Journal of Ovarian Research

BRIEF REPORT Open Access

The melanocortin receptor genes are linked to and associated with the risk of polycystic ovary syndrome in Italian families

Rongling Wu[1,2] and Claudia Gragnoli[1,3,4*]

Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder occurring in women of reproductive age. The disease is caused by a complex interplay of genetic and environmental factors including genes encoding components of the hypothalamic-pituitary-adrenal (HPA) axis. We have recently reported the association of melanocortin receptor genes (*MC1R*, *MC2R*, *MC3R*, *MC4R*, and *MC5R*) with the risk of type 2 diabetes (T2D) and/or major depressive disorder (MDD). The latter 2 disorders are comorbid with PCOS. In this study, we used microarray to test 12 single nucleotide polymorphisms (SNPs) in the *MC1R* gene, 10 SNPs in the *MC2R* gene, 5 SNPs in the *MC3R* gene, 6 SNPs in the *MC4R* gene, and 4 SNPs in the *MC5R* gene in 212 original Italian families with PCOS. We identified 1 SNP in *MC1R*, 1 SNP in *MC2R*, 2 SNPs in *MC3R*, and 2 SNPs in *MC5R* significantly linked and/or associated to/with the risk of PCOS in Italian families. This is the first study to report the novel implication of melanocortin receptor genes (*MC1R*, *MC2R*, and *MC5R*) in PCOS. *MC3R* and *MC4R* were previously reported in PCOS. However, functional studies are needed to validate these results.

Keywords Polycystic ovary syndrome, PCOS, Melanocortin receptor gene, Melanocortin receptor 1 gene, MC1R, Melanocortin receptor 2 gene, MC2R, Melanocortin receptor 3 gene, MC3R, Melanocortin receptor 4 gene, MC4R, Melanocortin receptor 5 gene, MC5R, Hypothalamic-pituitary-adrenal axis, HPA axis, Linkage disequilibrium, LD, Association, Novel, Variant, Single nucleotide polymorphism, SNP, Microarray, Type 2 diabetes, Depression, Anxiety, Insomnia

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, characterized by hyperandrogenism, irregular menses, anovulation, and polycystic ovaries. It is associated with an increased risk of infertility [1], type 2 diabetes (T2D) [2], hypertension [3], depression, anxiety [4, 5], and insomnia [6], and insulin resistance (IR) [7], which may be linked to impaired stress response [8]. In addition to metabolic and hormonal factors, the neuroendocrine system plays an essential and central role in the pathophysiology of PCOS and remains up-to-date in recent research. An imbalance in the pattern of gonadotropin-releasing hormone

*Correspondence:
Claudia Gragnoli
claudia.gragnoli@gmail.com
[1]Department of Public Health Sciences, Penn State College of Medicine, Hershey, PA 17033, USA
[2]Department of Statistics, Penn State College of Medicine, Hershey, PA 17033, USA
[3]Division of Endocrinology, Department of Medicine, Creighton University School of Medicine, Omaha, NE 68124, USA
[4]Molecular Biology Laboratory, Bios Biotech Multi-Diagnostic Health Center, Rome 00197, Italy

production can lead to a disruption in the hypothalamic-pituitary-ovarian or adrenal axis, which in turn has been associated with the development of PCOS [9].

The hypothalamic-pituitary-adrenal (HPA) axis, which regulates stress response [10], shows increased activation, pro-inflammatory mediators, and psychological distress and maladaptive stress-driven HPA axis activation in women with PCOS [11]. HPA dysfunction is implicated in the metabolic and inflammatory aspects of PCOS, including IR [12, 13].

In some publications on PCOS, genetic mechanisms are particularly emphasized, and it is stated that these genetic changes may be related to some metabolic consequences of PCOS [13, 14]. Genes potentially contributing to HPA axis-related predisposition to T2D, major depressive disorder (MDD), and possibly PCOS include the corticotropin-releasing hormone receptors (*CRHR1* and *CRHR2*) [15, 16], melanocortin receptors (*MC1R*–*MC5R*) [17], glucocorticoid receptor (*NR3C1*) [18], and mineralocorticoid receptor (*NR3C2*) [19]. Melanocortin receptor genes encode key feeding and metabolic regulators (*MC3R*, *MC4R*, and *MC5R*) [20–23] and components of the HPA axis (*MC2R*) [24]. While *MC1R* is primarily known for its role in skin pigmentation [25], it also contributes to the inflammatory response [26] and obesity [27]. The *MC4R* gene is overexpressed in the hypothalamus of PCOS rat models [28], and variants in *MC3R* and *MC4R* have been identified in Turkish and Chinese individuals with PCOS [29, 30]. Mutations or variations in the *MC4R* gene have also been associated with obesity [31], a common comorbidity in PCOS [32]. The *MC4R* gene may influence the development of insulin resistance [33] and hyperandrogenism [34], key features of PCOS [35], by affecting metabolic pathways that regulate glucose and lipid metabolism [36, 37]. Dysfunction in the melanocortin signaling pathway can, therefore, exacerbate the metabolic and reproductive abnormalities seen in women with PCOS. This study aims to investigate the association of melanocortin receptor genes (*MC1R*–*MC5R*) with PCOS in Italian families.

Materials and methods

We used microarray to test 12 single nucleotide polymorphisms (SNPs) in the *MC1R* gene, 10 SNPs in the *MC2R* gene, 5 SNPs in the *MC3R* gene, 6 SNPs in the *MC4R* gene, and 4 SNPs in the *MC5R* gene in 212 original Italian families with familial history of T2D. The average age at T2D diagnosis was 47.85 years, ranging from 7 to 81, with a median age of 41. The male-to-female ratio was 1.04:1, and the average family size was 5.45. The families were additionally diagnosed with PCOS according to the Rotterdam diagnostic criteria (presence of at least two of the following three characteristics: chronic anovulation or oligomenorrhea, clinical or biological hyperandrogenism, and/or polycystic ovaries) [38]. To diagnose a subject with PCOS, conditions such as thyroid hormonal disorders, hyperprolactinemia, hypothalamic amenorrhea, and congenital adrenal hyperplasia were ruled out. Additionally, two or more of the following criteria had to be met: chronic anovulation or oligomenorrhea, clinical or biochemical hyperandrogenism, and/or the presence of polycystic ovaries. In the familial dataset with T2D, 11% of families had at least one member diagnosed with PCOS. The average BMI of PCOS patients at age 20 was 24.73 (range 19.53–34.08), with 30% classified as overweight (BMI≥25) and 13% as obese (BMI≥30). The average maximum lifetime BMI for these patients was 32.51 (range 20.57–69.85), with 74% being overweight (BMI≥25) and 39% obese (BMI≥30). The average increase in BMI from age 20 to maximum lifetime BMI was 8.91, with a mean BMI increment of 1.36. The SNPs were selected according to their genomic positions as well as minimum allele frequency (MAF) to ensure adequate representation of the gene. The average MAF was 0.06. Families were recruited based on Italian-only ancestry for at least 3 generations, inherited familial T2D, and characterized for additional phenotypes (e.g., PCOS) and traits (e.g., blood pressure). After the exclusion of Mendelian inheritance errors and/or uncertain paternity via the PLINK software [39], we tested the SNPs via the Pseudomarker software [40] for parametric linkage to and/or linkage disequilibrium (LD) – the latter testing linkage and association – with PCOS via the recessive model with complete penetrance (R1) and incomplete penetrance (R2). We then ran a secondary analysis under the dominant models with complete (D1) and incomplete penetrance (D2). The recessive and dominant models were selected based on the genetic architecture of the SNPs studied, as these models can help capture potential associations with PCOS by accounting for different modes of inheritance. We used these models to explore the effects of SNPs under varying genetic assumptions, enhancing the robustness of our findings. PLINK was used for its efficiency and widespread application in genetic studies' quality control and data management, making it particularly suitable to run quality checks and exclude bad inheritance, uncertain paternity, and errors in genotypes by also analyzing SNP data within a familial dataset [39]. Pseudomarker was chosen for its ability to perform combined linkage and association analysis, which is valuable in family-based studies, as it allows to leverage both linkage and association information to identify genetic variants potentially contributing to PCOS [40]. Tested variants were computed for the presence of LD blocks using correlations from SNPs available in the Toscani Italian population from the 1000 Genomes Project (https://www.internationalgenome.org/data-portal/population/TSI).

Table 1 Polycystic ovary syndrome (PCOS) melanocortin receptor genes risk single nucleotide polymorphisms (SNPs)

Gene	Model[1]	SNP	Position	REF	ALT	Risk allele	Consequence	LD block	Reported?
MC1R	D1,R1,R2	rs1805005	16:89919436	G	T	G	Missense (p.R151G)	Independent	T2D [14]
MC2R	R1,R2	rs28926173	18:13886720	G	A	A	Intronic	Independent	Novel
MC3R	D2,R1	rs3746619	20:56248749	C	A	C	5′-UTR	Set01	T2D [14]
	D2,R1,R2	rs3827103	20:56248973	G	A	G	Missense (p.V44I)	Set01	T2D [14]
MC5R	D2	rs59999658	18:13824728	T	G	T	Intronic	NA	Novel
	D1,R1	rs2236700	18:13826392	C	G	C	Missense (p.F209L)	Independent	MDD [14]

Legend: [1]Models: D1: dominant, complete penetrance, D2, dominant, incomplete penetrance: R1: recessive, complete penetrance, R2: recessive, incomplete penetrance. LD: linkage-disequilibrium block

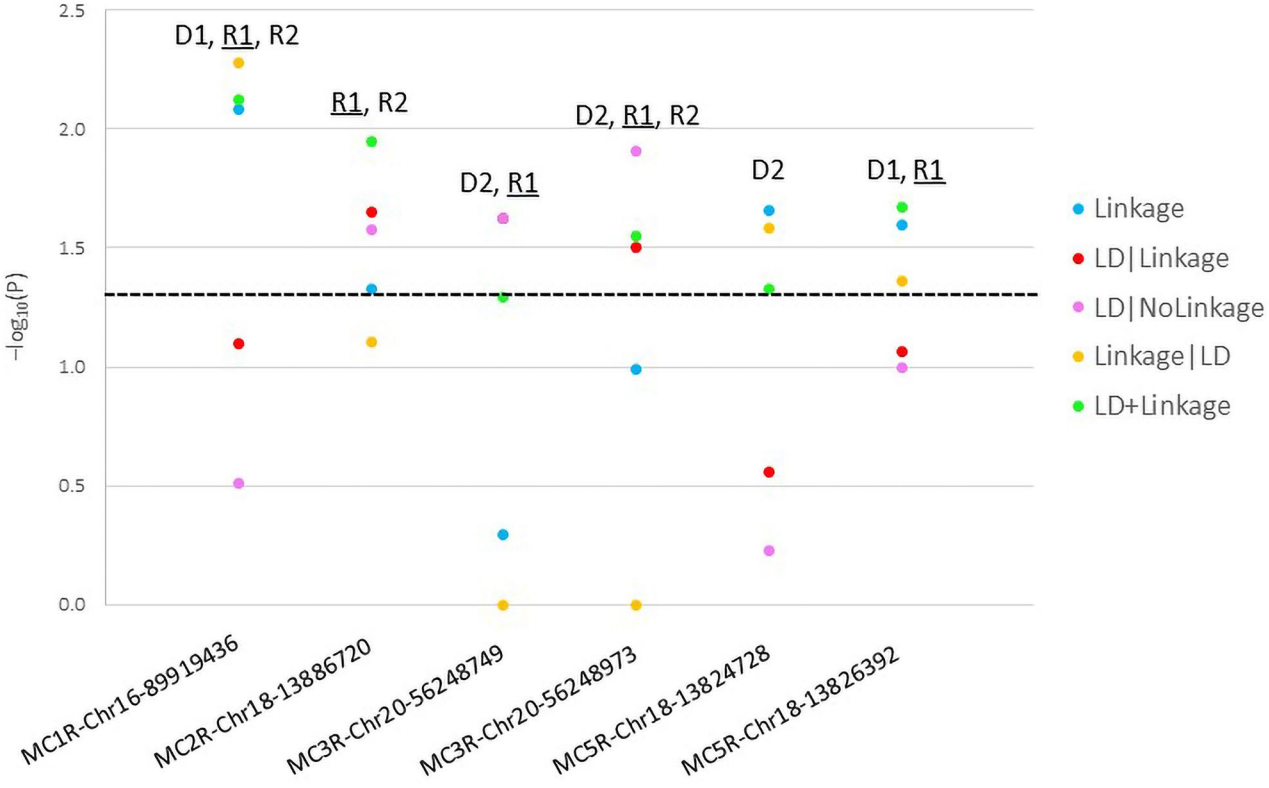

Fig. 1 Parametric analysis results of melanocortin receptor genes risk single nucleotide polymorphisms (SNPs) in polycystic ovary syndrome (PCOS)

We conducted a bioinformatic analysis to predict the significant variant's role in the expression or function of the corresponding proteins (transcription-factor binding (SNP2TFBS [41]), splicing (SNP-function prediction [42]), miRNA binding (mirSNP [43]), and regulatory potential (RegulomeDB [44]). We selected these tools because they are specifically designed to predict the functional impact of SNPs on transcription-factor binding sites (SNP2TFBS [41] and RegulomeDB [44]), and miRNA target sites (mirSNP [43]), and splicing (SNP-function prediction [42]) These analyses are particularly relevant for our study, given the focus on understanding how genetic variants in melanocortin receptor genes might affect gene regulation and contribute to PCOS pathogenesis.

Results

We identified 1 SNP in *MC1R*, 1 SNP in *MC2R*, 2 SNPs in *MC3R*, and 2 SNPs in *MC5R* significantly linked and/or associated to/with the risk of PCOS in Italian families (Table 1; Fig. 1). Table 1 summarizes the genetic variants studied, their positions, alleles, significant models, potential effects, presence or absence of LD block, and whether they have been previously documented, providing a detailed snapshot of the genetic factors considered in this research. Figure 1 presents the results of the parametric analysis of the risk-associated SNPs in melanocortin receptor genes related to PCOS. None of the variants in *MC4R* gene was significant. The two SNPs in the *MC3R* gene were in an LD block (Set01). None of the detected risk variants was previously reported with the risk of PCOS. However, the same risk allele of

the variants *MC1R*-rs1805005, *MC3R*-rs3746619, and *MC3R*-rs3827103 were previously associated with the risk of T2D, and the same risk allele of the variant *MC5R*-rs2236700 was previously associated with the risk of MDD in the same Italian families in this study [17].

Discussion

Our findings highlight the potential role of melanocortin receptor genes in PCOS, building on existing evidence of their involvement in other metabolic and psychological disorders due to their wide tissue distribution and pleiotropic roles [45] The melanocortin receptor genes (*MC1R*, *MC2R*, *MC3R*, *MC4R*, and *MC5R*) have been studied in various mental, metabolic, and endocrine disorders [46–48]. We have recently reported the implication of melanocortin receptor genes in the risk of T2D and MDD [17]. In this study, we report the additional implication of melaocortin receptor genes in the susceptibility to PCOS. We reported single variants in each of *MC1R* and *MC2R* genes, and two variants in each of *MC3R* and *MC5R* genes significantly linked to and associated with PCOS in Italian families. Only the *MC3R* and *MC4R* genes were previously reported in PCOS [29, 30]. Therefore, the association that we report in this study of *MC1R*, *MC2R*, and *MC5R* genes with PCOS is novel. Variants in these genes, however, were previously reported in patients with obesity [27, 49] and T2D [17]. Interestingly, no *MC4R* variant was associated with PCOS in our study despite its well-known involvement in several metabolic derangements such as obesity [49], T2D [50], BMI in PCOS patients [51]. The statistically non-significant association between *MC4R* and PCOS in our study does not constitute a contradiction but reflects a lack of replication within our dataset.

Comparing our results with studies from other populations reveals both overlaps and unique findings, suggesting potential genetic and environmental interactions influencing PCOS phenotypes [52]. For instance, studies in Asian and Middle Eastern populations have identified other gene variants associated with PCOS, such as *FTO* and *CAPN10*, reflecting distinct genetic risk profiles [53, 54]. Further research in diverse cohorts will be critical to validate our findings and understand the broader applicability of melanocortin receptor gene involvement in PCOS.

The functional role of the detected risk variants reported in our study is yet to be determined. No results were predicted frr in-silico functional analysis. Interestingly all risk variants were mostly significant across the R1 model (except for *MC5R*-Chr18-13824728 [rs59999658]) (Fig. 1). This suggests that the disease mechanism is probably related to abnormal receptor density mediated by the recessive genotypes. Dysfunctional adrenal melanocortin receptor 2 could also possibly divert cellular steroids towards excess androgen production [55]. However, functional studies are needed to confirm these results.

Our study has potential therapeutic implications. A recent study has shown that metformin, which is effective in PCOS by improving metabolic control and ovulatory cycles [56], acts specifically through MC2R and MC3R, potentially mediating an anti-androgenic and weight control effect [57]. However, for clarity, the effect mediated by metformin has not been investigated in the MC2R and MC3R variants identified in our study. Understanding the genetic predisposition involving melanocortin receptors could guide personalized treatment strategies, especially targeting specific pathways affected by these genes. For example, therapies enhancing *MC2R* function could potentially modulate androgen excess, a core feature of PCOS.

However, our study has limitations. The sample size and familial recruitment approach may limit the robustness of associations observed to this Italian peninsular familial dataset, and replication in larger and ethnically diverse populations is warranted. Additionally, the lack of functional validation of the implicated variants restricts our understanding of their functional role in PCOS pathophysiology. Future studies should aim to explore these genetic associations in functional models and across varied populations to establish a more comprehensive picture of the role of melanocortin receptor genes in PCOS.

Acknowledgements

We thank the families who participated in the study, and we thank Bios Biotech Multi-Diagnostic Health Center, Rome, Italy, for data access and for financial, medical, and laboratory staff support.

Author contributions

C.G. conceived and supervised the project, including statistical analysis and manuscript drafting. R.W. critically helped in data interpretation and critical revision of the manuscript. All authors have approved the final manuscript.

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

Competing interests

The authors declare no competing interests.

Received: 21 October 2023 / Accepted: 26 November 2024
Published online: 05 December 2024

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