Author’s response to reviews

Title: Lack of genetic susceptibility in takotsubo cardiomyopathy A case-control study

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Author’s response to reviews:

Dear Editor,

I would like to thank you for the reviewers constructive comments and suggestions regarding our manuscript entitled “Lack of genetic susceptibility in takotsubo cardiomyopathy. A case-control study” (MGTC-D-17-00305). We feel that we have responded satisfactorily to the questions raised by the reviewers and now hope that the manuscript has a high enough quality to be published in BMC Medical Genetics. Please find enclosed a point-by-point response to the reviewers. Changes in text are marked in yellow.

Sincerely Yours

Per Tornvall, MD, PhD
Response to questions raised by the reviewers:

Reviewer 1:

Q1. Would it be more straightforward to move section 'the Genotype distribution of the studied polymorphisms' after 'Patient characteristics' and before 'Allele Frequencies in....'

A1. The section including genotype distribution has been moved forward as suggested.

Q2. In table 1, Please also include the ethnical information of the participants.

A2. The origin of the patients has been included in Table 1.

Q3. In conclusion section, restate the SNPs tested in the paper.

A3. The SNPs tested have been added to the conclusion section.

Reviewer 2:

Q1. The biggest concern is that the genotyping methods. As the flowchart of subject recruitment is not well described. We cannot assess the genotyping call rate in current study for the studied SNPs. If using 665, number of final studied subjects as the reference, the call rate of three SNPs were 94.3%, 97.6% and 94.6%, which were rather low and not acceptable for genetic analysis with relative small sample size. Could the authors clarify the overall call rate (per SNPs) in a clearer manner and report how many samples had clear genotype for all three SNPs as well.

A1. The call rates for the three SNPs are given per patient group in the revised version of the Figure. Possible causes of the low call rate are discussed in the limitation section of the revised manuscript; “The call rates of the studied SNPs were low, possibly due to the origin of DNA. Although DNA purified from saliva has been reported to be of similar quality as blood DNA it cannot be excluded that some of the samples were of poor quality”.

Q2. By saying that, a clearer flow-chart than the Figure is really helpful, especially the information about the participants who had bad DNA sample quality or missing genotype.

A2. Please see A1.
Q3. The second concern is the CAD control. The authors used the terms of "ACS" and "CAD" in the context. Clinically, "ACS" is a temporal diagnosis pending for the confirmation of myocardial infarction or unstable angina pectoris. The authors need to clarify if CAD as for control in current study was a composite definition of MI and angina pectoris or not. Would it change the results if only the MI patients were included as control?

A3. The SCAAR registry was used to select patients and their respective controls for the present study since this is the only available registry using a clear definition of takotsubo (Mayo Clinic definition). Unfortunately, the registry does not use the indication MI for coronary angiography, instead the indication ACS was used when inviting the patients for the present study. Therefore, it is not possible to do a sub-group analysis of MI-patients. The objective of having a patient-group consisting of patients with CAD was to distinguish the genetic susceptibility in patients with CAD from patients without CAD and was thus not depending of the final diagnosis. The focus was that the patients should have significant CAD, a criterion fulfilled by including only patients undergoing PCI.

Q4. The significance was set as $P \leq 0.05$ as conventionally without clarification of one-side or two-side assessment. Concerning the results of previous studies, I am wondering whether the results would be changed if one-side statistic is applied.

A4. Significance was tested using two-sided assessments, an information that has been added to the revised version of the manuscript. We did not apply one-side statistics, instead data were analysed using both odds ratios and chi2-tests to be sure of not missing out on differences between the groups. Applying a one-sided test is not expected to change the interpretation of the data drastically. The confidence interval for odds ratios are defined as $\log(\text{odds ratio}) \pm 1.96 \times \text{standard error}$. By applying a one-sided test the factor 1.96 is exchanged for a factor of 1.65, which would result in a narrower confidence interval. We have tried one-sided statistics with the expected result of a narrower confidence interval, please see Table below (not included in the revised version of the manuscript).

Q5. The allele and genotype frequency should be reported before the main results, could be table 2. The odd ratio estimate, then, could be table 3.

A5. The order of the tables is changed as suggested.

Q6. The main results reported in the text contained no summarised numeric information (OR and P value or CI and so on).
A6. ORs have been added to the text with the notion that there were no statistical differences.