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## A low frequency variant within the GWAS locus of *MTNR1B* affects fasting glucose concentrations: *Genetic risk is modulated by obesity*

L. F. Been<sup>1</sup>, J. L. Hatfield<sup>1</sup>, A. Shankar<sup>1</sup>, C. E. Aston<sup>1,2</sup>, S. Ralhan<sup>3</sup>, G. S. Wander<sup>3</sup>, N. K. Mehra<sup>4</sup>, J. R. Singh<sup>5</sup>, J. J. Mulvihill<sup>1</sup>, and D. K. Sanghera<sup>1,\*</sup>

<sup>1</sup> Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

<sup>2</sup> General Clinical Research Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

<sup>3</sup> Hero DMC Heart Institute, Ludhiana, Punjab, India

<sup>4</sup> All India Institute of Medical Sciences, New Delhi, India

<sup>5</sup> Central University of Punjab, Bathinda, Punjab, India

### Abstract

Two common variants (rs1387153, rs10830963) in *MTNR1B* have been reported to have independent effects on fasting blood glucose (FBG) levels with increased risk to type 2 diabetes (T2D) in recent genome-wide association studies (GWAS). In this investigation, we report the association of these two variants, and an additional variant (rs1374645) within the GWAS locus of *MTNR1B* with FBG, 2h glucose, insulin resistance (HOMA IR),  $\beta$ -cell function (HOMA B), and T2D in our sample of Asian Sikhs from India. Our cohort comprised 2,222 subjects [1,201 T2D, 1,021 controls]. None of these SNPs was associated with T2D in this cohort. Our data also could not confirm association of rs1387153 and rs10830963 with FBG phenotype. However, upon stratifying data according to body mass index (BMI) (low  $\leq 25$  kg/m<sup>2</sup> and high  $> 25$  kg/m<sup>2</sup>) in normo-glycemic subjects (n= 1,021), the rs1374645 revealed a strong association with low FBG levels in low BMI group ( $\beta = -0.073$ ,  $p = 0.002$ , Bonferoni  $p = 0.01$ ) compared to the high BMI group ( $\beta = 0.015$ ,  $p = 0.50$ ). We also detected a strong evidence of interaction between rs1374645 and BMI with respect to FBG levels ( $p = 0.002$ ). Our data provide new information about the significant impact of another *MTNR1B* variant on FBG levels that appears to be modulated by BMI. Future confirmation on independent datasets and functional studies will be required to define the role of this variant in fasting glucose variation.

### Keywords

*MTNR1B*; Fasting blood glucose; SNP-obesity interaction; Asian Indians

\*Corresponding Author: Dharambir K. Sanghera, PhD, Department of Pediatrics, Section of Genetics, University of Oklahoma Health Sciences Center, 940 Stanton Young Blvd., BMSB-317, Oklahoma City, OK- 73104, USA, Phone: 405–271–8001 ext. 43064, Fax: 405–271–6027, Dharambir-Sanghera@ouhsc.edu.

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## Introduction

Elevated levels of fasting blood glucose (FBG) are hallmarks of type 2 diabetes (T2D). Individual variability in FBG is heritable and around 50% of the variance in FBG levels could be attributed to genetic factors.<sup>1, 2</sup> Recent genome-wide association studies (GWAS) and meta-analysis have identified genes contributing to the variation of FBG levels in populations of European origin.<sup>3–6</sup> Two common variants in the melatonin receptor1-B (*MTNR1B*) were shown to have independent effects on FBG levels in normoglycemic (NG) individuals with an increased risk for T2D.<sup>3, 7</sup> These results were also later replicated in other independent studies from same or different ethnicities.<sup>8–10</sup> However, these variants explain only a small portion of the heritability of T2D, implying more variants in or around these GWAS signals need to be identified.<sup>11</sup> In this investigation, we report the association of these two previously known variants rs10830963, rs1387153, and another less common variant rs1374645 in *MTNR1B* with FBG, 2h glucose, homeostasis model assessment (HOMA) for insulin resistance (HOMA IR) and  $\beta$ -cell function (HOMA B), and T2D in this unique sample of Asian Sikhs from India.

## Methods

### Study Subjects

The study participants are part of our ongoing Sikh diabetes Study.<sup>12</sup> The DNA and serum samples of a total of 2,222 subjects including 1,201 T2D cases [628 male, 573 female, mean age (mean  $\pm$  SD) 53.9  $\pm$  10.7 yrs.], 1,021 NG controls [535 male, 486 female, mean age 51.9  $\pm$  13.9 yrs.] were used in this investigation. The diagnostic criteria used for recruiting T2D patient and NG control have been described in detail elsewhere.<sup>13</sup> Briefly, the diagnosis of T2D was confirmed by medical records, use of medication, and measuring fasting glucose levels following the guidelines of American Diabetes Association.<sup>14</sup> Impaired glucose tolerance (IGT) was defined as a FBG level > 100.8 mg/dL but < 126.1 mg/dL or a 2 hour oral glucose tolerance test (2h OGTT) > 141.0 mg/dL but  $\leq$  200 mg/dL. The 2h OGTTs were performed following the criteria of the World Health Organization (WHO) (75 g oral load of glucose). BMI was calculated as weight (kg)/height (meter<sup>2</sup>). The NG subjects were from the same Punjabi community and from the same geographic location where the T2D patients were recruited.<sup>15</sup> Majority of the subjects were recruited from the state of Punjab from Northern India. Individuals of South, East, and Central Indian origins, or with type 1 diabetes (T1D), or a family member with T1D, rare forms of T2D called maturity-onset diabetes of young (MODYs), and secondary diabetes (e.g., hemochromatosis, pancreatitis) were excluded from the study. The selection of controls was based on a fasting glycemia <100.8 mg/dL or a 2h glucose <141.0 mg/dL. In general, Punjabi Sikhs do not smoke for religious and cultural reasons and about 50% of participants were life-long vegetarians. Subjects with IGT were excluded from this study. All blood samples were obtained at the baseline visit. Insulin was measured by radio-immuno assay (Diagnostic Products, Cypress, USA). HOMA IR was calculated as (fasting glucose mg/dL  $\times$  fasting insulin  $\mu$ IU/mL)/405 and HOMA B (fasting insulin  $\mu$ IU/mL  $\times$  360/fastest glucose mg/dL - 63), as described.<sup>16</sup> All participants provided a written informed consent for investigations. The study was reviewed and approved by the University of Oklahoma Health Sciences Center's Institutional Review Board, as well as the Human Subject Protection Committees at the participating hospitals and institutes in India.

### DNA Sequencing

DNA was extracted from buffy coats using QiaAmp blood kits (Qiagen, Chatworth, USA) or by the salting out procedure.<sup>17</sup> In search of putative functional variants in the *MTNR1B*

gene, we explored 5' UTR region around rs1387153 using PCR and DNA sequencing techniques. A variant detected through sequencing using Applied Biosystems' automatic sequencer 3730 (ABI, Foster City, USA) was a common point mutation (C→T) detected at position 92313529, was only 53 bases downstream from rs1387153 and was later found to have a dbSNP number rs1374645 by 1000 genomes with a rare minor allele frequency (MAF) in Caucasians as 0.028. Figure 1 shows the location of three investigated SNPs in the *MTNR1B* gene. We further explored 5' UTR region to discover more variants using DNA samples from 30 T2D and 30 NG controls from our SDS cohort. However, our initial sequencing results did not reveal the presence of any further common variant (allele frequency >0.05) in this region. Another mutation (G→A) discovered in exon 2 was also not polymorphic and was not further pursued.

### SNP Genotyping

Genotyping for all three SNPs was performed using TaqMan pre-designed or TaqMan made-to order SNP Genotyping Assays from Applied Biosystems Inc. (ABI, Foster City, USA). Genotyping reactions were performed on an ABI 7900 genetic analyzer using 2 ul of genomic DNA (10 ng/ul), following manufacturers' instructions. For quality control, 8–10% replicative controls and 4–8 negative controls were included in each 384 well plate to match the concordance, and the discrepancy rate of duplicate genotyping was <0.2%. Genotyping call rate was 96% or more in all the SNPs genotyped.

### Statistical Analysis

Data quality for SNP genotyping was checked by establishing reproducibility of control DNA samples. Departure from Hardy-Weinberg equilibrium (HWE) in controls was tested using the Pearson chi-square test. The genotype and allele frequencies in T2D cases were compared to those in control subjects using the chi-square test. Statistical evaluation of genetic effects on T2D risk used multivariate logistic regression analysis with adjustments for age, gender, and other covariates. Continuous traits with skewed sampling distributions (e.g. glucose, insulin, HOMA IR, and HOMA B) were log-transformed before statistical analysis. However, for illustrative purposes, values were re-transformed into the original measurement scale. General linear models (GLM) were used to test the impact of genetic variants on transformed continuous traits (FBG, 2h glucose, HOMA IR, and HOMA B) in only NG subjects after adjusting for the effects of covariates. Significant covariates for each dependent trait were identified by using Spearman's correlation and step-wise multiple linear regression with an overall 5% level of significance using SPSS for Windows statistical package (version 18.0) (SPSS Inc., Chicago, USA). Age, gender, and BMI were used as significant covariates in un-stratified analysis while age and gender were significant covariates in data stratified by BMI. Homozygous minor allele carriers of rs1374645 were analyzed combined with heterozygotes because of low frequency of minor alleles (0.06). Mean values between cases and controls were compared by using an unpaired t-test. The portion of variance on each adjusted quantitative trait (e.g. FBG) attributable to each *MTNR1B* polymorphism was estimated as  $R^2$  for the SNP by comparing the complete model to a model in which that specific SNP was removed. SNP-BMI interaction effects on FBG levels were tested using all three SNPs by applying GLM procedures and using FBG as a dependent variable. Age and gender were included as covariates in the model. Interaction analysis was only performed in un-stratified sample of NG controls.

Haplotype analysis of three *MTNR1B* SNPs was performed using HAPLOVIEW (version 4.0) (<http://www.broadinstitute.org/haploview/haploview>) that uses an accelerated expectation maximization algorithm to calculate haplotype frequencies. Effect of three-site haplotypes on quantitative variables were determined using PLINK (version 1.0.6) (<http://pnu.mgh.harvard.edu/~purcell/plink/>). To adjust for multiple testing, we used Bonferroni's

correction (0.05/ number of observations). Statistical power was assessed using the Genetic Power Calculator.<sup>18</sup> Assuming an additive genetic model, 1,021 unrelated control subjects, and  $\alpha = 0.05$ , our study has 80% power to detect a variant (with allele frequency as small as 0.02) that is associated with a difference in FBG of as little as 1 mg/dL. This accounts for an effect size of 0.1, a small effect size for a quantitative trait like FBG corresponding to detecting as significant  $\beta$ s outside of the range of  $\pm 0.05$ . These effect sizes are determined by differences between the group means relative to within group standard deviation.

## Results

Demographics and clinical characteristics of study subjects are summarized in Table 1. The genotype distribution for all three investigated SNPs was in HWE in controls with respective  $p$  values as, rs1387153 ( $p = 0.170$ ), rs1374645 ( $p = 0.332$ ), and rs10830963 ( $p = 0.053$ ). The variant rs1374645, despite in tight linkage disequilibrium (LD) with rs1387153 ( $D' = 0.91$ ), was poorly correlated ( $r^2 = 0.03$ ) to rs1387153 and it had no correlation with rs10830963 ( $D' = 0.15$ ;  $r^2 = 0.00$ ) in this sample (Figure 1). The other two variants (rs1387153 and rs10830963) exhibited relatively stronger correlation with each other ( $D' = 0.74$ ,  $r^2 = 0.50$ ).

None of these SNPs revealed any association with T2D in this Asian Indian cohort (Table 2). In multiple linear regression analysis, a marginal increase in HOMA IR was observed among 'G' allele carriers in rs10830963. As shown in Table 3, mean levels of HOMA IR were increased from 1.6 (1.4–1.8) in CC to 1.7 (1.5–1.9) in CG to 2.0 (1.6–2.5) in GG carriers, showing effect size of  $\beta = 0.13$ ,  $p = 0.026$ , Bonferroni  $p = 0.005$ . However, our data failed to confirm significant association of rs10830963 and rs1387153 with FBG concentrations; [ $\beta = 0.00$  95%CI (–0.01 – 0.02),  $p = 0.586$ ] and [ $\beta = 0.01$  95%CI (–0.01 – 0.02),  $p = 0.338$ ], respectively, unlike seen in Caucasian studies. On the other hand, in rs1374645, mean levels of 2h glucose were significantly reduced [ $\beta = -0.06$  95%CI (–0.12 – –0.01),  $p = 0.026$ ] in minor 'T' allele carriers (CT+TT) 99.7 (94.9–104.8) compared to non-'T' allele carriers (CC) 106.0 (103.9–108.1) in combined (un-stratified) NG cohort after adjusting for age, BMI, and gender (Table 3). However, this association did not remain significant after applying Bonferroni correction.

On the other hand, upon stratifying data by low (BMI  $\leq 25$  kg/m<sup>2</sup>) and high (BMI  $> 25$  kg/m<sup>2</sup>), the minor 'T' allele carriers of rs1374645 revealed a strong association with lower FBG levels only in low BMI group [ $\beta = -0.073$  95%CI (–0.119 – –0.028),  $p = 0.002$ , Bonferroni  $p = 0.01$ ], as the mean levels of FBG were significantly higher among CC genotypes (non-'T' allele carriers) ( $94.5 \pm 12.5$  mg/dL) compared to CT+TT genotypes ('T' allele carriers) ( $88.0 \pm 11.4$  mg/dL) (Table 4, Figure 2A). However, this association was not observed among high BMI individuals [ $\beta = 0.015$  95%CI (–0.028 – 0.057),  $p = 0.50$ ], as the mean FBG did not differ among CC ( $96.2 \pm 13.3$  mg/dL) versus CT+TT individuals ( $97.5 \pm 13.1$  mg/dL). As shown in the Table 4, the observed association did not disappear even after including covariates (age and gender) and other two SNPs in the model. Similarly, upon analyzing the effect of this SNP rs1374645 on 2h glucose in BMI-stratified sample, the significance was again confined to the low BMI group. The mean levels of 2h glucose were also moderately higher among CC ( $105.3 \pm 18.8$  mg/dL) compared to CT+TT ( $97.7 \pm 20.4$  mg/dL) individuals in non-obese group ( $\leq 25$  kg/m<sup>2</sup>,  $p = 0.013$ , Bonferroni  $p = 0.02$ ), while there was no significant difference in 2h glucose among CC ( $108.3 \pm 18.5$  mg/dL) versus CT+TT ( $104.4 \pm 14.9$  mg/dL) individuals in high BMI group ( $> 25$  kg/m<sup>2</sup>,  $p = 0.279$ ) (Table 4, Figure 2B).

To examine, if the effect of genetic variation at the *MTNR1B* locus on FBG is modulated by obesity status, we tested SNP-BMI interactions of all three SNPs in NG controls using GLM procedure and including FBG as dependent variable. We also included age and gender as

covariates in the model. As shown in Table 5, a significant evidence of interaction was seen between BMI\*1374645 ( $p=0.002$ ), whereas no evidence of interaction was observed between BMI and other two SNPs (rs1387153 and rs10830963). In order to study the effect of all three SNPs together on T2D and other related quantitative traits, we performed haplotype analysis in the *MTNR1B* locus. No haplotype was associated with T2D or FBG, 2h glucose, insulin, HOMA IR, or HOMA B levels (data not shown).

## Discussion

The earlier reported associations of rs1387153 and rs10830963 with FBG concentrations could not be confirmed in our Asian Indian study.<sup>4, 5</sup> However, the 'G' (rs10830963) allele-associated non-significant increase in HOMA IR levels was observed in this sample [ $\beta=0.13$ ,  $p=0.026$ , Bonferroni  $p=0.005$ ] in the same direction, as reported previously.<sup>5, 19</sup> Another publication on Asian Indians from UK reported association of these *MTNR1B* SNPs with FBG levels.<sup>8</sup> Incidentally, the allelic distribution in rs1387153 in our Punjabi cohort was comparable with the UK cohort [0.38 vs. 0.38], respectively, while the frequency information on rs10830963 was not available on the UK cohort. Despite similar allele frequencies, we were unable to replicate the association of these SNPs with FBG levels in our Indian Sikh cohort. Perhaps, comparatively smaller size of our cohort could be the reason of non-replication or due to weak LD between these variants and causative SNP in this population.<sup>20</sup> However, considering the common occurrence of these SNPs (MAF in both being  $>0.35$ ) in our cohort, the sample of 1,021 NG controls would have captured the quantitative trait locus (QTL) effect with  $>85\%$  power, had the association been robust. Alternatively, the differences in the ethnic composition of the UK cohort, and phenotype heterogeneity and trait characterization could have lead to these differences as our non-smoking Punjabi Sikh cohort is relatively homogenous.

However, the most interesting observation in this study is the strong and independent association of another variant within the same LD block of a GWAS-guided *MTNR1B* locus for affecting the same trait (FBG) in the absence of obesity. These results suggest that the same loci with common variation may harbor additional independent variants (e.g. low frequency variants like rs1374645, previously not captured by GWAS) to also influence the same trait.<sup>21</sup> Furthermore, the observed association of this variant with FBG levels was again confirmed in SNP\*BMI interaction where a strong evidence of interaction was observed in rs1374645 with BMI ( $p=0.002$ , Bonferroni  $p=0.0055$ ). At the same time, the other two SNPs showed no evidence of interaction with BMI for affecting FBG levels. Even the association of rs1374645 with 2h glucose was only confined to the low BMI group ( $p=0.013$ , Bonferroni  $p=0.02$ ) and not in high BMI group ( $p=0.279$ ) when the NG sample was stratified by BMI. None of the remaining two SNPs revealed any association with 2h glucose levels when tested in the data stratified by obesity (data not presented). Nevertheless, the evidence of association of rs1374546 with 2h glucose was not as strong as seen with FBG in our stratified data. Likewise, no strong association with 2h glucose with *MTNR1B* locus has been reported in other studies published previously.<sup>6</sup>

From these results it appears that the rs1374645 SNP truly affects fasting glucose concentrations before the onset of obesity. Even adjustment of age and gender did not alter the observed association. Apparently, relatively small size of our sample may be a limitation in this study. However, our data have 80% power to detect a difference in FBG as small as 1mg/dL (corresponding to detecting as significant  $\beta$ s outside of the range of  $\pm 0.05$ ) between genotypes at allele frequency of 0.06 and  $\alpha=0.05$  even in our stratified NG cohort by low and high BMI. Also, this deeply phenotyped homogenous cohort is collected from one geographic location and possibility of false positive associations due to population structure

is less likely. Moreover, measurements on BMI and FBG levels are available in over 96% of our entire cohort, which would further strengthened the power.

Our study reveals, for the first time, a significant protective association of a less common variant in the *MTNR1B* with FBG levels in the absence of obesity. These findings are somewhat in agreement with a prior study where the genetic variants acting on insulin secretion in *GCK*, *HNFI1A*, and *SLC30A8* were associated with T2D only in non-obese individuals and the genetic variants linked with insulin resistance in *PPARG2*, *ADIPOQ* were associated with T2D among obese individuals in two European populations<sup>22</sup>. In fact, more recently, rare alleles of two variants in *PPARGC1A* were associated with lower glucose levels in subjects with low BMI and with higher glucose levels in subjects with high BMI.<sup>23</sup> Notably, *MTNR1B* receptor is abundantly expressed in hypothalamus and It is speculated that the *MTNR1B* receptor could indirectly regulate glucose levels and insulin secretion through brain control center of circadian clock.<sup>24</sup> A recent study also confirmed the expression of *MTNR1B* in both islets and sorted pancreatic  $\beta$  cells.<sup>5</sup> These studies support putative direct role of *MTNR1B* in regulation of insulin secretion. Therefore, it is possible that this variant rs1374645 (being located near 5' region of the *MTNR1B*) may be involved in affecting its expression, or the expression of other genes that may influence insulin signaling and insulin response mediated by obesity.<sup>25</sup>

As the overall trait variance explained by this SNP is 2.9%, perhaps a causal variant in this gene with a greater risk is yet to be identified. Comprehensive deep sequencing is necessary to identify common and rare putative functional variants in this locus. Our data provide new information about the significant impact of a less common variant in *MTNR1B* on FBG levels that appears to be modulated by BMI. Future confirmation on independent datasets and functional studies will be required to define the role of this variant in fasting glucose variation, and whether this gene-interaction with obesity status can over time predispose to T2D.

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## Abbreviations

<b>T2D</b>	Type 2 diabetes
<b>MTNR1B</b>	Melatonin receptor 1-B
<b>GWAS</b>	Genome-wide association study
<b>HOMA IR</b>	Homeostasis model assessment for insulin resistance
<b>HOMA B</b>	Homeostasis model assessment for $\beta$ cell function
<b>SNP</b>	Single nucleotide polymorphism
<b>FBG</b>	Fasting blood glucose
<b>BMI</b>	Body mass index

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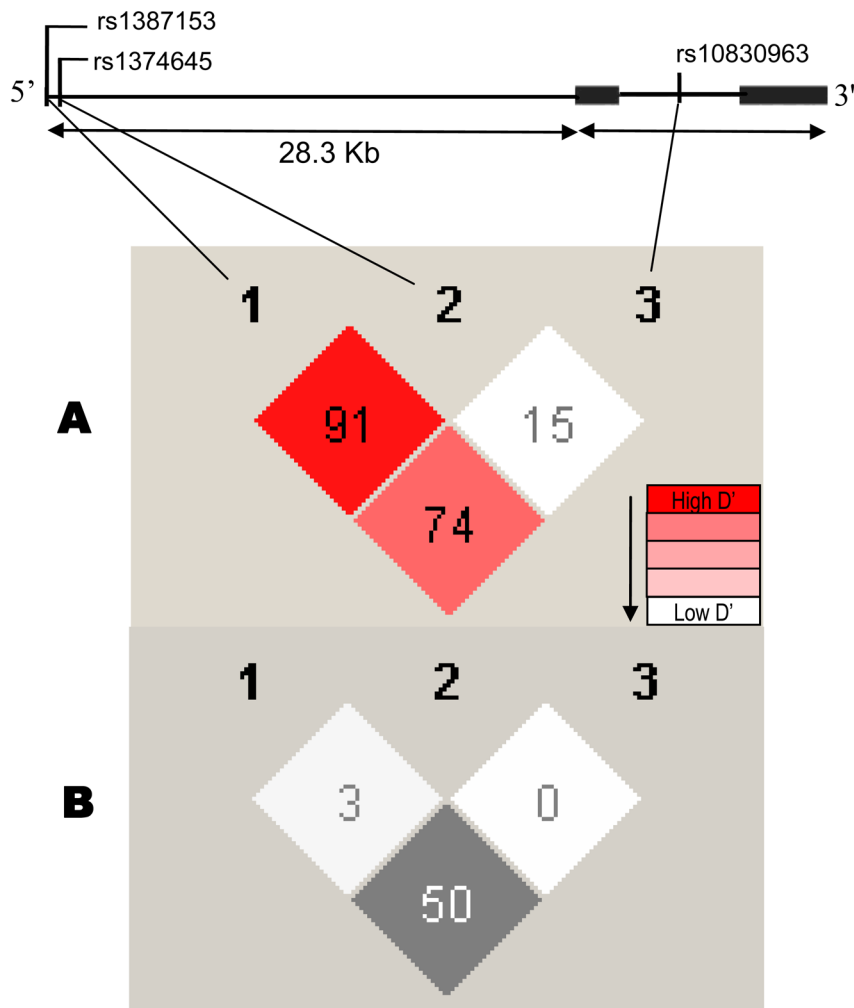
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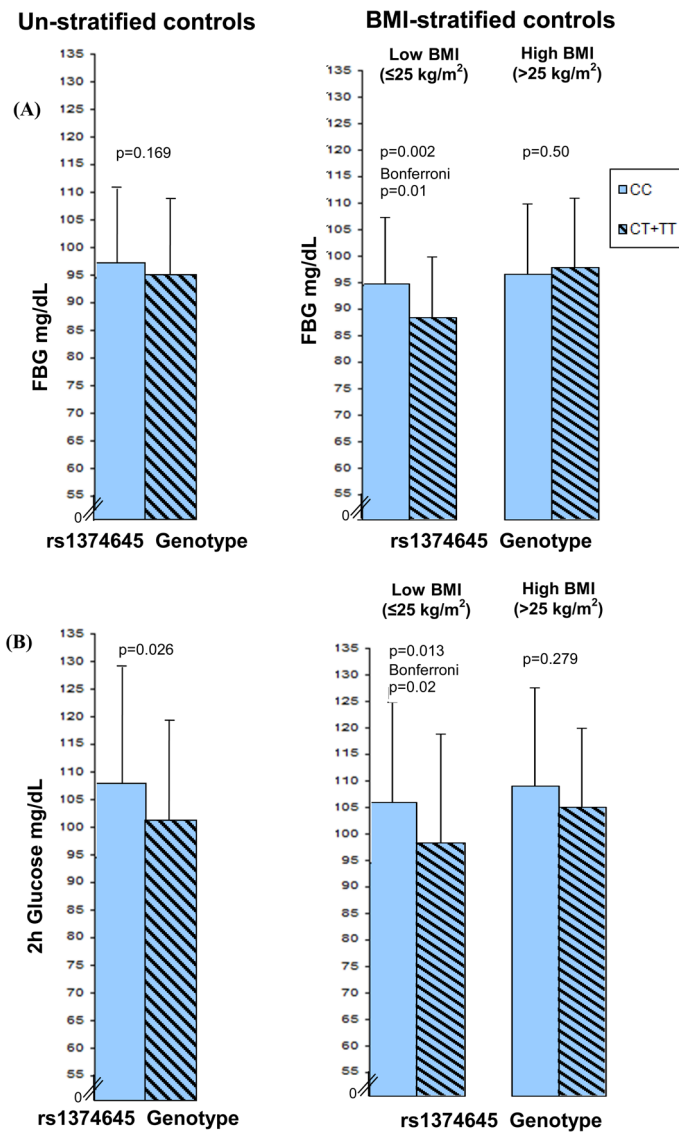


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# MTNR1B LOCUS



**Figure 1.** Figure 1 describes human *MTNR1B* locus showing genomic structure of *MTNR1B* and 28.3 kb 5' un-translated region harboring rs1387153, rs1374645, and rs10830963 variants. These variants reside within 62.1 kb linkage disequilibrium (LD) block (CEU, HapMap Phase II) on chromosome 11 near 5' UTR of *MTNR1B*. Top portion of the Figure 1 shows the position of three *MTNR1B* SNPs genotyped in this study. Figure 1(A) shows pair-wise LD between SNPs and Figure 1(B) shows pair-wise correlation ( $r^2$ ) between SNPs.



**Figure 2.** Figure 2(A) shows distribution of FBG in combined controls (un-stratified) and stratified by obesity (low BMI  $\leq 25$  kg/m<sup>2</sup> and high BMI  $> 25$  kg/m<sup>2</sup>). Analysis was performed using BMI, age and gender as covariates in un-stratified sample and age and gender as covariates in BMI-stratified sample. Figure 2(B) shows the distribution of 2h glucose in combined controls (unstratified) and stratified by obesity status. *P* values are from linear regression models.

**Table 1**Clinical characteristics of study population stratified by gender and disease (Mean  $\pm$  SD)

	Gender*	NG <sup>†</sup> Controls (n=1,021) (535 M/486 F)	T2D <sup>‡</sup> Cases (n=1,201) (628 M/573 F)
Age (yr)	M	51.3 $\pm$ 15.2	54.1 $\pm$ 10.2
	F	50.1 $\pm$ 13.3	53.7 $\pm$ 9.8
Age at Diagnosis (yr)	M	-	45.9 $\pm$ 10.2
	F	-	47.4 $\pm$ 9.4
Duration of Diabetes (yr)	M	-	8.0 $\pm$ 6.9
	F	-	6.5 $\pm$ 5.7
BMI (kg/m <sup>2</sup> )	M	25.7 $\pm$ 4.8	26.6 $\pm$ 4.4
	F	27.1 $\pm$ 6.7	28.4 $\pm$ 5.4
FBG (mg/dL) <sup>€</sup>	M	95.4 $\pm$ 11.2	163.8 $\pm$ 60.7
	F	93.7 $\pm$ 10.9	163.3 $\pm$ 64.9
2h Glucose (mg/dL)	M	104.0 $\pm$ 22.0	206.8 $\pm$ 67.3
	F	106.6 $\pm$ 17.9	193.9 $\pm$ 67.2
Fasting Insulin( $\mu$ IU/mL)	M	7.5 $\pm$ 7.1	5.9 $\pm$ 5.8
	F	7.7 $\pm$ 7.4	6.3 $\pm$ 5.6
HOMA IR <sup>*</sup>	M	1.7 $\pm$ 1.6	2.3 $\pm$ 2.2
	F	1.7 $\pm$ 1.6	2.5 $\pm$ 2.3

\* M- Males, F- Females;

<sup>†</sup> Normoglycemic;<sup>‡</sup> type 2 diabetes;<sup>€</sup> fasting blood glucose;<sup>\*</sup> homeostasis model assessment for insulin resistance

**Table 2**

Genotype distribution and association of candidate SNPs with type 2 diabetes

SNP	Position	Genotype**	NG (%)	T2D (%)	Odds Ratios (OR)* (95%CI)	P value
rs1387153	92313476	CC	363 (37)	459 (40)	1.0 (0.9-1.1)	0.668
		CT	479 (50)	558 (48)		
		TT	131 (13)	147 (12)		
		<b>C/T</b>	0.62/0.38	0.63/0.37		
rs1374645	92313529	CC	869 (87.9)	1054 (88.2)	1.0 (0.8-1.2)	0.801
		CT	118 (11.9)	137 (11.5)		
		TT	2 (0.2)	4 (0.3)		
rs10830963	92348358	<b>C/T</b>	0.94/0.06	0.94/0.06	1.0 (0.9-1.2)	0.458
		CC	393 (40)	435 (37)		
		CG	445 (44)	560 (48)		
		GG	163 (16)	174 (15)		
		<b>C/G</b>	0.61/0.39	0.61/0.39		

\* ORs were adjusted for age, sex, and BMI; Position of SNPs on chromosome has been taken from NCBI (Genome Build 36.3);

\*\* risk allele is bold faced

**Table 3**  
 Association of SNP genotypes with quantitative traits affecting glucose homeostasis in NG Controls (un-stratified for obesity)<sup>‡</sup>

rs1387153	CC	CT	TT	Effect $\beta$ (95% CI)	P value
Total (N)	363	479	131	--	--
FBG (mg/dL) <sup>€</sup>	95.0 (93.5 – 96.5)	94.9 (93.7 – 96.2)	96.2 (93.4 – 99.2)	0.01 (-0.01 – 0.02)	0.338
2h Glucose (mg/dL)	105.8 (102.8 – 108.8)	104.6 (101.5 – 107.7)	105.8 (101.5 – 110.3)	0.00 (-0.03 – 0.02)	0.841
Fasting Insulin( $\mu$ IU/mL)	7.5 (6.6 – 8.5)	7.6 (6.8 – 8.4)	7.2 (5.7 – 9.2)	-0.01 (-0.13 – 0.10)	0.844
HOMA IR <sup>*</sup>	1.6 (1.4 – 1.8)	1.7 (1.5 – 1.9)	1.8 (1.3 – 2.3)	0.04 (-0.08 – 0.16)	0.538
HOMA B <sup>£</sup>	84.5 (72.5 – 98.5)	86.6 (76.5 – 98.2)	82.2 (60.8 – 111.2)	-0.01 (-0.15 – 0.12)	0.813
rs1374645 <sup>‡</sup>	CC	CT + TT	GG	Effect $\beta$ (95% CI)	P value
Total (N)	869	118 + 2	--	--	--
FBG (mg/dL)	95.4 (94.5 – 96.4)	93.4 (90.9 – 96.1)	--	-0.02 (-0.05 – 0.01)	0.169
2h Glucose (mg/dL)	106.0 (103.9 – 108.1)	99.7 (94.9 – 104.8)	--	-0.06 (-0.12 – -0.01)	0.026
Fasting Insulin( $\mu$ IU/mL)	7.5 (6.9 – 8.2)	7.5 (6.1 – 9.2)	--	-0.03 (-0.26 – 0.21)	0.826
Homa-IR	1.7 (1.5 – 1.8)	1.6 (1.3 – 2.0)	--	-0.01 (-0.25 – 0.23)	0.921
Homa B	84.5 (76.7 – 93.2)	88.6 (68.4 – 114.8)	--	0.00 (-0.25 – 0.27)	0.935
rs10830963	CC	CG	GG	Effect $\beta$ (95% CI)	P value
Total (N)	393	445	163	--	--
FBG (mg/dL)	95.2 (93.8 – 96.7)	94.6 (93.3 – 96.0)	95.8 (93.7 – 98.0)	0.00 (-0.01 – 0.02)	0.586
2h Glucose (mg/dL)	106.4 (103.6 – 109.3)	105.3 (102.2 – 108.5)	102.3 (97.9 – 107.0)	-0.01 (-0.04 – 0.01)	0.367
Fasting Insulin( $\mu$ IU/mL)	7.3 (6.4 – 8.2)	7.6 (6.8 – 8.5)	8.4 (6.9 – 10.1)	0.08 (-0.03 – 0.19)	0.151
Homa-IR	1.6 (1.4 – 1.8)	1.7 (1.5 – 1.9)	2.0 (1.6 – 2.5)	0.13 (0.02 – 0.24)	0.026
Homa B	80.3 (69.3 – 92.9)	88.2 (77.1 – 101.0)	95.0 (75.4 – 119.5)	0.05 (-0.05 – 0.20)	0.232

<sup>€</sup> Fasting blood glucose;

<sup>\*</sup> homeostasis model assessment for insulin resistance;

<sup>£</sup> homeostasis model assessment for  $\beta$  cell function;

<sup>‡</sup> homozygous minor allele carriers were analyzed combined with heterozygotes because of small number;

<sup>‡</sup> Analysis was performed using age, BMI, and gender as covariates in multiple linear regression.

**Table 4**

Multiple linear regression analysis showing the impact of *MTNR1B* genotypes on FBG and 2h glucose levels in NG cohort in data stratified by obesity

	Non-Obese (BMI < 25)				Obese (BMI >25)				
	FBG in NG Controls				FBG in NG Controls				
	$\beta$	Lower	Upper	P value	$\beta$	Lower	Upper	P value	
Age	0.001	0.001	0.002	0.001	Age	0.002	0.000	0.003	0.004
Gender	-0.011	-0.040	0.018	0.444	Gender	-0.003	-0.029	0.024	0.839
rs1387153	0.020	-0.009	0.048	0.173	rs1387153	0.013	-0.015	0.040	0.367
rs1374645	<b>-0.073</b>	<b>-0.119</b>	<b>-0.028</b>	<b>0.002</b> *	rs1374645	0.015	-0.028	0.057	0.500
rs10830963	-0.024	-0.063	0.015	0.224	rs10830963	0.003	-0.034	0.039	0.884
Age	0.161	0.000	0.002	0.002	Age	0.119	0.000	0.002	0.011
Gender	-0.052	-0.042	0.013	0.315	Gender	0.007	-0.023	0.027	0.880
rs1374645	<b>-0.152</b>	<b>-0.102</b>	<b>-0.021</b>	<b>0.003</b> *	rs1374645	0.038	-0.022	0.052	0.413
<b>2h glucose in NG controls</b>									
Age	0.079	-0.001	0.002	0.226	Age	0.081	0.000	0.003	0.171
Gender	0.052	-0.028	0.066	0.424	Gender	0.095	-0.007	0.075	0.108
rs1387153	0.119	-0.022	0.087	0.239	rs1387153	-0.058	-0.065	0.034	0.538
rs1374645	-0.129	-0.146	0.006	0.070	rs1374645	-0.080	-0.112	0.023	0.198
rs10830963	-0.060	-0.065	0.035	0.550	rs10830963	0.044	-0.036	0.059	0.636
Age	0.071	-0.001	0.002	0.269	Age	0.080	0.000	0.003	0.162
Gender	0.059	-0.025	0.068	0.361	Gender	0.110	-0.001	0.079	0.057
rs1374645	<b>-0.160</b>	<b>-0.154</b>	<b>-0.018</b>	<b>0.013</b> *	rs1374645	-0.062	-0.097	0.028	0.279

FBG- fasting blood glucose; NG- normoglycemic;

\* p-value are significant after Bonferroni correction

**Table 5**Intracation between *MTNR1B* SNPs and BMI for affecting FBG levels

Model	Sum of Squares	Mean Square	F	P value
BMI	0.275	0.275	15.159	0.000
Gender	0.007	0.007	0.408	0.523
Age	0.257	0.257	14.189	0.000
rs1387153	0.046	0.023	1.270	0.281
rs1374645	0.199	0.199	11.002	<b>0.001</b>
rs10830963	0.021	0.010	0.570	0.566
rs1387153 * BMI	0.059	0.029	1.625	0.198
rs1374645 * BMI	0.170	0.170	9.408	<b>0.002</b>
rs10830963 * BMI	0.019	0.010	0.533	0.587

Bonferroni p=0.0055