taking antihypertensive medication as covariate. In response to reviewer’s comment, we added this in discussion as follows; Additionally, we did not investigate the class and period of antihypertensive drugs (Page 10; line 13).

In statistics, the author stated “Multiple linear and logistic regressions with an additive model were performed using PLINK 1.07 software (http://pngu.mgh.harvard.edu/purcell/plink) [26], adjusted for covariates, such as age, gender, BMI, systolic BP, heart rate and antihypertensive medication.”. Some other factors, such as serum Cr also affect LVH. Why these factors are neglected in this study? Also I would like to see how the authors use antihypertensive medication as confounder factors?

Response) We thanks the reviewer for the insightful comment. In response to reviewer’s comment, we added serum creatinine into one of covariates (Table 4 and Table 5). We added this in method as follows; adjusting for age, gender, BMI, serum creatinine, systolic BP, heart rate, and antihypertensive medication (Page 6; line 21-22).

We adjusted the effect of antihypertensive medication on LVH by coding (0: no antihypertensive medication, 1: antihypertensive medication). However, we did not
investigate the class of antihypertensive medication.

Although this is a validation study, the name and function of selected gene should be briefly mentioned in discussion to make the readers easier to follow.

Response) In response to reviewer’s comment, we added this in discussion as follows;

Among other eight SNPs, the rs765529 on chromosome 20 was intragenic, which is located in the potassium voltage-gated channel, Shab-related subfamily, member 1 gene (KCNB1). Its protein product is dephosphorylated by calcineurin, which is associated with LVH in human study [33]. RAI14 (retinoic acid induced protein 14) gene may contribute to the inhibition of adipogenesis by retinoic acid [34]. MIER3 (mesoderm induction early response 3) gene has been suggested to be candidate breast cancer susceptibility gene [35]. RP1-272J12.1 is an uncharacterized gene. Maria et al. presented that CD36 may impact cardiovascular disease [36]. However, all had no significant association with LVH in our study (Page 9; line 20-Page 10; line 3).

Minor----

1. The author stated in method: “3 SNPs from the HyperGEN study (rs1833534, rs4129000 and rs238688) were filtered out because they had a minor allele frequency (MAF) lower than 0.05 in the Chinese and Japanese HapMap database”. Reference is needed here to support this statement.

Response) In response to reviewer’s comment, we added the reference as follows;

three SNPs from the HyperGEN study (rs1833534, rs4129000 and rs238688) were filtered out because they had a minor allele frequency (MAF) lower than 0.05 in the Chinese and Japanese HapMap database (The International HapMap Project. Nature 2003, 426(6968):789-796.) (Page 5; line 25-Page 6; line 2).

2. Genotyping methods should be briefly described.

Response) In response to reviewer’s comment, we added the method as follows;
Polymerase chain reaction (PCR) primers were designed in a region of approximately 100 base pairs around the SNP of interest and an extension primer was designed immediately adjacent to the SNP. After PCR amplification, Shrimp Alkaline Phosphatase (SAP) was added, along with the primer extension mixture after a brief incubation. After a standardized PCR program, SpectroCLEAN resin was added to the mixture to prepare it for spotting and detection of the PCR products using a 384-well SpectroChip® and a Compact TM MALDI-TOF mass-spectrometer automatically (Page 6; line 5-11).