Author's response to reviews

Title: MTNR1B rs10830963 is associated with fasting plasma glucose, HbA1C and impaired beta-cell function in Chinese Hans from Shanghai

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Author's response to reviews: see over
Dear Dr. Scott Edmunds,

Attached please find the manuscript titled “MTNR1B rs10830963 is associated with fasting plasma glucose, HbA1C and impaired beta-cell function in Chinese Hans from Shanghai” that has been revised according to the reviewers' comments. We resubmit this manuscript for consideration as a General Article of The BioMed Central, and the itemized response to each reviewer’s comments is attached with this letter. Hopefully, the revised manuscript will be eligible for being published in The BioMed Central.

Thank you very much for giving us the opportunity to revise the manuscript.

With my best wishes,

Yours sincerely,

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For your guidance, itemized response to each reviewer’s comments is appended below.

Reviewer's report (Dr. Ehm Astrid A Andersson):

The authors investigate associations between MTNR1B rs10830963 and fasting plasma glucose (FPG), HOMA-indices T2D and sleep patterns/disorders in Chinese Hans from Beijing and Shanghai. The study confirms previous findings on FPG and HOMA-B in European and Asian populations in the Shanghai population, but describes differences in associations between the two geographical regions. Of novel interest, no associations with sleep patterns and disorders are observed.

Major revisions

1. In the background, the authors should explain in more detail that MTNR1B variants have been investigated in Asian populations before, including Chinese Hans. This should be clarified even in the abstract section.

   We appreciate the reviewer’s comment and, accordingly, have updated the Abstract section by modifying “Genome-wide association studies (GWAS) in White Europeans have suggested that genetic variation rs10830963 in melatonin receptor 1B gene (MTNR1B) is associated with fasting glucose and type 2 diabetes. However, the associations need further replication in different ethnic populations, and as a variant in the gene regulates circadian rhythms, the effect of the variant on sleep status remains unknown.” to “Genome-wide association studies (GWAS) in White Europeans have shown that a common variant (rs10830963) in the melatonin receptor 1B gene (MTNR1B) is associated with fasting glucose levels and type 2 diabetes, which has also been replicated in Asian populations. As a variant in the gene involved in the regulation of circadian rhythms, its effect on sleep status remains unknown.” (Abstract).

   We also added more information on the studies in Asian population by modifying “Similar results were observed in several subsequent studies [4, 8, 9]” with “Several replication studies in European [5, 9-11] and Asian populations [12, 13] showed reproducible associations for MTNR1B- rs10830963. A case-control study including 1165 case and 1105 control of Chinese Hans from Shanghai [13] confirmed the associations of MTNR1B- rs10830963 with increased risk of type 2 diabetes and increasing fasting glucose, while another study in general Japanese and Sri Lankan populations [12] reported association between the variant and fasting glucose with effect sizes similar to those observed in Chinese Hans [13].” (Background).

   We also replaced “We replicated the association of MTNR1B rs10830963 with fasting glucose, HbA1C and HOMA-B in Chinese Hans from Shanghai, with similar effect size for fasting glucose as observed in White Europeans, but haven’t found any association between MTNR1B rs10830963 and sleep status. Our findings strengthen the previous evidence for a role of genetic variation in MTNR1B in fasting glycemia and impaired beta-cell function.” with “A common variant in MTNR1B was associated with fasting glucose, HbA1C and HOMA-B but not with sleep status in Chinese Hans from Shanghai, strengthening the role of MTNR1B rs10830963 on fasting glycemia and impaired beta-cell function.” in the conclusion of Abstract section to cut the word in Abstract.
2. Throughout the manuscript a meta-analysis with other studies is described (abstract results section line 4; main text result section line 21; discussion line 7). This meta-analysis is not found anywhere in the manuscript, making it difficult to interpret the results. However, the authors seem to perform a meta-analysis with only data from Prokopenko et al (ref 6) and Rönn et al (ref 9) and their own data (from Shanghai Hans) and conclude that no heterogeneity is observed.

Why were all other previously published studies and the Beijing Hans not included in this meta-analysis?

The authors state that the effect in Shanghai Hans is slightly higher than previously reported (0.11 vs 0.07), but was it significantly higher? It seems as if, it is not, which suggests that the effect is not different. This is a very simple test to perform.

The meta-analysis described does not add additional information and should not be used to state that effects are similar, when only a few studies are included. It should therefore not be included in the manuscript, unless all studies are included.

As suggested we have performed the meta-analysis again, now including data of all previously published studies available to date on white Europeans and East Asians (reported by Prokopenko et al., Thomas Sparso et al., Valeriva Lyssenko et al., E. Reiling et al., Langenberg C et al., Rönn et al., F. Takeuchi et al.) and of the Beijing and Shanghai subpopulations in the present study. The results are presented in Figure 1 showing the effect sizes of rs10830963 on fasting glucose for each of the individual studies, grouped by ethnicity, as well as the effect size of all studies combined (meta-analyzed).

The results of meta-analysis were as follows (Figure 1):
The data in the Asian populations are from the studies in Chinese (Chinese Hans from SH1 [12], Chinese Hans from SH2 and BJ in this population), Japanese [13] and Sri Lankan [13], and data in Europeans are from studies of CoLaus [7], deCODE [7], DGI [7], Framingham [7], FUSION [7], NFBC1966 [7], NTR/NESDA [7], Rotterdam [7], Sardinia [7], TwinUK [7], DESIR [11], Haguenau [11], Inter99 [11], NFBC86 [11], Botnia PPP [5], Botnia prospective [5], Helsinki Birth Cohort [5], METSIM [5], New Hoorn Study [10] and RISC [9]. The black circles and horizontal lines represent the point estimated beta and 95% CI for of each study, respectively. The overall 95% CI for the meta-analyses is represented by diamond. SH, Shanghai; BJ, Beijing.

We appreciate the reviewers concern and our updated meta-analysis now provides the actual statistics on differences between studies and between ethnicities. As such, the effect size of the association between MTNR1B rs10830963 and fasting glucose in Shanghai Hans of the present study was not significantly different from that previously reported by Rönn et al. (0.11 vs 0.07, \( P \) for heterogenetity = 0.33). We observed no heterogeneity among the Asian populations \( (P \) for heterogeneity = 0.37). While the effect sizes tended to be slightly smaller in Asians than in white Europeans, the difference didn’t reach statistical significance \( (P \) for heterogeneity = 0.06). We therefore updated the Abstract section by replacing “A meta-analysis of the current and published results suggests the effect size of MTNR1B rs10830963 on fasting glucose in Shanghai Chinese Hans is the same as that reported for white Europeans” with “The effect size of MTNR1B rs10830963 on fasting glucose in Shanghai Chinese Hans was comparable to that reported for other Asian populations. Combining all data available shows that the effect size in Asians tends to be smaller than in white Europeans \( (P=0.06) \).” (Abstract).

In the Result section we changed “The observed effect size of MTNR1B rs10830963 G allele on fasting glucose in Shanghai participants of this study (beta=0.11 mmol/l) was slightly higher than those reported for Shanghai Chinese Hans by Ronn et al. (beta =0.07 mmol/l) [9] and White Europeans by Prokopenko et al. (beta =0.07 mmol/l) [6]. However, meta-analysis of the current Chinese data and the published data, including both White Europeans [6] and Chinese Hans from Shanghai [9], suggested no heterogeneity in the genetic effect of MTNR1B rs10830963 G allele on fasting glucose levels either between Shanghai Chinese Hans of this study and the previously reported (0.11 vs. 0.07 mmol/l, \( P \) for heterogeneity =0.33) or between Shanghai Chinese Hans and White Europeans (0.07 vs. 0.07 mmol/l, \( P \) for heterogeneity =0.86)” to “The observed effect size of MTNR1B-rs10830963 on fasting glucose in Shanghai participants of this study was similar to that reported for Shanghai Chinese Hans by Ronn et al. [13] \( (P \) for heterogeneity = 0.33). Meta-analyses of our data in Chinese and the published data, including all studies in White Europeans [5, 7, 9-11] and Asians [12, 13], showed no heterogeneity in effect size of MTNR1B-rs10830963 on fasting glucose levels among Asian populations, including the current Beijing and Shanghai Chinese Hans \( (P \) for heterogeneity =0.37). However, the overall effect size in Asians (0.06 mmol/l / per allele) tended \( (P \) for heterogeneity =0.061) to be smaller than in White Europeans (0.08 mmol/l / per allele) (Figure 1).”

We also replaced “Although the effect size of the MTNR1B rs10830963 on fasting glucose in our Shanghai Hans was slightly higher than those reported for white Europeans, a meta-analysis of the current data and published data showed no evidence of heterogeneity between East Asians and white Europeans.” with “Meta-analyses of the MTNR1B rs10830963 effect size on fasting glucose showed no evidence for heterogeneity among Asian populations. The overall effect size among Asians tended to be smaller than in white Europeans.” in the Discussion section.
3. The power calculation for sleep disorders seems to be overestimated. Please re-calculate and report the effect that can be excluded with 80% power in the separate and/or in the combined study samples.

As suggested, we re-calculated the power for sleep disorder in separate and in the combined samples, and modified the Discussion section accordingly; i.e. we replaced “Our study had 80% power to detect odd ratios (ORs) ≥ 1.09 for self-reported sleep disorder at a significance of 5%” with “Our study had 80% power to detect odd ratios (OR) ≥ 1.27, 1.32 and 1.20 for self-reported sleep disorder at a significance of 5% in Beijing, Shanghai subpopulations and whole population, respectively.” (Discussion).

Minor revision

4. In the abstract background section, it is stated that “a variant in the gene regulates circadian rhythms,” this needs elaboration and a reference in the main text, otherwise it should be rewritten.

We appreciate the reviewer’s comment. As a major regulator of circadian rhythms, the melatonin effects on sleep and circadian phase are mainly mediated by activation of its two receptors: melatonin receptor 1A and melatonin receptor 1B. Therefore, MTNR1B which encoded melatonin receptor 1B was involved in the regulation of circadian rhythms. To clarify the background section in the abstract we changed the word “regulates” to “involved in the regulation of”. In the background of the main text, we cited one additional reference (Sleep Med 2007, 8 Suppl 3:34-42) which describes the role of melatonin receptors on circadian rhythm regulation (Background).

5. Effect sizes and 95% confidence intervals should be represented in the abstract for the positive associations.

Corrections have been made accordingly.

6. Results line 5 and discussion line 4, all appropriate references should be added.

Relative references have been cited accordingly.

7. Why is data not shown when adjustments for sleep duration or disturbances are made? Although no associations change, these analyses are novel and could be presented. Interaction analyses with sleep (SNP x environment [sleep]) on FPG, homa-b etc. could also be of interest.

We appreciate the reviewer’s comment and have updated Table 1 by including also data on sleep duration, self-reported sleep disorders and physical activity (which was evaluated by using “MET-h/wk” rather than classifying it into “low/middle/high”).

We modified Table 2 by including the suggested information and by using the raw beta (SE) to represent the effect size of log-transformed HOMA-S. We also updated the Result section by replacing “Moreover, adjustment for sleep duration or quality did not materially change the associations of rs10830963 with those diabetes-related quantitative traits in Shanghai subpopulation (data not shown).” with “Moreover, further adjustment for sleep duration (Table 2, model 2) or self-reported sleep disorders (Table 2, model 3) did not materially change the associations of rs10830963 with the diabetes-related quantitative traits in either Shanghai or Beijing subpopulations, and no significant gene-sleep status
interaction (P for interaction ≥ 0.22) was observed (Table 2).” (Results).
<table>
<thead>
<tr>
<th>MTNR1B rs10830963</th>
<th>Beijing (1389)</th>
<th>Shanghai (1497)</th>
<th>Total population (2886)</th>
<th>P for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>P_{add}</td>
<td>β (SE)</td>
<td>P_{add}</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.03 (0.06)</td>
<td>0.59</td>
<td>0.11 (0.04)</td>
<td>0.005</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.03 (0.05)</td>
<td>0.58</td>
<td>0.11 (0.04)</td>
<td>0.005</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.03 (0.06)</td>
<td>0.59</td>
<td>0.11 (0.04)</td>
<td>0.005</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.003 (0.04)</td>
<td>0.93</td>
<td>0.07 (0.02)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.004 (0.04)</td>
<td>0.92</td>
<td>0.07 (0.03)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.004 (0.04)</td>
<td>0.93</td>
<td>0.07 (0.03)</td>
<td>0.004</td>
</tr>
<tr>
<td>HOMA-B (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.88 (1.58)</td>
<td>0.58</td>
<td>-5.01 (1.65)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.91 (1.57)</td>
<td>0.56</td>
<td>-5.01 (1.65)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.88 (1.58)</td>
<td>0.58</td>
<td>-5.01 (1.65)</td>
<td>0.003</td>
</tr>
<tr>
<td>Log-transformed HOMA-S (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.004 (0.02)</td>
<td>0.82</td>
<td>0.008 (0.02)</td>
<td>0.66</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.005 (0.02)</td>
<td>0.82</td>
<td>0.008 (0.02)</td>
<td>0.66</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.004 (0.02)</td>
<td>0.82</td>
<td>0.008 (0.02)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Participants previously diagnosed with type 2 diabetes or receiving glucose-lowering treatment (n=264) were excluded from the analyses. HOMA-S values were log-transformed before analyses. P values and beta (SE) for Beijing and Shanghai subpopulations were calculated using linear regression model assuming an additive model. The combined P values and beta (SE) were calculated in meta-analyses using random model.
Model 1: Adjusted for age, sex and BMI.
Model 2: Adjusted variables in model 1 plus sleep duration.
Model 3: Adjusted variables in model 1 plus self-reported sleep disorder.

*P* values for interaction were tested in linear regression model using terms of SNP × Region (Beijing/Shanghai), SNP × Sleep duration or SNP × Sleep disorder.

8. The lack of association in the Beijing population is speculated to be due to differential lifestyle. Did you try to adjust for the available lifestyle factors (drinking, smoking etc)?

We have adjusted for the available lifestyle factors such as drinking, smoking, physical activity, education, diet etc. However, these adjustments did not materially change the associations.

9. Lack of statistical power should also be discussed as a potential explanation for the lack of association and power calculations for quantitative traits should be performed.

We agree with the reviewer that insufficient power may be a reason for the lack of association. We recalculated the power and updated the Discussion by changing “we had 20.3% power to detect the previously reported OR of 1.09 at *P*<0.05. Thus, absence of association with type 2 diabetes in our study is possibly due to insufficient power,” to “we had less than 35% power to detect the previously reported OR of 1.09 [7] for type 2 diabetes and beta value of 0.072 mmol/l [7] for fasting glucose at *P*<0.05. Thus, absence of association with type 2 diabetes and related quantitative traits in our study could be due to insufficient power,” (Discussion).

10. Table 2; to be consistent HOMA-S may as well be calculated using a random model.

We appreciate the reviewer’s comment and we redid the analyses as suggested in Table 2.

11. Table 3 should be more consistent in the way results are presented. The odds ratio per allele should be presented when applying logistic regression together with a *P*-value for the additive model (diabetes and sleep disorders). Only for two traits the models used are described, please specify the models used for the remaining traits.

We agree and updated table 3 to present the odd ratio per allele for sleep disorder with a *P* value for the additive model. We have also updated the Result section by replacing “No significant association between SNP rs10830963 and sleep duration or quality was found in either Beijing or Shanghai subpopulations (*P*≥0.44) (Table 3)” with “No significant associations of MTNR1B-rs10830963 with sleep duration, siesta frequency or sleep disorder were found in either Beijing or Shanghai subpopulations (*P* ≥ 0.44) (Table 3).” (Results).

The associations with sleep duration, siesta frequency and self-reported sleep disorder were carried out using nonparametric test (Kruskal-Wallis), *χ*² test and logistic regression, respectively. We specify the models for all traits or phenotypes in the Methods and the footnote of Table 3.

12. How were the associations analyses with siesta frequencies carried out? Please describe in the statistical methods section.

The associations analyzed with siesta frequencies were carried out using *χ*² test, which have been described in statistical methods and footnote of Table 3.
The associate editor commented:

The first reviewer has thoroughly assessed and critiqued the statistical analyses at the heart of this manuscript and provided well reasoned suggestions for changes which would greatly improve the it. I am in agreement with the suggested changes.

The following major revisions are necessary:
1. To provide context for this study, additional information should be added to the background section, providing some details on previous studies of MTN1RB variants in Asian populations, including Han Chinese, and the specific reason for selecting SNP rs10830963. The other studies should also be briefly noted in the abstract.

We appreciate the associate editor’s comments and, accordingly, have updated the Abstract section by modifying “Genome-wide association studies (GWAS) in White Europeans have suggested that genetic variation rs10830963 in melatonin receptor 1B gene (MTNR1B) is associated with fasting glucose and type 2 diabetes. However, the associations need further replication in different ethnic populations, and as a variant in the gene regulates circadian rhythms, the effect of the variant on sleep status remains unknown.” to “Genome-wide association studies (GWAS) in White Europeans have shown that a common variant (rs10830963) in melatonin receptor 1B gene (MTNR1B) is associated with fasting glucose levels and type 2 diabetes, which has also been replicated in Asian populations. As a variant in the gene involved in the regulation of circadian rhythms, its effect on sleep status remains unknown.” (Abstract).

We also added more information on the studies in Asian population by modifying “Similar results were observed in several subsequent studies [4, 8, 9]” with “Several replication studies in European [5, 9-11] and Asian populations [12, 13] showed reproducible associations for MTNR1B- rs10830963. A case-control study including 1165 case and 1105 control of Chinese Hans from Shanghai [13] confirmed the associations of MTNR1B- rs10830963 with increased risk of type 2 diabetes and increasing fasting glucose, while another study in general Japanese and Sri Lankan populations [12] reported association between the variant and fasting glucose with effect sizes to those observed in Chinese Hans [13].” (Background). We also replaced “We replicated the association of MTNR1B rs10830963 with fasting glucose, HbA1C and HOMA-B in Chinese Hans from Shanghai, with similar effect size for fasting glucose as observed in White Europeans, but haven’t found any association between MTNR1B rs10830963 and sleep status. Our findings strengthen the previous evidence for a role of genetic variation in MTNR1B in fasting glycemia and impaired beta-cell function.” with “A common variant in MTNR1B was associated with fasting glucose, HbA1C and HOMA-B but not with sleep status in Chinese Hans from Shanghai, strengthening the role of MTNR1B rs10830963 on fasting glycemia and impaired beta-cell function.” in the conclusion of Abstract section to cut the word in Abstract.

We choose SNP rs10830963 since it showed the most significant association signal with fasting glucose [7] in GWAS reported by Inga Prokopenko et al.. This study refined the location of MTNR1B association signal by extending the meta-analysis to all SNPs (genotyped and imputed from the HapMAP) within the 1-Mb region flanking the gene, and the strongest signal was detect at rs10830963. Furthermore, it is the only SNP located in the only intron of MTNR1B. Other SNPs identified by GWAS were near the gene MTNR1B. This is mentioned in the Background section.

2. Given that the meta-analysis described in the manuscript does not seem to provide additional
information, it should be dropped, unless all studies are included. The meta-analysis referred to several times in the manuscript should be fully described, in terms of methodology, contributing studies and results. A statistical test should be performed to determine whether the effect size of SNP rs10830963 is significantly different than that previously reported.

We performed the meta-analysis again, including data of all previously published studies in white Europeans and in Asians and including our own results. More specifically, the meta-analysis includes data of twenty European populations (reported by Prokopenko et al., Thomas Sparso et al., Valeriya Lyssenko et al., E. Reiling et al. and Langenberg C et al.) and five Asian populations (three populations reported by Rönn et al. and F. Takeuchi et al., the two subpopulations in the present study). The forest plot that summarizes the meta-analyses is presented in Figure 1. As this meta-analysis includes all available data to date, allowing to compare results of Asian and European population, we think that this figure provides additional information to our own results and will be of interest to the reader. Therefore, we would prefer to include the figure in our paper.

As recommended, we have provided more details on the contributing studies in the footnote of Figure 1. We used inverse variance weighting Cochran Q test in Stata to conduct the meta-analyses. A more detailed description of the methodology of the meta-analyses and studies included has been provided in the Methods section by replacing “We conducted meta-analyses using the inverse variance method implemented in meta.summaries function of R RMETA package to evaluate the combined effect sizes” with “We conducted meta-analyses applying Cochran Q test in Stata to evaluate the combined effect sizes of subpopulations and the heterogeneity of effect size across different ethnic populations. The meta-analyses were carried out using inverse variance weighting and random model.” (Methods)

The effect size of the association between MTNR1B rs10830963 and fasting glucose in Shanghai Hans of the present study was not significantly different from that previously reported by Rönn et al. (0.11 vs 0.07 , P for heterogeneity = 0.33). We observed no heterogeneity among the Asian populations (P for heterogeneity = 0.37). While the effect sizes tended to be slightly smaller in Asians than in white Europeans populations the difference didn’t reach statistical significance (P=0.06).

We therefore updated the Abstract section by replacing “A meta-analysis of the current and published results suggests the effect size of MTNR1B rs10830963 on fasting glucose in Shanghai Chinese Hans is the same as that reported for white Europeans” with “The effect size of MTNR1B rs10830963 on fasting glucose in Shanghai Chinese Hans was comparable to that reported for other Asian populations. Combining all data available shows that the effect size in Asians tends to be smaller than in white Europeans (P=0.06).” (Abstract). In the Result section we changed “The observed effect size of MTNR1B rs10830963 G allele on fasting glucose in Shanghai participants of this study (beta=0.11 mmol/l) was slightly higher than these reported for Shanghai Chinese Hans by Ronn et al.(beta =0.07 mmol/l) [9] and White Europeans by Prokopenko et al. (beta =0.07 mmol/l) [6]. However, meta-analysis of the current Chinese data and the published data, including both White Europeans [6] and Chinese Hans from Shanghai [9], suggested no heterogeneity in the genetic effect of MTNR1B rs10830963 G allele on fasting glucose levels either between Shanghai Chinese Hans of this study and the previously reported (0.11 vs. 0.07 mmol/l, P for heterogeneity =0.33) or between Shanghai Chinese Hans and White Europeans (0.07 vs. 0.07 mmol/l, P for heterogeneity =0.86)” to “The observed effect size of MTNR1B-rs10830963 on fasting glucose in Shanghai participants of this study was similar to that
reported for Shanghai Chinese Hans by Ronn et al. [13] (P for heterogeneity = 0.33). Meta-analyses of our data in Chinese and the published data, including all studies in White Europeans [5, 7, 9-11] and Asians [12, 13], show no heterogeneity in effect size of MTNR1B-rs10830963 on fasting glucose levels among Asian populations, including the current Beijing and Shanghai Chinese Hans (P for heterogeneity =0.37). However, the overall effect size in Asians (0.06 mmol/l / per allele) tended (P for heterogeneity =0.061) to be smaller than in White Europeans (0.08 mmol/l / per allele) (Supplementary Figure 1).” (Results). We also replaced “Although the effect size of the MTNR1B rs10830963 on fasting glucose in our Shanghai Hans was slightly higher than those reported for white Europeans, a meta-analysis of the current data and published data showed no evidence of heterogeneity between East Asians and white Europeans.” with, “Meta-analyses of the MTNR1B rs10830963 effect size on fasting glucose showed no evidence for heterogeneity among Asian populations. The overall effect size among Asians tended to be smaller than in white Europeans.” in the Discussion section.

The results of meta-analysis are summarized in Figure 1.
The data in the Asian populations are from the studies in Chinese (Chinese Hans from SH 1 [12], Chinese Hans from SH2 and BJ in this population), Japanese [13] and Sri Lankan [13], and data in Europeans are from studies of CoLaus [7], deCODE [7], DGI [7], Framingham [7], FUSION [7],...
NFBC1966 [7], NTR/NESDA [7], Rotterdam [7], Sardinia [7], TwinUK [7], DESIR [11], Haguenau [11], Inter99 [11], NFBC86 [11], Botnia PPP [5], Botnia prospective [5], Helsinki Birth Cohort [5], METSIM [5], New Hoorn Study [10] and RISC [9]. The black circles and horizontal lines represent the point estimated beta and 95%CI for of each study, respectively. The overall 95% CI for the meta-analyses is represented by diamond.

SH, Shanghai; BJ, Beijing

3. The power analysis for sleep disorders should be re-calculated as suggested. The lack of statistically significant association in the Beijing population is puzzling in light of the Shanghai results and requires further explanation.

As suggested, we re-calculated the power for sleep disorder in separate and in the combined samples, and modified the Discussion section accordingly; i.e. we replaced “Our study had 80% power to detect odd ratios (ORs) ≥ 1.09 for self-reported sleep disorder at a significance of 5%” with “Our study had 80% power to detect odd ratio (OR) ≥ 1.27, 1.32 and 1.20 for self-reported sleep disorder at a significance of 5% in Beijing, Shanghai subpopulations and in whole population, respectively” (Discussion).

To explore the potential reason for the discrepancies between Beijing and Shanghai, we adjusted associations for the available lifestyle factors such as drinking, smoking, physical activity, education, diet etc. However, these adjustments did not materially change the associations. We also stratified analyses by drinking, smoking, physical activity, education, diet etc., but no significant association was observed in Beijing subpopulation. Assuming that we have insufficient power to detect gene-environment interaction and there may be some unmeasured factors not included in this study, we can only speculate the discrepancies be due to “unmeasured” environmental/lifestyle factors and to limited power, in particular in subpopulations.

Minor revisions:
4. The statement linking variation in the gene with circadian rhythm should be expanded and supported with a reference.

As suggested we have no expanded and provided better support for our statements. As a major regulator of circadian rhythms, the melatonin effects on sleep and circadian phase are mainly mediated by activation of its two receptors: melatonin receptor 1A and melatonin receptor 1B. Therefore, MTNR1B which encoded melatonin receptor 1B was involved in the regulation of circadian rhythms. To clarify the background section in the abstract we changed the word “regulates” to “involved in the regulation of”.

In the background of the main text, we cited one additional reference (Sleep Med 2007, 8 Suppl 3:34-42) which describes the role of melatonin receptors on circadian rhythm regulation (Background section line 10).

5. For positive associations, the effect sizes and confidence intervals should be included in the abstract.

Changes have been made as suggested.

6. Additional references should be included as suggested.
Changes have been made as suggested.

7. Some additional information on the sleep analyses would be useful. Tables 2 and 3 should be improved as suggested.

We have improved the tables as suggested by including data on sleep disorder using the odds ratio per allele, assuming an additive model. The models used for association analyses of all traits have now been described in more detail in the Methods and in the footnote of Table 3.

In the Methods section, we have added more detailed information by replacing “Generalized linear regression was used for associations with diabetes-related quantitative traits and gene-geographical regions (Beijing/Shanghai) interactions in which participants with known diabetes or receiving glucose-lowering treatment (n=276) were excluded.” to “Generalized linear regression was used to test for association with diabetes-related quantitative traits and gene-geographical regions (Beijing/Shanghai) or gene-sleep status interactions in which participants with known diabetes or those receiving glucose-lowering treatment (n=276) were excluded.”. We also changed “The associations with sleep duration and self-reported sleep disorder were carried out using nonparametric test (Kruskal-Wallis) and logistic regression, respectively” to “The associations with sleep duration, siesta frequency and self-reported sleep disorder were carried out using nonparametric test (Kruskal-Wallis), $\chi^2$ test and logistic regression, respectively.” in the Methods section.

We also modified the way we present results of sleep status by replacing “No significant association between SNP rs10830963 and sleep duration or quality was found in either Beijing or Shanghai subpopulations (P ≥ 0.44) (Table 3).” with “No significant associations of MTNR1B-rs10830963 with sleep duration, siesta frequency or sleep disorder were found in either Beijing or Shanghai subpopulations (P ≥ 0.44) (Table 3).” in the Results section.

We modified Table 2 according to the reviewer’s comments. Models with further adjustment for sleep duration and disturbances were tested and included in the table. Interaction analyses are also shown in the table. Table 2 was modified as follows:
Table 2  a Associations of rs10830963 with fasting glucose, HbA1C, HOMA-B and HOMA-S.

<table>
<thead>
<tr>
<th>MTNR1B rs10830963</th>
<th>Beijing (1389)</th>
<th>Shanghai (1497)</th>
<th>Total population (2886)</th>
<th>P for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (SE)</td>
<td>P add</td>
<td>β (SE)</td>
<td>P add</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.03 (0.06)</td>
<td>0.59</td>
<td>0.11 (0.04)</td>
<td>0.005</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.03 (0.05)</td>
<td>0.58</td>
<td>0.11 (0.04)</td>
<td>0.005</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.03 (0.06)</td>
<td>0.59</td>
<td>0.11 (0.04)</td>
<td>0.005</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.003 (0.04)</td>
<td>0.93</td>
<td>0.07 (0.02)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.004 (0.04)</td>
<td>0.92</td>
<td>0.07 (0.03)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.004 (0.04)</td>
<td>0.93</td>
<td>0.07 (0.03)</td>
<td>0.004</td>
</tr>
<tr>
<td>HOMA-B (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.88 (1.58)</td>
<td>0.58</td>
<td>-5.01 (1.65)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.91 (1.57)</td>
<td>0.56</td>
<td>-5.01 (1.65)</td>
<td>0.003</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.88 (1.58)</td>
<td>0.58</td>
<td>-5.01 (1.65)</td>
<td>0.003</td>
</tr>
<tr>
<td>Log-transformed HOMA-S (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.004 (0.02)</td>
<td>0.82</td>
<td>0.008 (0.02)</td>
<td>0.66</td>
</tr>
<tr>
<td>Model 2</td>
<td>-0.005 (0.02)</td>
<td>0.82</td>
<td>0.008 (0.02)</td>
<td>0.66</td>
</tr>
<tr>
<td>Model 3</td>
<td>-0.004 (0.02)</td>
<td>0.82</td>
<td>0.008 (0.02)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Participants previously diagnosed with type 2 diabetes or receiving glucose-lowering treatment (n=264) were excluded from the analyses. HOMA-S values were log-transformed before analyses. P values and beta (SE) for Beijing and Shanghai subpopulations were calculated using linear regression model assuming an additive model. The combined P values and beta (SE) were calculated in meta-analyses using random model.
Model 1: Adjusted for age, sex and BMI.
Model 2: Adjusted variables in model 1 plus sleep duration.
Model 3: Adjusted variables in model 1 plus self-reported sleep disorder.

\( P \) values for interaction were tested in linear regression model using terms of SNP × Region (Beijing/Shanghai), SNP × Sleep duration or SNP × Sleep disorder.

Since HOMA-B was log-transformed before analyzes, in the submitted manuscript we used the antilogarithm of the beta (SE) to represent the effect size. However, SE cannot be transformed back using the antilogarithm for log-transformed traits. We revised this in the updated Table 2 and used the raw beta (SE) to represent the effect size of log-transformed HOMA-S.

Accordingly, we replaced “Moreover, adjustment for sleep duration or quality did not materially change the associations of rs10830963 with those diabetes-related quantitative traits in Shanghai subpopulation. (data not shown)” with “Moreover, further adjustment for sleep duration (Table 2, model 2) or self-reported sleep disorders (Table 2, model 3) did not materially change the associations of rs10830963 with the diabetes-related quantitative traits in either Shanghai or Beijing subpopulation, and no significant gene-sleep status interaction \((P \text{ for interaction} \geq 0.22)\) was observed (Table 2).” (Results).

We added data for sleep duration and self-reported sleep disorder in both subpopulations and total population in Table 1. We also used “MET-h/wk” to evaluate physical activity in stead of classifying it into “low/middle/high”.