Author's response to reviews

Title: Common Polymorphisms of Calpain-10 and the Risk of Type 2 Diabetes in a Tunisian Arab Population: a case-control study

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Author's response to reviews: see over
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Author's response to reviews: see over
February 20, 2010

Scott Edmunds, Ph.D.
Senior Scientific Editor
BMC-series Journals
BioMed Central
Floor 6, 236 Gray's Inn Road
London, WC1X 8HL

Dear Dr. Edmunds,

Please find enclosed a revised version of the manuscript entitled:

**Common Polymorphisms of Calpain-10 and the Risk of Type 2 Diabetes in a Tunisian Arab Population: a case-control study**

Intissar EZZIDI, Safia MESSAOUDI, Molka CHAIEB, Maha KACEM, Ghada M AL-KHATEEB, Touhami MAHJOUB, Wassim Y ALMAWI and Nabil MTIRAOUI, which we submit for consideration for publication in BMC Medical Genetics, after having addressed all reviewer’s comments. Attached also is our point-by-point response to reviewer’s comments. The changes made in the text were marked in blue.

**None of the authors has any potential financial conflict of interest related to this manuscript**

All authors have carefully examined and approved of the changes made to the manuscript, and understand and accept that in the event of its publication, all copyright shall be transferred to BMC Medical Genetics. We look forward to hear from you favorably in due course.

Sincerely,

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RESPONSE TO REVIEWERS' COMMENTS

Reviewer No. 1. Kazuki Yasuda

Reviewer: There was no information on Arab or non-Arab subjects in the panel, although the title said “Tunisian Arab populations”. This point should be clearly stated in the text and discussed.

Authors: Will do.

Changes: The reference to the Arab population was discussed in the revised text. Two new references (Cherni et al., 2005 and Hajjej et al., 2006) were added to the references list.

Reviewer: From the haplotype frequencies, the authors concluded their subjects were ethnically more close to Europeans than Africans, Does this hold true with other SNPs examined formerly in the same panel (ref-18)?

Authors: As pointed out by the reviewer, the association of specific gene variants to T2D pathogenesis depends on ethnicity. In a recent report which investigated the association of key variants identified through GWAS to T2D (TCF7L2, HHEX, GCK, ENPP1 and KCNJ11) (Ref. 18), we documented variability (association, lack of association) in the association of these compared to Caucasians, African Americans, or even another cohort living in the northern Tunisia (47%) [Bouhaha et al., 2008].

Changes: The Discussion section was modified as per the reviewer's comment.

Reviewer: ....... then the authors should compare their LD block structure and risk haplotypes with those reported in Caucasians. What seemed to be the reason for discrepancies, especially for the association of 111 rather than 121 haplotypes with diabetes?

Authors: In the absence of validated reports, explanation for this remains speculative. The heterogeneity of CAPN10 in the magnitude of LD between CAPN10 variants is strongly attributed to ethnic differences (i.e., population-specific LD). In addition, sample size differences between the different studies, coupled with altered frequencies of CAPN10 gene variants, and in the failure to control for possible confounding variables (gender, familial history of diabetes, obesity, etc.) by some of the studies, likely which may have modulated potential effects of CAPN10 gene variants on T2D.

Changes: Appropriate modification to the Discussion was made as per the reviewer's comment.
Reviewer: Another candidate SNP, UCSNP-44, near SNP43 has been associated with diabetes in several populations. This SNP should be also examined.

Authors: That was for purely technical considerations. Will consider running this SNP, and other CAPN10 SNPs in future studies.

Changes: None for now.

Reviewer: How was the association of UCSNP-19 after adjustment for BMI, since 2R/2R was associated with increased body weight in patients? What could be the reason for this association with obesity in their panel?

Authors: A point well-taken and appreciated.

Changes: The impact of BMI changes, along with hypertension and altered lipid profile on the association of UCSNP-19 with T2D was addressed by multivariate regression analysis (Table 3). Appropriate changes were made throughout the text (section: Results and Table 3).

Reviewer: Minor Essential Revisions: The Abstract should be more carefully written. PCR-RFPL (Methods) should be PCR-RFLP and fully spelled out.................2. P4. Lines21#22: the sentence is mixed up. “Dipoltypes” should be read as “Diplotypes”.......... English should be corrected throughout the manuscript.

Authors: We thank the reviewer for pointing out these typographical errors.

Changes: The typographic mistakes were corrected, English was thoroughly checked out throughout the manuscript.
**Reviewer No. 2, Simin Liu**

**Reviewer:** How can the authors justify the sufficiency of the three SNPs to capture the genetic variability of CAPN10 in Tunisian population?

**Authors:** This was based on the assumption that a limited haplotype diversity is present within each block, a minimal set of haplotype-tagging SNPs is thought to be sufficient to capture the LD variation in a population, as was suggested (Johnson *et al.* 2001). Our choice of the three SNPs was based on previous studies, on Tunisian (Kifagi *et al.*, 2008) and other populations (Horikawa *et al.* 2000), which demonstrated the contribution of these variants to T2D risk in large cohorts. Future extension of this study will definitely include testing other CAPN10 SNPs as potential T2D risk variants.

**Changes:** Justification for inclusion of the three CAPN10 SNPs in the study was referred to in the revised text.

**Reviewer:** The author may consider providing more details about subjects selection. (i) How were the cases and controls enrolled? Were they recruited by mailed questionnaires? (ii) What were the exclusion and inclusion criteria? (iii) What were the participation rates for cases and controls? (iv) Because the mean duration of T2D was 12.6 years (table1), it appears that most cases are prevalent cases. Would there be an problems of using prevalent cases?

**Authors:** Will do.

**Changes:** More information on subjects' selection was added to the revised text (Methods section; Study Population)

**Reviewer:** What were the matching criteria and the number of controls per case used?

**Authors:** We acknowledge this comment.

**Changes:** More information on case/control ratio and matching criteria were added to the revised text (Results section; Study Subjects).

**Reviewer:** More details should be provided in the statistical analysis .... (i) What specific genetic models did the authors use ...... (ii) What variables were included in the multivariate regression analysis apart from the genotype or haplotype? ...... (iii) The authors should consider including odds ratios in Table 6. ... odds ratios should be presented together with the p-values ...... the author may consider including confidence intervals, ..... What methods did they use to compute p-values and odds ratios?

**Authors:** The odds ratio in table 2, 3 and 5 were estimated under additive model. Since there were no significant differences in the diplotype distribution between cases and controls, we felt that inclusion of OR (95% CI) were not justified. To accommodate the reviewer's comment, we have included the OR (95% CI) in modified Table 6.
Changes: Variable in the regression model, OR (95% CI), and methods of calculation of OR were included in the revised text.

Reviewer: Minor Essential Revisions: 1. The authors should consider including the following: (i) was genotyping done blind to case-control status in this study? (ii) discuss more about the biology of CAPN10 (ii) possibility of population stratification (iii) issues identified in the previously reported meta-analysis (Song et al AJHG 2004) (iv) limitations of their work (v) sample size in Table 3 and 5

Authors: We acknowledge all (minor) comments raised by the reviewer here.

Changes: Appropriate changes as per the reviewer's comment were made in the revised text.

Reviewer: 2. Briefly explain what kind of expectation maximization algorithm was used to determine the haplotype and diplotype frequencies.

Authors: An expectation-maximization (EM) algorithm is used for finding maximum likelihood estimates of parameters in probabilistic models, where the model depends on unobserved latent variables. EM is an iterative method which alternates between performing an expectation (E) step, which computes an expectation of the log likelihood with respect to the current estimate of the distribution for the latent variables, and a maximization (M) step, which computes the parameters which maximize the expected log likelihood found on the E step.

Changes: A brief explanation of the EM algorithm used was added to the revised text.

Reviewer: 3. The definition of hypertension described in table 2 is not consistent with the one in the method section.

Authors: We apologize for this typographical error. The correct definition is BP reading of 145/90 mmHg or higher on 2 separate occasions, and/or use of anti-hypertension medications.

Changes: The definition of hypertension was corrected in the text.

Reviewer: Discretionary Revisions 1....Song et al. have also shown, both in a meta-analysis (Song et al AJHG, 2004) and a prospective cohort of multi-ethnic Americans, essentially the same null findings (Song et al, HMG, 16:23, 2007). The authors should also consider citing this article to further support their findings. 2. Typographical errors in Introduction section: (i) dipolotypes and dipolotypes -> diplotype (ii) (diplotype) ->(diplotype)

Authors: We acknowledge comments raised by the reviewer here and we apologize for the typographical errors.

Changes: Appropriate changes as per the reviewer's comment were made in the revised text (Discussion section).
Reviewer: Abstract: .... What does the UC in UCSNP stand for? I also find the minus sign .... this looks like promoter polymorphisms..... rs numbers should be mentioned upfront, preferably already in the abstract. Typo: genotyping were done = genotyping was done.

Authors: The naming of the SNPs as per: UCSNP-xx is the standard monenclature and thus can not be altered. We did include the rs numbers of each SNP as recommended by the reviewer in the abstract.

Changes: Changes to the abstract, as suggested by the reviewer, were made.

Reviewer: ..........Has a meta-analysis been done? Please discuss either here or in the discussion what the evidence from GWA studies tells us about this locus. Typos: was been shown to function = was shown to function; while whereas did not = while some did not.

Authors: We thank the reviewer for his suggestion of meta-analysis; will look into it in the future. We also thank the reviewer for pointing out the typos.

Changes: Appropriate changes as per the reviewer's comment were made in the text.

Reviewer: Methods: Were controls matched to cases in terms of geographic area? ... distribution (south, central, north) ...... information on family history of T2D ...... Was 140/90 used as cut-off for hypertension or 145/90 as stated in Table 1? None of the subjects were taking medication = None of the control subjects were taking medication.

Authors: Yes, controls were matched to patients in terms of geographical area (family origin). Family history of diabetes in cases (36.0%) but not controls (0.0%) was also addressed. As for the definition of hypertension, kindly refer to our response to the last comment of Reviewer 2 (above). Thank you for pointing out the omission of the word "control".

Changes: Appropriate changes as per the reviewer's comment were introduced into the revised text.

Reviewer: Genotyping: please state genotyping success/failure rate and genotyping accuracy rate based on genotyping of duplicates.... provide more detail on measures taken to ensure high quality genotyping results.

Authors: Will do.

Changes: Information on success/failure rate, and quality control measures were added to the revised text.

Reviewer: Statistical analysis: covariates such as age and sex should be included in the multiple regression analyses including those for haplotypes.
Authors: Actually no. We did not include them in the model, since they were not significantly different between cases and controls. Other covariates that were significantly different were included (BMI, hypertension, systolic and diastolic BP, lipid profile).

Changes: None.

Reviewer: Results: Per genotype only one overall chi-2 p-value for comparison of genotype distributions should be provided (Table 2) ..... The authors state that SNP19 was associated with body weight. The authors should include BMI in their multiple regression models to investigate whether the effect on T2D may be explained by the effect on BMI.

Authors: We agree with the reviewer that only one P value should be present in comparing genotype distributions (2-way ANOVA). As for the BMI, we did include BMI along with other coavriates in the regression analysis model (see comment above).

Changes: Appropriate modifications as per the reviewer's comment were made in the revised text.

Reviewer: It is unclear what the delta coefficient is, it doesn’t seem to be D’or r2?.

Authors: Both D’ and r$^2$ (along with P value) were included. These were computed using LDA v.1.0 software.

Changes: None.

Reviewer: Discussion: Please carefully check accuracy of citations. It seems at the end of both the 1st and 2nd paragraph the study on Mexican Americans (ref.11) should have been cited? In the 3rd paragraph, OR should be 1.61 instead of 2.17'; Is ref. 20 really on Scandinavians?

Authors: We acknowledge these comments.

Changes: We made the appropriate changes to Discussion section as suggested