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The melatonin receptor genes are linked and associated with the risk of polycystic ovary syndrome

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Abstract

Polycystic ovarian syndrome (PCOS) is a genetically complex disorder that involves the interplay of multiple genes and environmental factors. It is characterized by anovulation and irregular menses and is associated with type 2 diabetes. Neuroendocrine pathways and ovarian and adrenal dysfunctions are possibly implicated in the disorder pathogenesis. The melatonin system plays a role in PCOS. Melatonin receptors are expressed on the surface of ovarian granulosa cells, and variations in the melatonin receptor genes have been associated with increased risk of PCOS in both familial and sporadic cases. We have recently reported the association of variants in *MTNR1A* and *MTNR1B* genes with familial type 2 diabetes. In this study, we aimed to investigate whether *MTNR1A* and *MTNR1B* contribute to PCOS risk in peninsular families. In 212 Italian families phenotyped for PCOS, we amplified by microarray 14 variants in the *MTNR1A* gene and 6 variants in the *MTNR1B* gene and tested them for linkage and linkage disequilibrium with PCOS. We detected 4 variants in the *MTNR1A* gene and 2 variants in the *MTNR1B* gene significantly linked and/or in linkage disequilibrium with the risk of PCOS ($P < 0.05$). All variants are novel and have not been reported before with PCOS or any of its related phenotypes, except for 3 variants previously reported by us to confer risk for type 2 diabetes and 1 variant for type 2 diabetes-depression comorbidity. These findings implicate novel melatonin receptor genes' variants in the risk of PCOS with potential functional roles.

Keywords Polycystic ovarian syndrome, PCOS, Melatonin, Melatonin receptor, Melatonin receptor 1B, *MTNR1B*, Melatonin receptor 1A, *MTNR1A*, Gene, Variant, Linkage disequilibrium, Association, Ovary

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Background

Melatonin is a pineal hormone known for its role in the regulation of circadian [1] and seasonal rhythms [2], in addition to glucose and lipid metabolism [3, 4] with a role in obesity [5], anti-inflammation and antioxidation [6–8].

Melatonin exerts its roles through two melatonin G protein-coupled receptors: melatonin receptor 1 A (encoded by *MTNR1A* gene) and melatonin receptor 1B (encoded by *MTNR1B* gene) [9, 10]. The two receptors are expressed on the nervous system, pancreas, liver, skeletal muscle, adipose tissue, and ovaries [6, 11]. The two melatonin receptors have been implicated in the risk of mental and metabolic disorders such as type 2 diabetes (T2D) [12–14] and depression (MDD) [14, 15].

Of interest, polycystic ovarian syndrome (PCOS), a complex and common hormonal disorder affecting women of reproductive age and characterized by anovulation, hyperandrogenism, and polycystic ovaries, is commonly associated with type 2 diabetes (T2D) [16] and mental traits, including anxiety [17, 18] and depression [19, 20]. PCOS, which affects approximately 6-18% of women worldwide [21] and can lead to long-term health consequences, including infertility, metabolic syndrome, cardiovascular disease, is a genetically complex disorder that involves the interplay of multiple genes and environmental factors [22]. PCOS is linked to a variety of possible pathogenetic impairments, distinct or overlapping, including the neuroendocrine pathways, and ovarian and adrenal hormonal secretions [23]. The circadian rhythm and melatonin system have been implicated in PCOS [24]. Candidate gene studies have associated PCOS with several genes, including the insulin receptor gene (*INSR*) [25], insulin-like growth factor (IGF) system genes, luteinizing hormone (LH) /chorionic gonadotropin receptor gene (*LHCGR*) [26], genes involved in androgen biosynthesis and steroid hormone metabolism (*CYP11*) [25], and the melatonin receptor genes (*MTNR1A* and *MTNR1B*) [27]. Melatonin receptors are expressed on the surface of ovarian granulosa cells [28] and variations in the melatonin receptor genes have been associated with increased risk of PCOS in both familial and sporadic cases [27]. We have recently reported the linkage and linkage plus association of variants in *MTNR1A* with familial type 2 diabetes [13] and in *MTNR1B* [14] with familial type 2 diabetes and type 2 diabetes-depression comorbidity. In this study, we aimed to investigate whether the *MTNR1A* and *MTNR1B* genes are in linkage to and/or linkage disequilibrium (LD, i.e., association joint to linkage) with PCOS in Italian families.

Materials and methods

We originally recruited for a type 2 diabetes (T2D) study 212 Italian families, which were later phenotyped for PCOS according to the phenotypes for PCOS necessary to meet the Rotterdam diagnostic criteria (presence of at least two of these three characteristics: chronic anovulation or oligomenorrhea, clinical or biochemical hyperandrogenism, and/or polycystic ovaries) [29]. We amplified by microarray 14 variants in the *MTNR1A* gene and 6 variants in the *MTNR1B* gene and tested them for linkage and LD with PCOS, using Pseudomarker [30] with dominant and recessive models with complete or incomplete penetrance, after excluding genotyping and Mendelian errors via PLINK [31]. The first tool (Pseudomarker) offers a robust method to simultaneously examine linkage and LD in a combination of family and singleton samples, utilizing the true pedigree relationships without relying on artificial assumptions to rectify linkage effects in statistics [30]. The second tool (PLINK) is a well-known toolset for whole genome association analysis that is both free and open-source, engineered to efficiently conduct various fundamental, large-scale analyses. We used the correlation coefficient of variants with data from the 1000 Genomes Project (https://www.internationalgenome.org/data-portal/population/TSI) to estimate the presence of LD blocks.

In-Silico Analysis. We used different bioinformatics tools to predict the risk variants' roles in transcription factor (TF) binding (SNP Function Prediction) [32], miRNA binding (mirSNP) [33], splicing (SpliceAI) [34], and regulatory potential (RegulomeDB) [35].

Results and discussion

We detected 4 variants in the *MTNR1A* gene and 2 variants in the *MTNR1B* gene significantly linked and/or associated (LD) with the risk of PCOS ($P<0.05$) (Table 1). The variants were significant across different inheritance models (Fig. 1). Two variants (rs2119883 and rs13147179) were within an LD block (Set01). All variants are novel and have not been reported before with PCOS or any of its related phenotypes (i.e., T2D, obesity, insulin resistance, metabolic syndrome, hyperglycemia, oligomenorrhea, anovulation, irregular menses, hyperandrogenism, MDD, male-pattern baldness, acne, hirsutism, infertility), excluding T2D for 3 risk variants and T2D-MDD for 1 risk variant. Within peninsular families, the same risk alleles of the two variants (*MTNR1B*-rs61747139 and *MTNR1A*-rs2119883) were previously linked to and associated with the risk of T2D [13, 14], confirming the interrelatedness of these complex phenotypes. On the other hand, the non-risk alleles of the two variants (*MTNR1A*-rs13147179 and *MTNR1B*-rs4601728) were linked and associated with T2D and T2D-MDD comorbidity respectively [13, 14], indicating multiple association at the allelic level and possibly the presence of LD with other contributing yet undetected variants. Via our bioinformatic analyses, we found that the risk allele (A) of the variant *MTNR1A*-rs13147179 disrupts the binding

Table 1 Polycystic Ovarian Syndrome (PCOS) *MTNR1A*- and *MTNR1B*-Risk Single Nucleotide Polymorphisms (SNPs)

Gene	Model[1]	SNP	Position	Ref	Alt	Risk Allele	Consequence	LD block	Reported in PCOS or related phenotype?[2]
MTNR1A	D1, D2, R1, R2	rs6820205	186,543,713	T	C	C	Intronic	Independent	Novel
	D1, D2, R1, R2	rs2119883	186,547,921	C	T	T	Intronic	Set01	T2D[3] [13]
	D1	rs4862706	186,552,540	G	A	A	Intronic	Independent	Novel
	D1, D2	rs13147179	186,554,365	G	A	A	Intronic	Set01	T2D[3] [13]
MTNR1B	D1, R1	rs4601728	92,971,992	A	G	G	Intronic	Independent	T2D-MDD[4] [14]
	D1, D2, R1	rs61747139	92,981,951	A	G	G	Missense	Independent	T2D[3] [14]

[1]Models: D1: dominant, complete penetrance, D2: dominant, incomplete penetrance, R1: recessive, complete penetrance, R2: recessive, incomplete penetrance. [2](i.e., type 2 diabetes, obesity, insulin resistance, metabolic syndrome, hyperglycemia, oligoamenorrhea, anovulation, irregular menses, hyperandrogenism, male-pattern baldness, acne, hirsutism, infertility). [3]T2D=type 2 diabetes, [4]MDD=major depressive disorder)

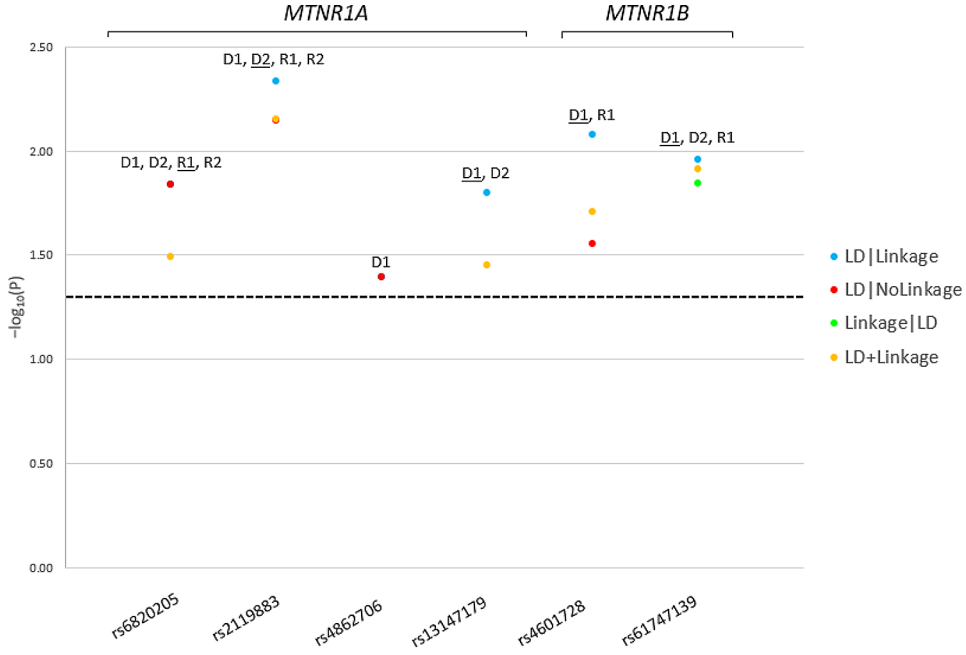

Fig. 1 Parametric Analysis Results of Polycystic Ovarian Syndrome (PCOS) *MTNR1A*- and *MTNR1B*-Risk Single Nucleotide Polymorphisms (SNPs)

of Kruppel-like factor 5 (KLF5) which is hypomethylated in the ovarian tissue in PCOS [36], potentially extending the role of this variant to epigenetic mechanisms. We also found that the risk allele (G) of the variant *MTNR1B*-rs61747139 affects the binding of transcription factor AP2A (TFAP2A) which is expressed in the brain, liver, pancreas, and ovaries [37] and forms a part of the signaling network of PCOS at least in vitro [38]. PCOS patients in our study could therefore be at higher risk due to altered expression of genes in PCOS pathways. Our study therefore implicates novel melatonin receptor genes' variants in the risk of PCOS with potential functional roles. It also offers the possibility of inhibitors of melatonin metabolism (e.g., coumarins [39]) as novel therapeutic modalities in the treatment of PCOS. However, more studies are needed to validate these results.

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Author contributions
C.G. (https://orcid.org/0000-0002-3873-6617) conceived and performed the study and critically revised the manuscript. T.T.P. (https://orcid.org/0000-0001-6056-4244) helped with the data interpretation and manuscript critical revision. Q.M.A.T drafted the manuscript and helped with literature search.

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

Declarations

Ethics approval
Families were recruited following the Helsinki declaration guidelines, and individuals provided written informed consent prior to participation. The Bios Ethical Committee approved this study (Prot.PR/Mg/Cg/311,708).

Competing interests
The authors declare no competing interests.

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