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Genetic variation in *MTNR1B* is associated with gestational diabetes mellitus and contributes only to the absolute level of beta cell compensation in Mexican Americans

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Abstract

Aims/hypothesis —*MTNR1B* is a type 2 diabetes susceptibility locus associated with cross-sectional measures of insulin secretion. We hypothesised that variation in *MTNR1B* contributes to the absolute level of a diabetes-related trait, temporal rate of change in that trait, or both.

Methods—We tested rs10830963 for association with cross-sectional diabetes-related traits in up to 1,383 individuals or with rate of change in the same phenotypes over a 3–5 year follow-up in up to 374 individuals from the family-based BetaGene study of Mexican Americans.

Results—rs10830963 was associated cross-sectionally with fasting glucose ($p = 0.0069$), acute insulin response (AIR; $p = 0.0013$), disposition index ($p = 0.00078$), glucose effectiveness ($p =$

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Contribution statement

The study was designed by AHX, TAB and RMW. Data collection and assays were carried out by ET, EB, JML, JH, JMR and HA and statistical analysis was performed by JR and MT. JR and RMW prepared the manuscript. Critical edits and intellectual contributions to the manuscript were contributed by AHX, ET, MT, EB, JML, JH, JMR, HA, and TAB. The final manuscript was reviewed and approved by all the authors. Overall study management by AHX, TAB and RMW. RMW is responsible for the integrity of this work.

Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

0.018) and gestational diabetes mellitus (OR 1.48; $p = 0.012$), but not with OGTT 30 min insulin (the difference between the 30 min and fasting plasma insulin concentration) or 30 min insulin-based disposition index. rs10830963 was also associated with rate of change in fasting glucose ($p = 0.043$), OGTT 30 min insulin ($p = 0.01$) and AIR ($p = 0.037$). There was no evidence for an association with the rate of change in beta cell compensation for insulin resistance

Conclusions/interpretation—We conclude that variation in *MTNR1B* contributes to the absolute level of insulin secretion but not to differences in the temporal rate of change in insulin secretion. The observed association with the rate of change in insulin secretion reflects the natural physiological response to changes in underlying insulin sensitivity and is not a direct effect of the variant.

Keywords

Association study; Beta cell function; Families; Genetics; Gestational diabetes mellitus; Insulin secretion; Longitudinal; Mexican Americans; *MTNR1B*

Introduction

Genome-wide association studies have identified hundreds of loci associated with type 2 diabetes or type 2 diabetes-related quantitative traits (T2DQTs). Discovery of these loci has mostly relied upon large cross-sectional samples under the assumption that genetic variants associated with risk for type 2 diabetes or altered levels of a T2DQT individually have small effect. However, progression towards disease varies among individuals, reflecting a combination of both genetic and environmental factors and the complex feedback regulation of phenotypes. Thus, any cross-sectional sample captures a snapshot of individuals at differing temporal points along their specific trajectories towards disease. We have hypothesised that genetic variation can contribute to the absolute level of a T2DQT or the rate at which a given T2DQT changes over time, or possibly both. These subsequently contribute to risk for disease.

We tested this hypothesis by examining rs10830963 in *MTNR1B*, previously shown to be associated with type 2 diabetes in samples of northern European ancestry [1, 2]. Variation in *MTNR1B* is also associated with increased fasting glucose and reduced insulin secretion [1–3]. *MTNR1B* is one of two known receptors for melatonin, which has been implicated in regulation of circadian rhythms. Insulin secretion follows a circadian pattern inverse to that of circulating melatonin levels [4] and there is increasing evidence which links disruption in circadian sleep with risk for obesity and type 2 diabetes [5–8]. However, the mechanistic link between melatonin and insulin secretion has yet to be elucidated. We speculate that in addition to determining the absolute level of T2DQTs, variation in *MTNR1B* may affect long-term temporal change in insulin secretion or beta cell compensation for insulin resistance via differences in circadian regulation.

We tested these hypothesised effects using data from the BetaGene study, which is a family-based study of Mexican Americans designed to examine the association between genetic variation and T2DQT. Families were ascertained on probands with or without a previous diagnosis of gestational diabetes mellitus (GDM), ensuring a sample of at-risk individuals

and a wide range of phenotype values. A unique characteristic of BetaGene is that we obtained quantitative estimates of body composition, insulin sensitivity (S_I), acute insulin response (AIR) to glucose, glucose effectiveness (S_G) and beta cell compensation for insulin resistance (disposition index [DI]). A subset of our study population was recalled for re-phenotyping approximately 4 years after their baseline phenotyping in the latest phase of BetaGene. We took advantage of the totality of BetaGene to test our hypothesis regarding cross-sectional vs longitudinal phenotypes using variation in *MTNR1B* as an example.

Methods

Recruitment and recall of participants

Details of the baseline studies for BetaGene have been previously described [9]. Briefly, participants are Mexican Americans who are either probands with GDM diagnosed within the previous 5 years using Third International GDM Workshop criteria [10] and their family members or non-GDM probands with normal glucose levels in pregnancy in the past 5 years. The baseline BetaGene sample consists of 2,157 individuals from 526 families with available genotype data, 1,838 individuals with fasting blood measurements, 1,668 individuals with detailed body composition data gathered by dual-energy x-ray absorptiometry (DXA) scanning, 1,564 individuals with OGTT results with blood samples drawn at -10, 0, 30, 60, 90 and 120 min, and 1,126 individuals with frequently-sampled intravenous glucose tolerance tests (FSIGTs) with minimal modelling. The second phase of BetaGene (BetaGene II) successfully recalled and phenotyped 374 individuals approximately 3–5 years after baseline testing. Individuals who developed type 2 diabetes during the follow-up period or whose fasting glucose was > 7 mmol/l (126 mg/dl) were not included in the study. All protocols for BetaGene have been approved by the Institutional Review Boards of participating institutions and all participants provided written informed consent before participation.

Clinical protocols

Phenotyping was performed, on two separate visits, at the University of Southern California (USC) General Clinical Research Center or the Clinical Trials Unit of the Southern California Clinical and Translational Sciences Institute. The first visit consisted of a physical examination, DNA collection and a 75 g OGTT, as previously described [9]. Participants with fasting glucose < 7 mmol/l (126 mg/dl) were given an accelerometer (Actigraph, Pensacola, FL, USA) to be worn for 7 days to assess physical activity and were invited back for a second clinic visit. The second visit consisted of a DXA scan for body composition (per cent body fat) and an insulin-modified FSIGT performed as previously described [16].

Assays

Plasma glucose was measured on an auto-analyser using the glucose oxidase method (YSI Model 2300; Yellow Springs Instruments, Yellow Springs, OH, USA). Insulin was measured by a two-site immunoenzymometric assay (TOSOH) that has $< 0.1\%$ cross-reactivity with proinsulin and intermediate split products.

Molecular analysis

rs10830963 was genotyped using the TaqMan system (Applied Biosystems) [11, 12]. The genotyping assay was selected through the 'Assays on Demand' database (<https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=OnlineOrderingPageDisplay>; accessed July 2009).

Phenotype definitions

We calculated two measures of insulin response to glucose: the difference between the 30 min and fasting plasma insulin concentration from OGTT (30 min insulin) and the incremental area under the insulin curve for during the first 10 min of the FSIGT (AIR). The insulinogenic index was calculated as 30 min insulin divided by the 30 min change in glucose (30 min glucose – fasting glucose). FSIGT glucose and insulin data were analysed using the minimal model (MINMOD Millennium V5.18, Minmod, Los Angeles, CA, USA) [13] to derive estimates of S_G and S_I . The DI, a measure of beta cell compensation for insulin resistance, was computed as the product of S_I and early insulin response ($DI = S_I \times AIR$). We also computed a similar index of beta cell compensation from the OGTT (DI_{30}) using the 30 min insulin from the OGTT ($DI_{30} = S_I \times 30 \text{ min insulin}$) [9].

We computed the rates of change in T2DQTs among the recalled individuals as:

$$y_{\text{Rate of change}} = \frac{y_{\text{Follow-up}} - y_{\text{Baseline}}}{\text{Follow-up years}}$$

Follow-up years were computed as the number of years between the follow-up and baseline visit dates for the specific trait.

Data analysis

rs10830963 was tested for non-Mendelian inheritance using PEDSTATS V0.6.10 (<http://www.sph.umich.edu/csg/abecasis/PedStats/>) [14]. Allele frequencies and deviation from Hardy–Weinberg equilibrium were determined using all available data, taking into account relatedness using SOLAR V4.3.1 (<https://www.txbiomed.org/departments/genetics/genetics-detail?r=37>) [15,16]. The missing rate for rs10830963 was 4.7%.

rs10830963 was tested for association with both the cross-sectional measurement and rates of change in a given T2DQT. Previous studies showed rs10830963 in *MTNR1B* to be associated with measures of insulin secretion [3, 17, 18]. We therefore tested 30 min insulin, AIR, DI and DI_{30} as traits of primary interest in our association analyses, as they have not been previously tested in Mexican Americans. We then tested our remaining nine T2DQTs for association in a series of secondary analyses: BMI, percentage body fat, fasting and 2 h glucose, fasting and 2 h insulin, insulinogenic index, S_G and S_I . We also tested rs10830963 for association with GDM in 271 GDM cases and 215 non-GDM controls.

All data were statistically transformed to approximate univariate normality before association analyses by inverse normal scores. Association between single-nucleotide polymorphisms (SNPs) and T2DQTs or GDM status was tested using likelihood ratio testing

under a variance components framework as implemented in SOLAR [15, 16]. To be consistent with previous reports, we assumed an additive genetic model with the G allele as the reference. We assumed a dominant genetic model for the analysis of rates of change in T2DQTs, given the smaller sample size. All models were adjusted for age and sex, except for GDM status where the model only included adjustment for age. We also included baseline trait values as covariates in the analysis of rates of change in T2DQTs. We assessed the effect of adiposity on our association results by testing models with the additional adjustment for percentage body fat in the cross-sectional analysis or rates of change in percentage body fat for the longitudinal analysis. Results for models with the additional inclusion of body fat as a covariate were similar to those with only age and sex adjustment, so only results for the latter are reported.

We estimated a priori power to assess our ability to detect association with our traits of interest. We have 80% power to detect a quantitative trait locus that accounts for 0.7% of the variation in the quantitative trait at a minor allele frequency (MAF) < 0.3 given an additive genetic model and a sample size of 1,100 for our cross-sectional analysis. Similarly, we have 80% power to detect a quantitative locus that accounts for 2.1% of the variation in rate of change in quantitative trait at MAF < 0.3 given an additive genetic model and sample size of 375 for our longitudinal analysis.

The p values were Bonferroni corrected for multiple comparisons to account for the number of quantitative traits tested (four traits in the primary analysis, nine traits in the secondary analysis). Bonferroni correction assumes independence among tests and therefore would be overly conservative when applied to correlated tests as in the case of these analyses. Figures showing the rates of change in T2DQTs were generated by using the actual baseline genotype-specific median values and projecting those values 5 years later based upon the estimated β s from the test of association. We report medians and interquartile ranges, unless specified otherwise.

Results

We report results from 2,157 individuals in 526 families; 374 of these individuals were rephenotyped an average of 4.2 years after their baseline study. Characteristics for the cross-sectional and follow-up samples are shown in Table 1. The baseline characteristics of the subset of individuals who underwent follow-up were not different from the complement that did not participate in the follow-up. Several metabolic variables measured at follow-up were different from baseline, generally demonstrating a deterioration of metabolic status: fasting glucose, 2 h glucose, fasting insulin, 2 h insulin, 30 min insulin, insulinogenic index, S_I and DI (Table 1). rs10830963, in the lone intron of *MTNR1B*, had an estimated MAF of 22.5% in our sample and was associated with baseline AIR (corrected $p = 0.0013$) and DI (corrected $p = 0.00078$) (Table 2), with both measures decreasing with each copy of the G allele and consistent with results observed in samples of northern European ancestry [3]. It is of interest to note that this variant showed no evidence for an association with either baseline 30 min insulin (corrected $p = 0.580$) or DI30 (corrected $p = 0.960$) in our cross-sectional sample. We replicated the previously known association between rs10830963 and baseline fasting glucose (corrected $p = 0.0069$), with fasting glucose increasing with each

copy of the G allele. We also observed a novel association between rs10830963 and baseline S_G (corrected $p = 0.018$) where S_G adjusted for age and sex decreased 0.00093/min with each copy of the G allele.

The results of the test of association between rs10830963 and the rate of change in T2DQTs are shown in Table 3. We observed an association between rs10830963 and the rate of change in 30 min insulin (corrected $p = 0.010$), in contrast to the lack of association in the cross-sectional analysis. Individuals homozygous for the C allele had a rate of change in 30 min insulin of 28.2 pmol/l per year, while individuals with at least one G allele had a rate of change of 4.5 pmol/l per year (Fig. 1a). We also observed an association between rs10830963 and the rate of change in AIR (corrected $p = 0.037$); C/C individuals had a rate of change in AIR of 68.6 (pmol/l) $\times$ 10 min per year while those with at least one G allele had a rate of change in AIR of -64.8 (pmol/l) $\times$ 10 min per year (Fig. 1b). The rate of change in fasting glucose was also modestly associated with rs10830963 (corrected $p = 0.043$) and the rate of change in S_I showed a trend for association (corrected $p = 0.081$, Fig. 1c). This SNP did not show evidence for association with rates of change in other T2DQTs.

The consequence of these associations is characterised in Table 4, which shows the contrast between baseline and follow-up phenotype traits and their relative changes stratified by rs10830963 genotype. Both genotype groups showed significant increases in adiposity as reflected by BMI and percentage of body fat, but the relative increase was greater in C/C homozygotes (2.9% for BMI and 2.5% for percentage of body fat) compared with those individuals carrying at least one G allele (1.3% for BMI and 0.7% for percentage of body fat; $p < 0.05$ for both traits). S_I decreased in both genotype groups, as might be expected given the increase in adiposity, but despite the differences in the relative change in adiposity, the relative change in S_I was similar between the genotype groups (-20.8% for C/C vs -13.1% for G/*; $p = 0.173$).

The 30 min insulin increased 23.7% in C/C homozygous individuals ($p = 1.3 \times 10^{-6}$), but the increase was not significant in individuals with at least one G allele (4.9%, $p = 0.285$). Thus, the relative change in 30 min insulin was significantly greater in individuals with the C/C vs G/* genotype ($p = 0.0027$). In contrast, AIR did not significantly change during the follow-up period in C/C homozygous individuals (0.2%, $p = 0.967$) and showed a trend to decrease in individuals with at least one G allele (-8.1%, $p = 0.069$) and the relative changes were not significantly different between the two genotype groups ($p = 0.263$).

Finally, fasting glucose did not significantly change during the follow-up period among C/C homozygous individuals (relative change 1.5%, $p = 0.10$), but significantly increased among individuals with at least one G allele (relative change 5.7%, $p = 8.3 \times 10^{-7}$). The relative changes in fasting glucose differed significantly between the two genotype groups ($p = 0.0039$).

The significant cross-sectional associations with T2DQTs and the association between rs10830963 and rates of change in 30 min insulin and AIR led us to test whether rs10830963 was associated with GDM. rs10830963 showed evidence for association with

GDM (OR 1.48, 95% CI 1.09, 2.01, $p = 0.012$). The results were similar assuming a dominant genetic model (OR 1.64, 95% CI 1.13, 2.38, $p = 0.009$).

Discussion

MTNR1B is a known type 2 diabetes susceptibility locus associated with both fasting glucose [1, 2] and measures of insulin secretion [3]. The majority of the reported genetic associations have been cross-sectional in nature and examined primarily in samples of northern European [1, 2, 17, 18] or Asian ancestry [19–25]. *MTNR1B* has been reported to be associated with GDM in the Korean population [22, 25] and in other populations [21, 26–29]. However, few have examined the association of this gene with rates of change in T2DQTs and it is equivocal whether genetic variation in *MTNR1B* contributes to the rate at which a given phenotype changes over time. Lyssenko and colleagues examined the association between T2DQTs and known type 2 diabetes risk variants [30] (in particular, *MTNR1B* rs10830963 [3] was examined in two large prospective studies) but did not directly test the association between the rates of change in T2DQTs and genetic variants. Rather, they tested the cross-sectional association at baseline and follow-up visits and because the cross-sectional pattern of association was consistent across visits, inferred a longitudinal association. Our study differs in that we calculated the rate of change in T2DQTs, thus formally testing whether rs10830963 affected the rate at which specific phenotypes changed over our study period. We show that rs10830963 is associated with the absolute level of insulin secretion and beta cell compensation for insulin resistance at baseline and also with the rate of change in insulin secretion. Furthermore, we replicated in Mexican Americans the association between rs10830963 and risk for GDM first reported in the Korean population [22, 25] and subsequently in other populations [21, 26–29]. The initial study of 928 Korean women with GDM and 990 without GDM reported an OR of 1.35 [22]; a similar OR was observed when these samples were included in a genome-wide meta-analysis for GDM (1,399 GDM cases and 2,025 non-GDM controls; OR 1.45) [25]. The OR observed in BetaGene is consistent with these studies (OR 1.48). Interestingly, Kwak et al only observed a marginal association between rs10830963 and fasting glucose and found no evidence for association with other T2DQTs [25]. The studies examining the association between *MTNR1B* and GDM included limited phenotypes with which to assess the underlying physiological effects of genetic variation contributing to risk for type 2 diabetes or GDM. We showed that the rs10830963 G allele had strong effects on measures of insulin secretion, fasting glucose and S_G that likely contribute to susceptibility to GDM. The association with S_G is of particular interest, as we previously showed that this variable plays a critical role in the maintenance of glucose tolerance and in the pathogenesis of type 2 diabetes [31].

Unique to our study, we show that rs10830963 is associated with rates of change in 30 min insulin from the OGTT and AIR from the FSIGT (Table 3 and Fig. 1). Two copies of the C allele resulted in a positive rate of change in insulin secretion (30 min insulin or AIR) over the 4–5 year follow-up period, whereas the presence of at least one G allele was associated with a substantially lower rate of change over the same period. Taken together with the observation made in the cross-sectional analysis that the G allele is associated with lower absolute insulin secretion, one might make the conclusion that variation in *MTNR1B*

contributes to risk for type 2 diabetes or GDM via defective insulin secretion and subsequent poor beta cell compensation via both absolute and temporal effects. However, the G allele was not associated with the rate of change in DI or DI30 (Table 3 and Fig. 1 d, e), indicating that variation in *MTNR1B* does not appear to directly affect the rate of change in beta cell compensation for insulin resistance. In fact, both genotype groups showed a remarkably similar relative change in DI (C/C -17.9% vs G/* -16.9%) and in DI30 (C/C -7.1% vs G/* -7.7%), when comparing baseline to follow-up, consistent with the rate of change being parallel between the two genotype groups (Fig. 1 d, e). The only difference between the groups was the fact that the C/C homozygotes had a higher baseline DI and DI30 than the individuals with at least one G allele, a pattern that remained at follow-up. We speculate that the apparent association between rs10830963 and the rate of change in 30 min insulin and AIR simply reflects the fact that C/C homozygous individuals, having better absolute beta cell compensation, robustly compensate for insulin resistance. This results in a greater change in insulin secretion, translating into a greater positive rate of change over time. Such speculation is supported by the findings that the relative change in S_I over the follow-up period was similar between the two genotype groups (C/C -20.8% vs G/* -13.1%) and that individuals with a G allele had a reduced ability to compensate for this change in S_I as reflected in their lower absolute DI. This pattern may also account for the association between rs10830963 and both baseline fasting glucose and the rate of change in fasting glucose. The *MTNR1B*-associated inability to appropriately compensate for insulin resistance results in a significantly greater increase in fasting glucose in individuals with a G allele compared with C/C homozygotes (G/* 5.7% vs 1.5%; $p = 0.0039$).

Our findings suggest a critical contribution of *MTNR1B*, and possibly melatonin, to absolute levels of intravenous-based glucose-stimulated insulin secretion and beta cell compensation, but not to their rates of change over time. We previously showed that *MTNR1B* co-localises with insulin in both rodent and human pancreatic islets [3], suggesting that melatonin may have a direct effect on the pancreatic beta cell. Additional support comes from studies showing that melatonin therapy appeared to preserve or improve pancreatic beta cell integrity in the face of streptozotocin in rodents [32–34]. Also, long-term melatonin treatment of individuals with type 2 diabetes resulted in a reduction in HbA_{1c} levels [35], suggesting that melatonin had a beneficial effect on glycaemia. It is interesting to note that rs10830963 was associated with AIR but not with 30 min insulin or insulinogenic index in our cross-sectional analysis (Table 2). This suggests that the effect of rs10830963 involves pathways independent of the incretin component of insulin secretion, an observation that requires additional study.

Changes in circadian patterns and sleep disruption have been implicated in risk for obesity and type 2 diabetes; however, there have been few studies elucidating the mechanisms underlying these associations. The melatonin system plays an important role in the regulation of circadian rhythms, which is why we had hypothesised that variation in *MTNR1B* may have both cross-sectional and longitudinal effects. We observed greater increases in BMI and percentage of body fat in C/C homozygotes than in individuals with at least one G allele over the follow-up period ($p = 0.039$ and $p = 0.045$, respectively), but rs10830963 showed no evidence for association with BMI or percentage of body fat or with

the rates of change in these phenotypes. This suggests that variation in *MTNR1B* does not play a role in the regulation of adiposity in Mexican Americans. The longitudinal component of our study is limited in sample size and, coupled with the MAF of 22% for rs10830963, limited our ability to examine the association between rs10830963 and rates of change in T2DQTs. We were forced to use a dominant genetic model to test the association, which limited our ability to fully examine the effect of the G allele on the rates of change in T2DQTs using the additive model. Also, our analysis is based on a single 5 year follow-up visit after baseline. Thus, our estimate of the rate of change in phenotype may not reflect the actual rate of change over a longer follow-up period. In fact, it is likely that the rate changes as an individual approaches the state of diabetes (i.e. the rate of change may be nonlinear over time).

In summary, we tested rs10830963 in *MTNR1B* for association with both absolute levels of and rates of change in T2DQTs in the BetaGene sample of Mexican Americans. We demonstrate that rs10830963 is strongly associated with fasting glucose, S_G , AIR and DI cross-sectionally, but is also associated with the rate of change in insulin secretion. However, evidence suggests that the association with the rate of change in insulin secretion may reflect the natural physiological response to changes in underlying insulin sensitivity and is not a direct effect of the variant on longitudinal changes in insulin secretion. The differences in absolute insulin secretion and beta cell compensation could contribute to risk for type 2 diabetes or GDM.

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Abbreviations

AIR	Acute insulin response
DI	Disposition index
DXA	Dual-energy x-ray absorptiometry
FSIGT	Frequently-sampled intravenous glucose tolerance test
GDM	Gestational diabetes mellitus
MAF	Minor allele frequency
MTNR1B	Melatonin receptor 1B

S_I	Insulin sensitivity index
S_G	Glucose effectiveness
SNP	Single-nucleotide polymorphism
T2DQT	Type 2 diabetes-related quantitative trait
USC	University of Southern California

References

1. Bouatia-Naji N, Bonnefond A, Cavalcanti-Proenca C, et al. A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. *Nat Genet.* 2009; 41:89–94. [PubMed: 19060909]
2. Prokopenko I, Langenberg C, Florez JC, et al. Variants in MTNR1B influence fasting glucose levels. *Nature Genetics.* 2009; 41:77–81. [PubMed: 19060907]
3. Lyssenko V, Nagorny CL, Erdos MR, et al. Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nature Genetics.* 2009; 41:82–88. [PubMed: 19060908]
4. Boden G, Ruiz J, Urbain JL, Chen X. Evidence for a circadian rhythm of insulin secretion. *Am J Physiol.* 1996; 271:E246–E252. [PubMed: 8770017]
5. Lucassen EA, Rother KI, Cizza G. Interacting epidemics? Sleep curtailment, insulin resistance, and obesity. *Ann N Y Acad Sci.* 2012; 1264:110–134. [PubMed: 22827862]
6. Ong CW, O'Driscoll DM, Truby H, Naughton MT, Hamilton GS. The reciprocal interaction between obesity and obstructive sleep apnoea. *Sleep Med Rev.* 2012; 17:123–131. [PubMed: 22818968]
7. Sabanayagam C, Teppala S, Shankar A. Markers of sleep disordered breathing and diabetes mellitus in a multiethnic sample of US adults: results from the National Health and Nutrition Examination Survey (2005–2008). *Int J Endocrinol.* 2012; 2012:879134. [PubMed: 22518133]
8. Lindberg E, Theorell-Haglow J, Svensson M, Gislason T, Berne C, Janson C. Sleep apnea and glucose metabolism - a long-term follow-up in a community-based sample. *Chest.* 2012; 142:935–942. [PubMed: 22499826]
9. Watanabe RM, Allayee H, Xiang AH, et al. Transcription factor 7-like 2 (*TCF7L2*) is associated with gestational diabetes mellitus and interacts with adiposity to alter insulin secretion in Mexican Americans. *Diabetes.* 2007; 56:1481–1485. [PubMed: 17317761]
10. Metzger BE. Summary and recommendation of the Third International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes.* 1991; 40:197–201. [PubMed: 1748259]
11. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal.* 1999; 14:143–149. [PubMed: 10084106]
12. Livak KJ. SNP genotyping by the 5'-nuclease reaction. *Methods Mol Biol.* 2003; 212:129–147. [PubMed: 12491907]
13. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther.* 2003; 5:1003–1015. [PubMed: 14709204]
14. Wigginton JE, Abecasis GR. PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. *Bioinformatics.* 2005; 21:3445–3447. [PubMed: 15947021]
15. Blangero J, Almasy L. Multipoint oligogenic linkage analysis of quantitative traits. *Genet Epidemiol.* 1997; 14:959–964. [PubMed: 9433607]
16. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet.* 1998; 62:1198–1211. [PubMed: 9545414]

17. Stancakova A, Kuulasmaa T, Paananen J, et al. Association of 18 confirmed susceptibility loci for type 2 diabetes with indices of insulin release, proinsulin conversion, and insulin sensitivity in 5,327 nondiabetic Finnish men. *Diabetes*. 2009; 58:2129–2136. [PubMed: 19502414]
18. Ingelsson E, Langenberg C, Hivert MF, et al. Detailed physiologic characterization reveals diverse mechanisms for novel genetic Loci regulating glucose and insulin metabolism in humans. *Diabetes*. 2010; 59:1266–1275. [PubMed: 20185807]
19. Tam CH, Ho JS, Wang Y, et al. Common polymorphisms in MTNR1B, G6PC2 and GCK are associated with increased fasting plasma glucose and impaired beta-cell function in Chinese subjects. *PLoS One*. 2010; 5:e11428. [PubMed: 20628598]
20. Hu C, Zhang R, Wang C, et al. Effects of GCK, GCKR, G6PC2 and MTNR1B variants on glucose metabolism and insulin secretion. *PLoS One*. 2010; 5:e11761. [PubMed: 20668700]
21. Wang Y, Nie M, Li W, et al. Association of six single nucleotide polymorphisms with gestational diabetes mellitus in a Chinese population. *PLoS One*. 2011; 6:e26953. [PubMed: 22096510]
22. Kim JY, Cheong HS, Park BL, et al. Melatonin receptor 1 B polymorphisms associated with the risk of gestational diabetes mellitus. *BMC Med Genet*. 2011; 12:82. [PubMed: 21658282]
23. Li C, Shi Y, You L, Wang L, Chen ZJ. Association of rs10830963 and rs10830962 SNPs in the melatonin receptor (MTNR1B) gene among Han Chinese women with polycystic ovary syndrome. *Mol Hum Reprod*. 2011; 17:193–198. [PubMed: 20959387]
24. Song JY, Wang HJ, Ma J, et al. Association of the rs10830963 polymorphism in MTNR1B with fasting glucose levels in Chinese children and adolescents. *Obes Facts*. 2011; 4:197–203. [PubMed: 21701235]
25. Kwak SH, Kim SH, Cho YM, et al. A genome-wide association study of gestational diabetes mellitus in Korean women. *Diabetes*. 2012; 61:531–541. [PubMed: 22233651]
26. Vlassi M, Gazouli M, Paltoglou G, et al. The rs10830963 variant of melatonin receptor MTNR1B is associated with increased risk for gestational diabetes mellitus in a Greek population. *Hormones (Athens)*. 2012; 11:70–76. [PubMed: 22450346]
27. Mao H, Li Q, Gao S. Meta-analysis of the relationship between common type 2 diabetes risk gene variants with gestational diabetes mellitus. *PLoS One*. 2012; 7:e45882. [PubMed: 23029294]
28. Li C, Qiao B, Zhan Y, et al. Association between genetic variations in MTNR1A and MTNR1B genes and gestational diabetes mellitus in Han Chinese women. *Gynecol Obstet Invest*. 2013; 76:221–227. [PubMed: 24157813]
29. Stuebe AM, Wise A, Nguyen T, Herring A, North KE, Siega-Riz AM. Maternal genotype and gestational diabetes. *Am J Perinatol*. 2014; 31:69–76. [PubMed: 23456907]
30. Lyssenko V, Jonsson A, Almgren P, et al. Clinical risk factors, DNA variants, and the development of type 2 diabetes. *N Engl J Med*. 2008; 359:2220–2232. [PubMed: 19020324]
31. Bergman RN. Toward physiological understanding of glucose tolerance. Minimal model approach. *Diabetes*. 1989; 38:1512–1527. [PubMed: 2684710]
32. Andersson AK, Sandler S. Melatonin protects against streptozotocin, but not interleukin-1beta-induced damage of rodent pancreatic beta-cells. *J Pineal Res*. 2001; 30:157–165. [PubMed: 11316326]
33. Yavuz O, Cam M, Bukan N, Guven A, Silan F. Protective effect of melatonin on beta-cell damage in streptozotocin-induced diabetes in rats. *Acta Histochem*. 2003; 105:261–266. [PubMed: 13677620]
34. de Oliveira AC, Andreotti S, Farias TS, et al. Metabolic disorders and adipose tissue insulin responsiveness in neonatally STZ-induced diabetic rats are improved by long-term melatonin treatment. *Endocrinology*. 2012; 153:2178–2188. [PubMed: 22374967]
35. Garfinkel D, Zorin M, Wainstein J, Matas Z, Laudon M, Zisapel N. Efficacy and safety of prolonged-release melatonin in insomnia patients with diabetes: a randomized, double-blind, crossover study. *Diabetes Metab Syndr Obes*. 2011; 4:307–313. [PubMed: 21887103]

Fig. 1.

(a–c) Association between *MTNR1B* rs10830963 and rates of change in OGTT 30 min insulin (a), AIR (b) and S_I (c) assuming a dominant genetic model. Circles, C/C genotype; squares, combined C/G and G/G genotypes. Actual baseline values are shown (mean $\pm$ SE) and 5 year values were projected based on the regression model from the association analysis (see Methods for details). rs10830963 was significantly associated with the rate of change in OGTT 30 min insulin (corrected $p=0.01$) and AIR (corrected $p=0.037$), and there was a trend for an association with S_I (corrected $p=0.081$). (d, e) Rates of change in measures of beta cell compensation (DI30 [d], DI [e]) showed no evidence for an association with rs10830963 (corrected $p=1.0$ and 0.656 , respectively). These results suggest that the associations observed between rs10830963 and rates of change in 30 min insulin and AIR are a consequence of the natural physiological response to changes in S_I and are not a direct effect of the variant per se

Table 1

Characteristics of study participants

Characteristic	Cross-sectional sample	Follow-up subgroup		
		Baseline	Follow-up	<i>p</i> value ^a
Sex (female/male), <i>n</i>	1,305/852	281/97		
Age, years	34.7 (12.7)	34.8 (10.9)	39.4 (11.1)	
BMI, kg/m ²	28.1 (7.3)	28.5 (7.0)	29.0 (7.1)	0.0001
Body fat, %	34.5 (13.6)	36.1 (12.4)	36.0 (11.9)	0.0001
Fasting glucose, mmol/l	5.0 (0.7)	5.1 (0.7)	5.1 (0.8)	0.0001
2 h Glucose, mmol/l	7.1 (2.5)	7.0 (2.4)	7.4 (3.0)	0.0001
Fasting insulin, pmol/l	48.6 (48.6)	48.6 (48.6)	58.3 (58.9)	0.0001
2 h Insulin, pmol/l	410 (424)	396 (410)	506 (600)	0.0001
30 min insulin, pmol/l	375 (354)	372 (389)	443 (409)	0.0001
Insulinogenic index, $\mu\text{U/ml per mmol/l}^b$	1.06 (0.94)	1.09 (0.98)	1.19 (1.04)	0.0108
DI ₃₀ ^c	990 (835)	1,015 (786)	970 (755)	0.1409
$S_G \times 10^{-2}/\text{min}^c$	1.68 (0.81)	1.72 (0.79)	1.69 (0.82)	0.1448
$S_I \times 10^{-3} \text{ min}^{-1} (\text{pmol/l})^{-1c}$	2.75 (1.95)	2.80 (2.08)	2.31 (1.63)	0.0001
AIR, $(\text{pmol/l}) \times 10 \text{ min}^c$	2,942 (3,363)	3,174 (3,334)	3,056 (3,302)	0.1928
DI ^c	8,373 (7,949)	8,446 (7,615)	7,328 (6,706)	0.0001

Data are reported as median (interquartile range)

^aTwo-sample paired *t* test comparing baseline vs follow-up^bIndex is computed using non-SI units for insulin and SI units for glucose as this is how the values were calculated^cCross-sectional sample size for these traits is 818/308 (female/male sex)

Table 2Cross-sectional univariate association results for *MTNR1B* rs10830963 assuming an additive genetic model

Trait	<i>n</i>	β (SE) ^a	<i>p</i> value	Corrected <i>p</i> value ^b
Primary traits				
30 min insulin	1,560	−26 (20)	0.145	0.580
AIR	1,123	−720 (222)	3.15×10^{-4}	1.3×10^{-3}
DI	1,123	−1,608 (403)	1.96×10^{-4}	7.8×10^{-4}
DI30	1,098	−44 (53)	0.240	0.960
Secondary traits				
BMI	1,899	0.24 (0.28)	0.458	1
% Body fat	1,665	0.31 (0.27)	0.201	1
Fasting glucose	1,834	0.092 (0.033)	7.67×10^{-4}	6.9×10^{-3}
2 h Glucose	1,560	0.15 (0.10)	0.161	1
Fasting insulin	1,834	−3.4 (2.6)	0.498	1
2 h Insulin	1,560	26 (21)	0.152	1
Insulinogenic	1,444	−0.026(0.031)	0.035	1
index				
S _G	1,123	−0.12 (0.043)	2.03×10^{-3}	0.018
S _I	1,123	0.099 (0.10)	0.336	1

^a β values are based on raw phenotype values and adjusted for age and sex^b Bonferroni-corrected for the number of traits tested

Table 3

Univariate association between *MTNR1B* rs10830963 and rates of change (per year) in phenotypes assuming a dominant genetic model

Trait	<i>n</i>	β (SE) ^a	<i>p</i> value	Corrected <i>p</i> value ^b
Primary traits				
30 min insulin	361	-23.9 (7.3)	2.46×10^{-3}	0.010
AIR	351	-135 (63)	9.31×10^{-3}	0.037
DI	350	-135 (126)	0.164	0.656
DI30	347	-9.0 (17.1)	0.912	1
Secondary traits				
BMI	365	-0.084 (0.059)	0.140	1
% Body fat	362	-0.060 (0.072)	0.316	1
Fasting glucose	364	0.041 (0.017)	4.80×10^{-3}	0.043
2 h Glucose	364	-0.035 (0.057)	0.495	1
Fasting insulin	363	-0.22 (1.0)	0.396	1
2 h Insulin	363	-5.4 (13.2)	0.642	1
Insulinogenic index	360	-0.047 (0.021)	0.021	0.189
S _G	350	0.0063 (0.018)	0.961	1
S _I	350	0.10 (0.035)	9.02×10^{-3}	0.081

^a β values are based on raw phenotype values adjusted for age and sex

^bBonferroni-corrected *p* value

Table 4
Comparison of baseline and follow-up phenotypes stratified by *MTNR1B* rs10830963 genotype

Trait	C/C			C/G and G/G			p value ^d				
	n	Baseline ^b	Follow-up ^b	% Change	p value ^c	n		Baseline ^b	Follow-up ^b	% Change	p value ^c
Primary traits											
30 min 3insulin (pmol/l)	196	468	579	23.7	1.3×10^{-6}	165	434	456	4.9	0.285	2.7×10^{-3}
AIR, (pmol/l) $\times$ 10 min	190	4,544	4,553	0.2	0.967	161	3,530	3,244	-8.1	0.069	0.263
DI	190	11,076	9,098	-17.9	1.4×10^{-5}	160	8,589	7,139	-16.9	4.9×10^{-5}	0.350
DI30	186	1,231	1,144	-7.1	0.136	160	1,169	1,079	-7.7	0.278	0.980
Secondary traits											
BMI, kg/m ²	200	29.2	30.1	2.9	8.7×10^{-9}	165	29.4	29.8	1.3	0.031	0.039
Body fat, %	198	34.3	35.2	2.5	1.1×10^{-5}	164	35.0	35.3	0.7	0.302	0.045
Fasting glucose, mmol/l	199	5.0	5.1	1.5	0.100	165	5.0	5.3	5.7	8.3×10^{-7}	3.9×10^{-3}
2 h Glucose, mmol/l	200	7.1	7.8	10.6	3.5×10^{-7}	165	7.3	7.8	7.0	1.4×10^{-3}	0.258
Fasting insulin, pmol/l	198	58.1	71.2	22.6	9.1×10^{-6}	165	59.6	71.7	20.3	1.6×10^{-4}	0.812
2 h Insulin, pmol/l	199	474	681	43.7	2.4×10^{-8}	165	538	695	29.1	1.2×10^{-4}	0.345
Insulinogenic index	196	1.40	1.58	13.5	9.3×10^{-3}	164	1.22	1.24	2.1	0.635	0.071
S _G , $\times 10^{-2}$ min ⁻¹	190	1.81	1.73	-4.6	0.115	160	1.72	1.66	-3.4	0.368	0.764
S _I , $\times 10^{-3}$ min ⁻¹ (pmol/l) ⁻¹	190	3.01	2.38	-20.8	2.8×10^{-8}	160	3.18	2.76	-13.1	2.5×10^{-4}	0.173

^a p value comparing the change in phenotype between C/C and the combined C/G and G/G genotype groups

^b Median adjusted for age and sex

^c p value for the test of the difference between baseline and follow-up adjusted for age and sex