Author's response to reviews

Title: Common genetic variants are associated with type 2 diabetes in urban Ghana: a hospital-based case-control study

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Author's response to reviews: see over
Reviewer #1: Michele Ramsay

Reviewer’s report:

Minor Essential Revisions (some discretionary)

1. The title may be reconsidered – perhaps emphasising the positive result

We have changed the title into:

“Common genetic variants are associated with type 2 diabetes in urban Ghana: a hospital-based case-control study”

2. Abstract: Remove reference to hypertension, which allele has a frequency of 0.23 for the CAPN10 -19 (indel) \?, CAPN10 haplotypes are in fact haplotype combinations (the equivalent of genotypes as opposed to alleles).

For clarification, we have deleted the information on CAPN10 -19 (3 repeats) from the abstract. It now reads:

“Minor allele frequencies of \textit{CAPN10} were for -43 (A) 0.11 and for -63 (C) 0.46.”

3. The focus should be clear – there are two parts to the study

a. It is an association study for T2D (and therefore reference to the hypertensive cohort which was not analysed seems out of place in the methods section, though it makes sense to include the prevalence of hypertension in the T2D group)

b. It examines association with several quantitative traits among the control group using logistic regression

In the Methods section we aimed at describing the overall aims of the KDH Study itself. However, we acknowledge that the present manuscript presents a genetic analysis only. Therefore, we have rephrased the objectives as suggested (page 5, lines 2-3):

“The unmatched case-control study was designed to identify factors associated with type 2 diabetes. The present analysis aims at genetic associations with type 2 diabetes and with diabetic traits among the control group.”

4. The results for the KCNJ11 and PPAR\# should be given (even if there were only two observations of the variant allele)

We have included the results in Table 2 (page 19-20) as suggested and have added a respective reference in the Results section (page 8, lines 24-25):

“Due to the low MAFs of \textit{KCNJ11} and \textit{PPAR\gamma} (Table 2), the calculation of risk estimates did not yield meaningful results.”
5. Methods: Provide references for DNA extraction, genotyping protocols and explain “mutagenically separated PCR assays”

In the Methods section, we have added the reference protocol for DNA extraction (page 6, line 6), the genotyping protocols including primer sequences (Supplementary Table 1) and explained the mutagenically separated PCR method (page 6, lines 9-11):

“CAPN10 rs3792267 was detected by a mutagenically separated PCR method, which uses a common forward primer and two allele-specific reverse primers.”

6. Discussion – some over interpretation should be tempered (e.g. one SNP result suggesting a “worldwide distribution complies with the out-of-Africa migration”).

In the Discussion (pages 11-13), we have now mitigated our interpretations and wordings (marked in blue).

Paragraph 4 – consider clarifying and in last sentence did you mean “predisposes”?

Specifically, we have clarified in paragraph 4 that the PPARγ G allele is the minor allele that confers a protective effect and exhibits the highest frequencies in northern regions of the world. The paragraph now reads as follows (page 12, lines 5-17):

“Surprisingly, we are the first to investigate the importance of PPARγ rs1801282 and KCNJ11 rs5219 for type 2 diabetes in West Africa. Almost everybody in the present study population displayed the risk allele of PPARγ(C) while the risk allele of KCNJ11 (G) was almost absent. The worldwide allele frequency of PPARγ(G) is around 0.10. For SSA, associations with type 2 diabetes are conflicting [7, 9]. In Caucasian and Asian populations, the G variant commonly protects against type 2 diabetes (OR Caucasians, 0.9; OR Asians, 0.8) [7, 9]. In contrast to the TCF7L2 T allele, for which MAFs are decreasing from South to North, the frequencies of PPARγ(G) increase with geographic latitude. Possibly, this variant represents an “unthrifty allele”, meaning that it disposes for leanness. Such variant has been disadvantageous during the hunter-and-gatherer times, but may have become obstructive in the course of agricultural and economic development [28]. Particularly, in populations where lipids contribute >30% to energy intake, the protective G allele is most frequent and confers the highest effect [29].”

Paragraph 6 – not clear what relevance of “positional cloning” is in this context.

As it is irrelevant to present the history of exploration of a candidate locus in this context, we have deleted the respective word group in paragraph 6. We have changed into (page 13, lines 4-5):

“CAPN10 polymorphisms are associated with diabetic status in Mexican Americans (OR, 2.8), Botnian Fins (OR, 2.5) and Germans (OR, 5.0) [34].”

7. Conclusions – Last two sentences could be improved

We thank the reviewer for her suggestions and have now taken into account the potential drawbacks of single-locus replication studies and how these can be overcome in the future. We have changed the last two sentences accordingly (pages 13, line 25 – page 14, line 3):
"These results demonstrate the need for the identification of ethnicity-specific genetic associations with type 2 diabetes in SSA. The present replications of single but well-established loci from other populations can only serve as the first step to fine-map the candidate genes and explore their specific associations with type 2 diabetes in this region."

8. Careful editing is required – some examples – BMI is not kg/cm2, inconsistencies in allele frequency notation (0.1%, 0.001; 23%, 0.23 etc.), type 2 diabetes vs diabetes, with first use write out FPG

We have edited the manuscript as suggested.

Major compulsory Revisions

1. Since hypertension is so common among the cases, it should be examined as a confounder and adjusted for

In accordance with this helpful suggestion, we have now adjusted the association analyses for hypertension status in addition to age, gender and BMI (Tables 2 and 3).

2. Haplotype associations for CAPN10 need to be done (e.g. frequency and role of 112 in T2D – as opposed to 111-112)

We have performed haplotype analysis for CAPN10 as suggested and changed Table 3 (pages 21-22) accordingly. Also, in the Results section and in the Abstract, we have now clarified:

page 9, lines 2-6:
"Constructing haplotypes, the most frequent combination of alleles -43 (A), -19 (3 repeats), and -63 (T) was 112 at 0.53, followed by 111 (0.24), 121 (0.12), 221 (0.10), 222 (0.01), 122 (0.004), and 211 (0.003). None of these conferred a significant risk for type 2 diabetes. In contrast, two combinations were nominally associated with reduced odds for type 2 diabetes: 211 (aOR, 0.32; 95% CI, 0.03-2.92; p=0.31) and 221 (aOR, 0.73; 95% CI, 0.48-1.10; p=0.13) (Table 3)."

page 2, lines 16-18:
"Two CAPN10 haplotypes tended to protect against type 2 diabetes: 211 (aOR, 0.32; 95% CI, 0.03-1.92; p=0.31) and 221 (aOR, 0.73; 95% CI, 0.48-1.10; p=0.13)."
Reviewer #2: Per Medbøe Thorsby

Reviewer’s report:

Major concerns

1. To use unmatched controls may influence on the genetic risk observed. Since these controls were younger and may also differ from the cases in ethnicity and SES background that may influence heavily on diabetes prevalence.

We agree with the reviewer that the unmatched case-control design bears potential problems. Therefore, we have discussed respective limitations in the discussion. In multivariate analysis, we have accounted for the difference in mean age between cases and controls. Nevertheless, as stated in the discussion “we cannot rule out residual attenuation by an over-representation of young participants in the control group”. In the limitations section, we now acknowledge the potential influence of ethnic admixture as well as SES (page 10, lines 9-24). However, we believe that selection of our study participants is unlikely to be related to genetic background. This in turn makes a bias of the genetic associations with type 2 diabetes also unlikely.

2. Also to use only 50% of controls compared to cases may give rise to selection bias.

A respective statement has been added to the limitations section admitting this potential drawback of our study design (page 10, line 21):

“In addition, study participants recruited in hospital may not reflect the genetic make-up of the average Ghanaian population, particularly when the ratio of cases and controls is 2:1.”

3. Diagnosis of diabetes was mostly self-reported (97%) or on the basis of medication, but medication used is not stated in the paper.

It is correct that diabetes status was defined according to documented anti-diabetic medication in the hospital files or increased FPG (page 7, line 6). We did not rely on self-reported diabetes status. Important information on these characteristics has been added to the manuscript (page 8, lines 4-6) and the main medications prescribed are presented. We have added the reference of the original paper, including the detailed breakdown of all medications.

Page 8, lines 4-6:
“The majority of patients with type 2 diabetes (97%) regularly attended the diabetes center, presenting with a median duration of type 2 diabetes of 5.0 (interquartile range, 2.0-9.0) years.”

Page 8, lines 12-13:
“Metformin-based therapies (78%) and combinations with sulfonylureas (61%) were predominating [25].”

4. Since only one FBG sample was used to exclude diabetes in the controls, undiscovered diabetes among controls and some non-diabetics among cases may further contribute to selection bias between the groups.
As correctly stated by the reviewer, 97% of the patients with type 2 diabetes have already been known to the diabetes center and attended the regular diabetes clinics. It is therefore highly unlikely that there were healthy individuals among the type 2 diabetes cases. As for the opposite, we cannot fully exclude the possibility that type 2 diabetes cases were among the controls, but consider this highly unlikely. Also, single FPG measurements are common routine practice for screening for type 2 diabetes. We have added a statement in this regard in the limitations section (page 11, lines 1-2):

“We are aware that the definition of type 2 diabetes by single FPG measurement and known medications is sub-optimal. However, it corresponds to general practice in resource-poor settings and IDF consensus [24].”

5. FBG < 5 mmol/l rather than < 7 mmol/l should be used to exude diabetes, but preferably HbA1c <6.5% or OGTT.

In our study, we applied the global WHO definition of type 2 diabetes as FPG ≥7 mmol/l (Alberti & Zimmet 1998). We did not perform HbA1c measurements. The high prevalences of haemoglobinopathies and haemolytic conditions, such as malaria, may have obscured the results of glycated hemoglobin (Hinzmann et al. 2012). In addition and for practical and ethical reasons, we refrained from OGTT in this study setting. Nevertheless, the WHO definition was used for study purposes only. In case of any doubt, further laboratory tests for diagnosis may have been arranged by a diabetologist of the diabetes center. This information has been added to the limitations section (page 11, lines 3-5).


6. Did the control individuals all have BP below 120/80?

In accordance with the hypertension guidelines of the WHO and of the International Society of Hypertension, we applied the following definition of hypertension: systolic blood pressure (BP) ≥140 mmHg and/or diastolic BP ≥90 mmHg (WHO/ISH 1999). Controls were defined as negative for type 2 diabetes and hypertension (page 7, lines 7-9).


7. About the power calculation, dose it indicate that there should be 525 in each group? Then the study is underpowered.

We acknowledge that the description of power calculation has not been clear. Power calculation was performed using 2 cases for 1 control. We added this information to the Methods section (page 6, line 18-21):

“At an allele frequency of 0.30, given a significance level of $\alpha = 0.05$ and a 95% confidence interval (CI), the present sample size of 1052 (2 cases vs. 1 control) is sufficient to replicate this OR with a probability (power) of 84%.”
8. One should always address the question about multiple testing in such studies, when the P values are only mildly significant.

Reviewer #1 has asked us to re-run the analysis for haplotype associations for CAPN10. None of the results were significant (new Table 3) and no correction for multiple testing was applied. As for the only significant genetic association of the TCF7L2 T allele with type 2 diabetes, we have added the Bonferroni-corrected p-value to the Results section and have mitigated our interpretation in the Discussion section.

Page 8, line 24:
“In multivariate analysis adjusting for age, gender, BMI ≥25.0 kg/m² and hypertension status, TCF7L2 (T) was associated with increased odds of type 2 diabetes (aOR, 1.39; Bonferroni-corrected p=0.056).”

Page 9, lines 25-26:
“More than half of the individuals carried the TCF7L2 (T) allele, which was suggestive to increase the odds for type 2 diabetes by roughly 40%.”

Minor concerns

1. The description of possible additive effects of the risk allele of TCF7L2 is not clear

We have now clarified (page 7, lines 13-15):

“With respect to genetic polymorphisms, we assumed an additive effect of candidate alleles on type 2 diabetes risk (homozygous for non-risk allele, 0; heterozygous for risk allele, 1; homozygous for risk allele, 2).”

2. I think nearly 60 risk genotypes of type 2 diabetes is found so far (Page 3, 15 ref to 40 genotypes)

The references have been added and the respective sentence has been changed accordingly (page 3, lines 15-16):

“More than 60 susceptibility variants for type 2 diabetes have been identified, and their number increases continuously [5, 6].”

3. Was C-peptide or anti-GAD measured to exclude other forms of diabetes?

So far, we relied on the information of the patients’ clinical characteristics derived from the hospital files at Komfo Anokye Teaching Hospital (see above, 3. and 4.). However, we are planning to measure these important parameters as soon as the remaining aliquots will have been shipped from Ghana to Germany for laboratory analysis.

4. How was glucose measured? In plasma?

Fasting glucose was measured photometrically in fluoride whole blood according to the manufacturer’s instruction (Glucose 201+, HemoCue, Ängelholm, Sweden) and was automatically translated into plasma-equivalent values. We added a respective phrase to the Methods section for clarification (page 6, lines 2-4):
“FPG (fluoride whole blood, tubes cooled at +4°C) and urinary albumin were measured photometrically (Glucose 201+ & Albumin Systems; HemoCue, Angelholm, Sweden). Glucose concentration is presented as plasma equivalents.”

5. CV for the laboratory measurements should be mentioned

We have added this information as suggested to the Methods section (page 6, lines 4-5):

“The coefficients of variation ranged between 1.7-6.1% and 4.9-8.0%, respectively.“


6. Genotypes KCNJ11 and PPARG should be presented in the paper

These results have been added to the manuscript (Table 2 and page 8, lines 24-25).

7. Tab 3 and 4 is unnecessary

Table 3 has been changed as requested by reviewer #1 and is now more informative. Table 4 has been deleted as requested and the information has been included in the Results section (page 9, paragraph 2).

8. The discussion is much too long

We have shortened the Discussion where possible.