The association between the melatonin receptor 1B gene polymorphism rs10830963 and glucose levels in type 2 diabetes

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Introduction
Melatonin is a pineal hormone under the control of the biological clock, which is located in the hypothalamus and regulated by light exposure. Melatonin receptors have been found throughout the body in many tissues including pancreatic islet cells, reflecting the widespread effects of melatonin on physiological functions such as energy metabolism and the regulation of body weight. Several lines of evidence suggest that melatonin may play a role in glucose metabolism.

Aim of the work
To investigate the association between diabetes mellitus (T2D) and the variants rs10830963 in the melatonin receptor 1B gene (MTNR1B) locus in a sample of the Egyptian population.

Patients and method
This was a case–control study conducted in the internal medicine department at El-Kasr El-Aini Hospital, Cairo University.
It included 30 diabetic individuals (type 2) compared with 20 healthy individuals. All individuals included in the study were subjected to a detailed history taking, complete physical examination, body composition evaluation, and laboratory testing including blood picture, blood urea nitrogen, creatinine, lipid profile, and genotyping of melatonin receptor B1. Diabetic individuals were subtyped into three groups: (a) Diabetic patients without complications. (b) Diabetic patients with microvascular complications. (c) Diabetic patients with macrovascular complications.

Results
Statistical analysis revealed a significant positive correlation between the MTNR1B polymorphism rs10830963 and glucose levels in type 2 diabetes.

Conclusion
The study confirmed that individuals having the MTNR1B gene polymorphism are at a greater risk of developing type 2 diabetes and having higher blood glucose levels and are more prone to be dyslipidemic than others who have no polymorphism.

Keywords:
microvascular or macrovascular complications, melatonin receptor 1B gene, type 2 diabetes

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, the heart, and blood vessels [2].

Melatonin is a widely occurring neurotransmitter; chemically, it is *N*-acetyl-5-methoxytryptamine, a derivative of serotonin, which in turn is derived from the amino acid tryptophan [3]. Pineal melatonin production at night is synchronized by the light/dark cycle, in that melatonin synthesis is suppressed by light. Light signals to the retina are transduced through the retinohypothalamic pathway to the suprachiasmatic nucleus of the hypothalamus and by a multisynaptic pathway to the pineal body [4].

It was shown that melatonin inhibits the insulin secretion of pancreatic b-cells. However, it remained unclear for some time as to exactly how this happened [5]. Functional analysis of pancreatic b-cells finally proved that melatonin activates three major intracellular signaling cascades: the 3′–5′-cyclic AMP cascade, the 3′–5′-cyclic GMP cascade [6], and the inositol-1,4,5-triphosphate (IP3) cascade. The hypothesis that melatonin has a regulatory influence on the pancreatic b-cell has been strengthened by the detection of two melatonin receptors, MT1 and MT2 [7], in whole-tissue examinations of the pancreas in rats and humans [8].

Melatonin synthesis starts with the essential amino acid tryptophan. This amino acid is already found in much smaller amounts in the pineal glands of type 2 diabetic rats. In humans, there are also indications that the availability of tryptophan has a direct influence on melatonin synthesis [9].

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All intermediary substances on the way to melatonin synthesis, 5-hydroxytryptophan, serotonin, and *N*-acetylserotonin, are also significantly decreased in the pineal gland of type 2 diabetic animals. The especially large deficit in 5-hydroxytryptophan is notable. The hydroxylation of tryptophan to 5-hydroxytryptophan is catalyzed by tetrahydrobiopteridin (5,6,7,8-tetrahydropteridine) [10].

This factor is decreased in type 2 diabetic rats [11] and mice [12]. In addition, tetrahydrobiopteridin plays a crucial role in the synthesis of tyrosine hydroxylase, a key enzyme in noradrenaline synthesis [13]. Reduced noradrenaline levels in type 2 diabetes have been described not only in rats but also in humans [14]. Ultimately, these results point to insufficiencies of tryptophan and tetrahydrobiopteridin as causes of reduced pineal melatonin synthesis in type 2 diabetic organisms [15]. In addition, reduced activity of AA-NAT has been documented in type 2 diabetic rats, so that the immediate precursor of melatonin is likewise synthesized more slowly [16].

Patients and methods

This was a case–control study conducted to evaluate the association between the melatonin receptor 1B gene (MTNR1B) polymorphism rs10830963 and glucose levels in type 2 diabetes.

The study group included 30 patients with type 2 diabetes admitted at the El-Kasr El-Ainy university hospital internal medicine department, for assessing the presence of the polymorphism in the melatonin receptor 1B gene, compared with 20 healthy nondiabetic individuals as the control group.

The diabetic group was subdivided into three groups as follows:

(1) 10 diabetic persons with macroangiopathy complications (cerebrovascular stroke, CVD, peripheral vascular disease).
(2) Ten diabetic persons with microangiopathy complications (retinopathy, neuropathy, nephropathy).
(3) Ten diabetic persons with neither microcomlications nor macrocomplications.

Inclusion criteria

Male or female patients diagnosed to have diabetes (type 2).

Exclusion criteria

Diabetes types other than type 2:

(1) Diabetes type 1.
(2) Gestational diabetes.
(3) Maturity-onset diabetes of the young.
(4) Latent autoimmune diabetes of adults.
(5) Secondary diabetes (as a result of drugs, diseases, etc.).

All studied participants were subjected to detailed medical history taking and physical examination including measurement of the weight, the height, and the BMI, laboratory tests including, fasting blood sugar, PPBS, HbA1c, complete blood count, blood urea nitrogen, serum creatinine, the albumin/creatinine ratio, and an assay of the lipid profile.

Genotyping of melatonin receptor B1

DNA was extracted from EDTA blood for all patients and controls using the DNA extraction kit (Qiagen, Hilden, Germany). Primer probes for melatonin receptor B1 were supplied by Life technologies with Cat. No 4351379 (assay ID C-3256858-10) (Biomatik company, USA). The rs10830963 was genotyped by an allelic discrimination assay on real-time PCR (Qiagen). The Context Sequence was [VIC/FAM] GTGATGCTAAGAATTCACACCATCT[C/G] CTATCCAGAACCAGTAACTGCCTGG.

The SNP rs10830963 was genotyped using TaqMan assays (Applied Biosystems, Foster City, California, USA). The TaqMan genotyping reaction was amplified on a PCR 5 Qiaplex (Qiagen). The PCR condition was 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

Statistical analysis

Data were statistically described in terms of minimum, maximum, mean, SD, median, frequencies (number of cases), and relative frequencies (percentages) when appropriate. Comparison of quantitative variables was performed using the Kruskal–Wallis and the Mann–Whitney tests for unpaired samples. For comparing categorical data, the χ^2-test was performed. The exact test was used instead when the expected frequency was less than 5. Genotype and allele frequencies were compared between the disease and the control groups using χ^2-tests. The odds ratio with 95% confidence interval was calculated. A P value less than 0.05 was considered as statistically significant, and a value less than 0.001 as highly significant. All statistical calculations were performed using statistical package for the social science (SPSS Inc., Chicago, Illinois, USA) version 21.

Results

Table 1 shows the comparative study between patients and controls regarding the number and the percentage of clinical data.

Table 1 Comparison between diabetic patients and control individuals

Variable	Groups		*P* value
	DM (30) count [*n* (%)]	Control (20) count [*n* (%)]	
Sex			
Female	24 (80.0)	11 (55.0)	0.059
Male	6 (20.0)	9 (45.0)	
Smoking			
None	24 (80.0)	14 (70.0)	0.506
smoker	6 (20.0)	6 (30.0)	
HTN			
HTN	15 (50.0)	0 (0.0)	<0.001
None	15 (50.0)	20 (100.0)	
Fundus			
None	22 (73.3)	20 (100.0)	0.015
Retinopathy	8 (26.7)	0 (0.0)	
Neuropathy			
Neuropathy	7 (23.3)	0 (0.0)	0.033
None	23 (76.7)	20 (100.0)	
PVD			
None	24 (80.0)	20 (100.0)	0.069
Positive	6 (20.0)	0 (0.0)	
CVS			
None	23 (76.7)	20 (100.0)	0.033
Positive	7 (23.3)	0 (0.0)	
ECG changes			
None	21 (70.0)	20 (100.0)	0.007
Positive	9 (30.0)	0 (0.0)	

CVS, cerebrovascular stroke; DM, diabetes mellitus; HTN, hypertension; PVD, peripheral vascular disease.

Table 2 shows a comparison between both groups in which there was a significant difference regarding retinopathy, neuropathy, cerebrovascular stroke, ECG changes, HB, cholesterol, TG, LDL, and HDL.

It also shows a highly significant difference in the following parameters: HTN, ALB/CREAT, fasting blood sugar, BUN, CREAT, HA1C, and BMI.

Table 3 and Fig. 1 show a comparison between diabetic participants and the control group regarding the G allele. The G allele was found 25 times among diabetic individuals (41.7% of the diabetic population), whereas it was found 11 times among control individuals (27.5% of the control population).

Table 4 and Fig. 2 show that the G allele was found nine times among diabetic individuals with macrovascular complications (45%), whereas it was found seven times among diabetic individuals without complications (35%).

Table 5 and Fig. 3 show that the G allele was found nine times among diabetic individuals with microvascular complications (45%), whereas it was found seven times among diabetic individuals without complications (35%).

Table 6 and Figure 4 shows a positive correlation between fasting blood glucose and the G allele and the melatonin gene polymorphism.

Table 7 shows a highly significant positive correlation between glycated hemoglobin and the G allele and the melatonin gene polymorphism.

Table 2 Comparison between diabetic individuals and control individuals

Variable	Group										*P* value
	DM (30)					Control (20)					
	Mean	SD	Median	Minimum	Maximum	Mean	SD	Median	Minimum	Maximum	
Age (years)	52.90	7.44	54.50	42.00	67.00	47.50	13.24	48.00	30.00	78.00	0.102
ALB/CREAT	95.93	147.94	26.50	12.00	650.00	15.45	5.62	14.00	8.00	28.00	<0.001
HB	11.80	1.51	11.40	9.20	15.90	12.95	1.59	12.40	10.90	15.70	0.010
PLT	290.67	81.34	289.00	104.00	432.00	294.95	59.13	294.50	191.00	392.00	0.961
TLC	8.72	2.17	8.50	4.30	13.20	7.55	2.05	7.25	4.70	12.30	0.065
FBS	194.33	54.67	195.00	101.00	303.00	80.20	9.32	79.00	67.00	99.00	<0.001
BUN	55.60	35.24	43.50	11.00	157.00	20.15	8.39	20.50	7.00	38.00	<0.001
CREAT	1.75	1.01	1.25	0.60	4.10	0.77	0.17	0.70	0.50	1.20	<0.001
Cholesterol	218.67	50.50	208.50	109.00	351.00	169.90	43.76	174.00	102.00	253.00	0.002
TG	235.20	69.83	253.00	101.00	372.00	167.75	79.26	145.50	93.00	372.00	0.001
LDL	146.70	18.36	145.00	108.00	178.00	123.75	26.86	122.50	72.00	178.00	0.004
HDL	46.53	8.74	46.50	31.00	63.00	55.55	7.66	55.00	42.00	67.00	0.001
HA1C	9.92	2.24	9.85	6.50	15.00	5.57	0.57	5.65	4.70	6.60	<0.001
Weight (kg)	97.90	17.81	94.00	76.00	140.00	94.60	30.90	86.50	68.00	172.00	0.085
Height (cm)	165.00	9.04	163.50	151.00	189.00	153.90	35.59	165.00	59.00	188.00	0.945
BMI	35.98	6.57	36.15	25.90	47.70	29.01	4.31	28.35	23.00	35.70	<0.001

ALB/CREAT, albumin/creatinine ratio; BUN, blood urea nitrogen; CREAT, serum creatinine; DM, diabetes mellitus; FBS, fasting blood sugar; HA1C, glycohemoglobin; HB, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PLT, platelets; TG, triglyceride; TLC, total leukocytic count; $P < 0.001$, highly significant difference; $P < 0.05$, significant difference.

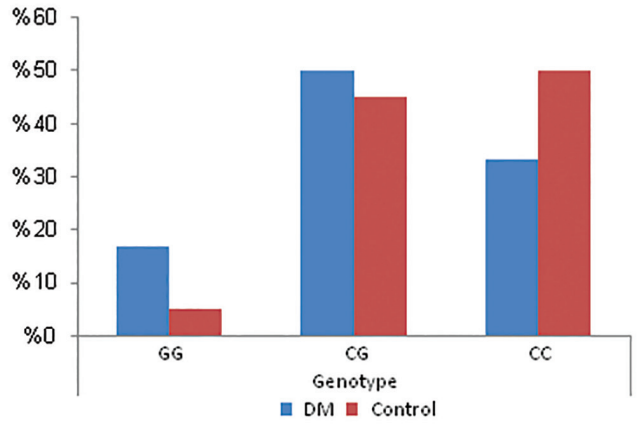
Table 8 shows a highly significant positive correlation between the BMI and the G allele and the melatonin gene polymorphism.

Discussion

Circadian rhythms are closely related to metabolism, and dysregulation of circadian rhythms may increase the risk of diabetes. The MTNR1B gene encodes a high-affinity receptor for melatonin, a hormone primarily secreted by the pineal gland to regulate the circadian rhythm and sleep cycles [16]. Plasma melatonin follows a circadian rhythm opposite to plasma insulin and glucose, increasing by night and decreasing by day. There are favorable evidences that the circadian rhythm of melatonin influences insulin secretion and glucose homeostasis through its islet-specific receptor [17].

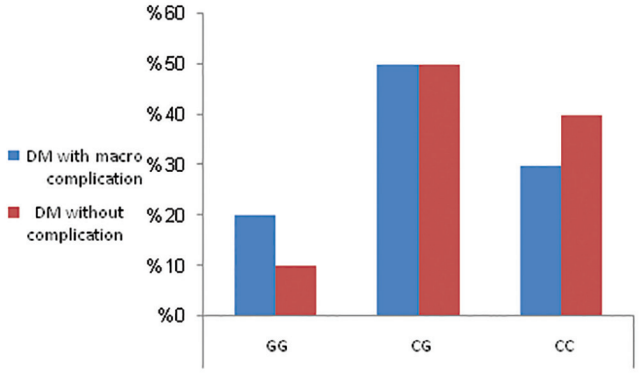
Consistently, melatonin secretion and the circadian rhythm are impaired in type 2 diabetes patients. More importantly, MTNR1B inhibits insulin secretion through its effect on CGMP formation when activated by melatonin [16–18]. Therefore, the MTNR1B gene might be involved in glucose homeostasis and type 2 diabetes.

Figure 1



Comparison between diabetics and control as regards G allele.

Figure 2



Comparison between diabetics with macrovascular complications and control as regards G allele.

Table 3 The percentage of diabetic patients and control individuals regarding the G allele

Variable	Groups		*P* value	OR (CI)
	DM (30) count [*n* (%)]	Control (20) count [*n* (%)]		
Genotype				
GG	5 (16.7)	1 (5.0)	0.197	5 (0.492–50.831)
CG	15 (50.0)	9 (45.0)	0.405	1.667 (0.5–5.559)
GG + CG	20 (66.7)	10 (50.0)	0.239	2 (0.627–6.377)
CC	10 (33.3)	10 (50.0)		Reference
Allele				
G	25 (41.7)	11 (27.5)	0.148	1.883 (0.794–4.464)
C	35 (58.3)	29 (72.5)		

CI, confidence interval; DM, diabetes mellitus; OR, odds ratio.

Table 4 Comparison between diabetic patients with macrovascular complications and diabetic patients without complications regarding the G allele

Variable	DM with macro (10) count [*n* (%)]	DM without complication (10) count [*n* (%)]	*P* value	OR (CI)
Genotype				
GG	2 (20.0)	1 (10.0)	1	2.667 (0.158–45.141)
CG	5 (50.0)	5 (50.0)	1	1.333 (0.191–9.311)
GG + CG	7 (70.0)	6 (60.0)	1	1.556 (0.244–9.913)
CC	3 (30.0)	4 (40.0)		Reference
Allele				
G	9 (45)	7 (35)	0.519	1.519 (0.425–5.426)
C	11 (55)	13 (65)		

CI, confidence interval; DM, diabetes mellitus; OR, odds ratio.

Table 5 Comparison between diabetic patients with microvascular complications and diabetic patients without complications regarding the G allele

Variable	DM with micro (10) count [*n* (%)]	DM without complication (10) count [*n* (%)]	*P* value	OR (CI)
Genotype				
GG	2 (20.0)	1 (10.0)	1	2.667 (0.158–45.141)
CG	5 (50.0)	5 (50.0)	1	1.333 (0.191–9.311)
GG + CG	7 (70.0)	6 (60.0)	1	1.556 (0.244–9.913)
CC	3 (30.0)	4 (40.0)		Reference
Allele				
G	9 (45)	7 (35)	0.519	1.519 (0.425–5.426)
C	11 (55)	13 (65)		

CI, confidence interval; DM, diabetes mellitus; OR, odds ratio.

Table 6 Correlation between the G allele and the melatonin gene polymorphism and fasting blood glucose

Variable	GG		CG		CC		*P* value
	Mean	SD	Mean	SD	Mean	SD	
FBS	242.2	48.45	192	57.87	173.9	40.41	0.076

FBS, fasting blood sugar.

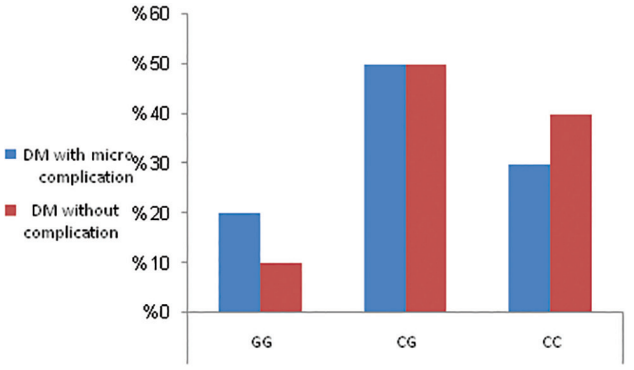
Table 7 Correlation between the G allele and the melatonin gene polymorphism and glycated hemoglobin

Variable	GG		CG		CC		*P* value
	Mean	SD	Mean	SD	Mean	SD	
HbA1c	13.06	1.17	10.43	1.37	7.58	0.8	<0.001

Table 8 Correlation between the G allele and the melatonin gene polymorphism and the body mass index

Variable	GG		CG		CC		*P* value
	Mean	SD	Mean	SD	Mean	SD	
BMI	45.16	2.3	36.73	4.08	30.26	5.18	<0.001

Figure 3



Comparison between diabetics with microvascular complications and control.

The aim of this study was to investigate the association between diabetes mellitus (T2D) and the variants rs10830963 in the MTNR1B locus in a sample of the Egyptian population.

In the current study, gene polymorphism was found in 20 diabetic individuals (five GG + 15 CG) (66.7% of the diabetic population), whereas it was found in 10 control individuals (one GG+nine CG) (50.0% of the control population). The G allele was found 25 times among diabetic individuals (41.7% of the diabetic population), whereas it was found 11 times among control individuals (27.5% of the control population).

In agreement with our results, a large-scale genome-wide association analysis demonstrated that common variants in or near the MTNR1B gene are associated with fasting glucose levels in European populations [8].

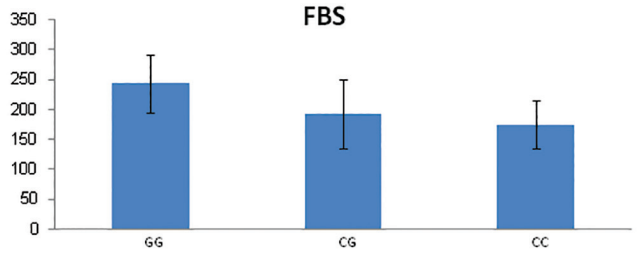
Our results are similar to several studies, including Sparsø *et al.* (Europe) [19], Chambers *et al.* (India) [20], and Takeuchi *et al.*(Sri Lankan and Japanese populations) [21], who confirmed that MTNR1B rs10830963 contributed to increased fasting glucose levels and an increased risk of type 2 diabetes.

Our results are similar to those of Rönn *et al.* [22], who found the association of MTNR1B rs10830963 with type 2 diabetes and fasting glucose in a case–control study including 1165 type 2 diabetes patients and 1105 normal glycemic controls.

Our results are also in agreement with Xia *et al.* [23], who found that that the rs10830963 polymorphism of MTNR1B is a risk factor for developing type 2 diabetes. In the stratified analysis by ethnicity, significant associations were found in Caucasians for the polymorphism in all genetic models. However, in contrast to our results, no significant associations were detected among East Asian and South Asian populations for rs10830963 and rs1387153 polymorphisms.

Furthermore, Lyssenko *et al.* [24] confirmed the presence of MTNR1B in human pancreatic islets and showed increased MTNR1B mRNA expression in carriers of the rs10830963 risk genotype, reporting a negative correlation between MTNR1B mRNA levels and insulin secretion.

Figure 4



Correlation between FBS and G allele.

Our study found a highly positive correlation between the presence of the G allele (MTNR1B gene polymorphism) and the BMI.

Also, Sparsø *et al.* [19] observed differences in the BMI when stratifying according to the rs10830963 among young healthy Danes and among elderly Danish twins.

Our results demonstrated a highly positive correlation between the presence of the G allele (MTNR1B gene polymorphism) and glycated hemoglobin.

Also, Semiz *et al.* [25] have demonstrated the association between the common MTNR1B rs10830963 variation and fasting plasma glucose levels in a BH population. Furthermore, the influence of this polymorphism on the HbA1c level was also shown in this study.

Conclusion

This study confirmed that individuals having the MTNR1B gene polymorphism are at a greater risk of developing type 2 diabetes and having higher blood glucose levels.

Acknowledgements

We appreciate the cooperation of our dear patients. I hope this work offers a chance for a better state of health, which they deserve.

Conflicts of interest

There are no conflicts of interest.

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