Author's response to reviews

Title: Functional polymorphism of the NFKB1 gene promoter is related to the risk of dilated cardiomyopathy

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Author's response to reviews: see over
Dear Editor and Reviewers:

Thanks very much for your attention to our paper.

We have revised our manuscript in a very careful way according to the reviewers’ comments. The revised manuscript was entitled “Marked manuscript” and all changes were marked in red.

According to the comments of the reviewers, we comment the statistical power using the Quanto software. An unmatched case-control design was used in this study (1.2 controls per case). The prevalence of the DCM in the population (Kp) is 1:2500 (ie. 0.0004), and the prevalence of the “ATTG₁” allele in the population is estimated to be \( q_A = 0.40 \). The relative risk for \( \text{ATTG}_2/\text{ATTG}_2 \) carriers is 0.55 (Rg) compared to normals. Then the case sample size is estimated to be 166 \( (166 \times 1.2 = 199 \) for control group) using the dominant model when desired power is 80% at a significance level of 0.05 with a 2-sided alternate hypothesis.

Response to Reviewers:

Reviewer #1:

Background
For the NFKB1 -94 insertion/deletion polymorphism its SNP-ID rs28362491 should be added. It is correct that this polymorphism has originally been associated with ulcerative colitis (ref 15). It is also correct that the results of several replication studies were inconsistent, but in part the authors cite these replication studies incorrectly. The single study which could confirm the original association (ref 21) should be already cited in this section. The study cited in ref 16 dealt with celiac disease and is not relevant in this context and can be deleted. In addition to the negative replication study in ref. 17 two further negative studies (Gut 54:1205-6,2005; Inflamm Bowel Dis 12:606-11, 2006) should also be cited. Furthermore significant associations of the NFKB1 -94 insertion/deletion polymorphism with other disease entities (inflammatory disorders, tumors etc.) should be listed.

Response:
Revised.

Methods/Determination of genotypes The criteria for diagnosis of DCM should described briefly. Have the results of genotyping been confirmed by sequence
analysis at least in a few samples?

Response:
The criteria for diagnosis of DCM are marked in red.
The results of genotyping have been confirmed by DNA sequencing.

Results:
The authors should address the power of their study providing a power calculation.
The comparisons of genotype frequencies, allele frequencies and phenotype (=carrier) frequencies should be described in more detail within the text. It should be pointed out more clearly that the comparison of ATTG1/ATTG2 + ATTG2/ATTG2 vs. ATTG1/ATTG1 refers to the ATTG2 phenotype. Additionally the comparison of the ATTG1 phenotype should also be given in the text and in table 1. In table 1 the three parts genotype frequencies, allele frequencies and phenotype (=carrier) frequencies should be separated more clearly and the comparison ATTG1/ATTG1 vs. ATTG2/ATTG2 should be removed from the table.

Response:
Revised.

Discussion:
The authors should discuss the main limitations of their study, particularly the relatively small size of their study population and the lack of replication of the significant associations in a second independent cohort of patients with DCM. Finally, it should also be mentioned that investigating a single or a few gene polymorphisms is rater an ineffective tool for unraveling the genetic background of a disorder with complex genetic background such as DCM and that currently the best strategy would be a genome-wide association study. Such genome-wide association studies could identify susceptibility genes for several disorders as Crohn's disease, diabetes, rheumatoid arthritis, multiple sclerosis etc., this is reviewed for example in Nat Rev Immunol 8:631-43, 2008.

Response:
We discussed the main limitation of our study and it is marked in red.
As this is a pilot study investigating the association between NFKB promoter polymorphism and DCM patients and this association was identified, further study is necessary to confirm the true association.

References:
Within the reference list the numbers in brackets after the volume numbers should be deleted.
Response:
The references were edited by EndNote.

Reviewer #2:
1. The introduction is perhaps too long. It could be shortened: Page 4, line 8: the list of the diseases in which NF-#B is involved should be replaced by “…for initiation and progression of pathogenesis of many autoimmune and inflammatory diseases” Delete the entire paragraph from line 11 to line 22 of page 4, in which NF-#B function is explained. The authors could simply cite a review article on that matter.

Response:
Revised.

2. Is the number of patients and controls included in the study large enough to achieve at least 80% statistical power? The authors should comment their statistical power. I have used the Quanto software (http://hydra.usc.edu/GxE/), and I am afraid that this study is underpowered to detect an association with an effect size similar to the one found in Karban et al.

Response:
Answered.

3. In the results section (page 7 line 17) it is stated that both patients and controls had no deviation from Hardy-Weinberg equilibrium. However, according to my calculations, the patient’s genotype distribution is slightly out of the HW equilibrium (P= 0.04). When allele frequencies are compared, no statistically significant differences were found. On the other hand, the authors found a significant decrease of the ATTG1/ATTG1 in patients compared with controls. Interestingly, the frequency of this genotype in patients is lower than that expected (from HW equilibrium calculations; maybe an excess of heterozygous due to genotype errors?). Could this have influenced the results obtained?

4. In order to clarify whether the NFKB1 variant is associated with DCM, an additional, well powered cohort could be analyzed. The results presented here are not robust enough, due to a lack of power and deviation from HWE.

Response:
We used “Modified-powerstate” to analysis the HW equilibrium and P=0.05. We think that our genotyping results are correct.
5. The decrease of the ATTG1/ATTG1 genotype found in DCM patients compared with controls is in contrast to the work of Karban et al, where an increase in this genotype was found for ulcerative colitis patients. The authors could comment this in the discussion, and provide a possible explanation for this phenomenon.

Response:
Results of several studies investing the association between this polymorphism and ulcerative colitis patients were inconsistent. Our study is about the association between this polymorphism and DCM patients. We think it is too intensive to comment that.

6. The paragraph about NF-κB and NFKB1 structure could be removed from the Discussion (page 8 line 20-page 9 line 6) in order to shorten this section.

Response:
Revised.

7. Discussion, page 9 line 15: The authors could cite a meta-analysis of the association of NFKB1 and UC, where no statistically significant differences were found (Latiano A, Palmieri O, Valvano MR, Bossa F, Latiano T, Corritore G, et al. Evaluating the role of the genetic variations of PTPN22, NFKB1, and FcGRIII A genes in inflammatory bowel disease: a meta-analysis. Inflamm Bowel Dis 2007;13:1212-9), and remove reference number 16, since it is from a study regarding celiac disease, not UC.

Response:
Revised.

Minor comments
1. Abstract, conclusion section: line 17-18 should be moved to the Results section of the Abstract.
2. Introduction, line 7. Should be “…severe symptoms, including heart failure AND sudden death, and asymptomatic individuals.”
3. Introduction, page 3, line 18: remove from this sentence the associated polymorphisms and keep only the names of the genes, as in the sentences it is stated: “Some susceptibility genes…, including…”

Response:
Revised.