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Melatonin receptor 1A *(MTNR1A)* gene linkage and association to type 2 diabetes in Italian families

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Abstract. – OBJECTIVE: Melatonin regulates the mammalian circadian rhythm and plays metabolic functions such as glucose homeostasis. Both melatonin receptors (MTNR1A and MTNR1B, encoded by the *MTNR1A* and *MTNR1B* genes, respectively) are expressed in pancreatic beta cells and mediate the glucometabolic roles of melatonin as well as insulin secretion. The *MTNR1B* gene is a well-known genetic risk factor in type 2 diabetes (T2D); however, little is known about the involvement of the *MTNR1A* gene in here T2D. We aimed to investigate whether *MTNR1A* is linked to and/or associated with familial T2D.

SUBJECTS AND METHODS: We genotyped 14 single nucleotide polymorphisms within the *MTNR1A* gene in 212 peninsular Italian families with T2D. We performed parametric linkage and linkage disequilibrium analyses to investigate the role of *MTNR1A* variants in conferring T2D risk. We considered variants statistically significant if conferring linkage or linkage disequilibrium with $p < 0.05$.

RESULTS: We found 3 novel variants (rs62350392, rs2119883, and rs13147179) significantly linked to and/or associated with T2D in multigenerational Italian families.

CONCLUSIONS: This is the first study to report *MTNR1A* as a novel risk gene in T2D. Functional studies are needed to confirm these results.

Key Words:
Melatonin, Melatonin receptor, Melatonin receptor 1A, MTNR1A, Melatonin receptor 1B, MTNR1B, Pineal gland, Type 2 diabetes, T2D, Metabolic, Risk, Variants, Gestational diabetes mellitus, GDM, Gene, Variant, Single nucleotide polymorphisms, SNP, Statistical, 2-Point parametric, Linkage disequilibrium, Association, Inheritance model, Recessive, Dominant, Complete penetrance: incomplete penetrance, Significant: LD block, Correlated, Uncorrelated, r2, Independent, Italian, Peninsular, Families, Familial, Tuscany, 1000 Genomes project, Glucose homeostasis, Metabolic disease, Mammalian circadian rhythm, Novel, rs62350392, rs2119883, rs13147179, Insulin resistance, SP1 transcription factor, Zinc-finger transcription factor, Krüppel-like factor 5 transcription factor, KLF5, Taurine up-regulated 1, TUG1, Hyperglycemia, Glucometabolic role, Pancreatic, Alpha cells, Glucagon, Pseudomarker, Brain, Skeletal muscle, Adipose tissue, Pancreatic islets, Insulin secretion, Beta cells, Human, Obese, Obesity, Polycystic ovarian syndrome, PCOS, Expressed, Knockout mice, Microarray, Multigenerational, Genotyping, Mendelian, Error exclusion, PLINK, In silico Analysis, Tools, Function prediction, RegulomeDB, SNP2TFBS, Binding, SpliceAI, Splicing disruption, Intronic, GC-Rich motifs, Promoters, Apoptosis, Cardiac, Renal complication, Implication, Significantly, Linked, Associated, study, Studies, Studied, Report, Reported, First, Located, Disrupt, Predicted, Proliferation, Interaction, Target, Adaptation, Mass, In vitro, Regulated, Mediated, Link, Photoreceptor cells, Development, Compromise, Setting, Represent, Brain-islets Circuity, Functional, Confirm, Pathogenesis.

Introduction

Melatonin is known to regulate mammalian circadian rhythm and plays various metabolic functions such as glucose homeostasis[1]. The effects of melatonin are mediated by its two receptors: melatonin receptor 1A (MTNR1A) and melatonin receptor 1B (MTNR1B). The two melatonin receptors, encoded by the *MTNR1A* and *MTNR1B* genes respectively, are expressed in various central and peripheral tissues such as the brain, skeletal muscles, adipose tissue, and pancreatic islets[2], and they play a role in insulin secretion[3]. MTNR1A is predominantly expressed in pancreatic alpha cells while MTNR1B is predominantly expressed in pancreatic beta cells[4]. The *MTNR1B* gene is a well-known ge-

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netic risk factor in type 2 diabetes (T2D)[5-9]. On the other hand, the *MTNR1A* gene has been less studied in humans and its involvement in metabolic disease is not known as well as for *MTNR1B*. However, it is known[10] that *MTNR1A*-knockout mice are obese and have severe insulin resistance. In humans, variants in the *MTNR1A* gene are associated with gestational diabetes[11] and polycystic ovary syndrome[12,13], especially related to obesity[14] and insulin secretion[15]. The *MTNR1A* gene is therefore a gene that potentially confers risk for T2D. Thus, we aimed to fill the gap by exploring whether the *MTNR1A* gene plays a role in the predisposition to T2D by testing peninsular Italian families. In this study, we report for the first time the novel linkage and association of the *MTNR1A* gene with the risk of T2D.

Subjects and Methods

We analyzed 14 microarray-based single nucleotide polymorphisms (SNPs) in the *MTNR1A* gene in 212 multigenerational Italian families with T2D. The families were previously recruited following the Helsinki declaration guidelines. Subjects provided written informed consent. The Bios Ethical Committee approved the study.

Statistical Analysis

Genotyping and Mendelian error exclusion were performed using the toolset PLINK[16] (available at: https://zzz.bwh.harvard.edu/plink/). The SNPs were analyzed for 2-point parametric-linkage to and linkage disequilibrium (i.e., LD, linkage + association) with T2D using the recessive model with complete penetrance (R1). Subsequently, we ran the analyses under the following models: recessive with incomplete penetrance (R2), dominant with complete penetrance (D1), and dominant with incomplete penetrance (D2). We considered significant the analyses reporting $p < 0.05$. LD blocks of the risk variants were computed using the data available in the Tuscany Italian population from the 1000 Genomes Project (available at: https://www.internationalgenome.org/data-portal/population/TSI). Single nucleotide polymorphisms (SNPs) that were significantly correlated ($r^2 \geq 0.9$) were considered to be within the same LD block. Uncorrelated SNPs were labeled "independent".

In Silico Analysis

We analyzed the significant variants for potential functional effects using the following *in silico* tools: SNP function prediction[17], RegulomeDB[18] and SNP2TFBS[19] for transcription-factor binding, and SpliceAI[20] for splicing disruption.

Results

We identified the significant linkage and association of 3 novel intronic variants in the *MTNR1A* gene (rs62350392, rs2119883, and rs13147179) with risk for T2D. Two variants (rs2119883 and rs13147179) were within an LD block (Set01). Detailed information for the statistically significant ($p \leq 0.05$) variants is reported in Table I. Results of the linkage and LD analyses are reported in Figure 1.

The variant rs13147179 affected the binding of SP1 transcription factor (TF), a zinc-finger TF which binds the GC-rich motifs of many promoters[21]. SP1 is involved in the apoptosis of pancreatic islets cells in T2D[22]. Also, the variant rs13147179 affected the binding of the TF Krüppel-like factor 5 (KLF5), which is associated with cardiac and renal complications of T2D[23,24].

Discussion

MTNR1A and MTNR1B are expressed in pancreatic alpha and beta cells respectively[4] and mediate the glucometabolic roles of melatonin[25]. The association of the *MTNR1B* gene with T2D has been documented in several reports[4-8] while the *MTNR1A* gene has only been linked to gestation-

Table I. *MTNR1A* type 2 diabetes risk single nucleotide polymorphisms..

Model[1]	SNP	Position	Ref	Alt	Risk Allele	Consequence	LD Block	Reported in T2D?
D1	rs62350392	186544982	G	A	G	Intronic	Independent	Novel
R1, R2	rs2119883	186547921	C	T	C	Intronic	Set01	Novel
R1	rs13147179	186554365	G	A	A	Intronic	Set01	Novel

[1]Models: D1: dominant complete-penetrance, R1: recessive complete-penetrance, R2: recessive incomplete-penetrance.

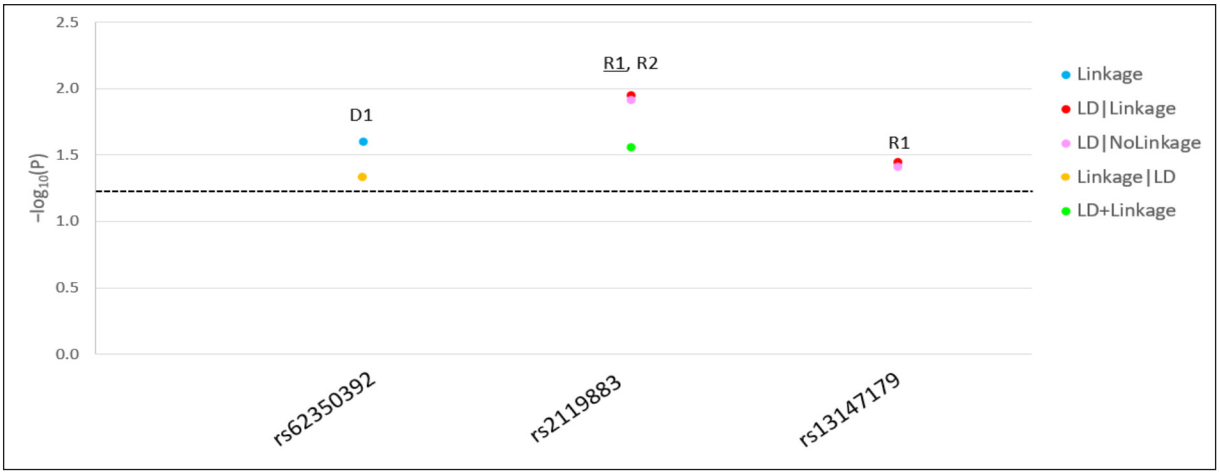

Figure 1. Linkage and Linkage Disequilibrium (i.e., Association) of *MTNR1A* type 2 diabetes risk single nucleotide polymorphisms. For each *MTNR1A*-risk SNPs in T2D, we present the -$\log_{10}$(P) as a function of each significant test statistic (Linkage, LD Linkage, LD NoLinkage, Linkage LD, and LD+Linkage) per inheritance model: D1: dominant, complete penetrance, R1: recessive, complete penetrance, R2: recessive, incomplete penetrance. The most significant model is underlined.

al diabetes[11]. In this study, we report for the first time the novel implication of the *MTNR1A* gene in the risk of T2D. We identified 3 novel variants in the *MTNR1A* gene that are significantly linked to and/or associated with the risk of T2D in multigenerational Italian families. The *MTNR1A* gene has been studied in gestational diabetes, but the association has been found in some studies[11] but not others[26]. This is therefore the first study to report *MTNR1A* as a novel risk gene in T2D. Two of the three risk variants in our study are located in an LD block (Set01); the risk allele (A) of the Set01 variant rs13147179 was predicted to disrupt the binding of transcription factors SP1 and KLF5, which are associated, respectively, with T2D and its complications[22-24]. As the TF SP1 regulates the proliferation and apoptosis of pancreatic beta cells in T2D *via* its interaction with the taurine up-regulated 1 (TUG1)[22], the disruption of its binding to target genes (e.g., *TUG1*)[22] might compromise the adaptation of beta cells mass to hyperglycemia[27,28]. Furthermore, as SP1 is regulated by glucagon in *in vitro* cells, and MTNR1A is predominantly expressed in alpha cells[4], it is possible that MTNR1A, SP1, and TUG1 may play a role within alpha cells and/or in the setting of hyperglycemia mediated by glucagon[29]. Of note, *TUG1* is predicted to act within photoreceptor cells' development (https://www.ncbi.nlm.nih.gov/gene/55000#gene-expression), which might represent a novel link of MTNR1A, SP1, and TUG1 with the circadian rhythm and alpha and beta cells effects, such as a brain-islets' circuity.

Limitations

This study has been conducted in a homogenous monoethnic population and it needs to be replicated in other ethnic groups in order to reach more solid conclusions. Furthermore, functional studies are needed to confirm the implication of the *MTNR1A* gene and its reported variants in the pathogenesis of T2D.

Conclusions

We are the first to report *MTNR1A* as a risk gene for T2D. However, functional studies are needed to confirm the implication of the *MTNR1A* gene and its reported variants in the pathogenesis of T2D.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Ethics Approval

Families were recruited following the Helsinki declaration guidelines. The Bios Ethical Committee approved this study (Prot.PR/Mg/Cg/311708).

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Authors' Contributions
C.G. conceived and supervised the project, including statistical analysis and manuscript drafting. M.A. helped with the bioinformatic analysis, literature search, and manuscript drafting. All authors have approved the final manuscript.

Availability of Data and Materials
The study data are available on reasonable request, and due to lacking specific patients' consent and privacy restrictions, they are not publicly available.

Informed Consent
Subjects provided written informed consent.

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