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Oxytocin receptor (*OXTR*) is a risk gene for polycystic ovarian syndrome

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Abstract. – OBJECTIVE: Oxytocin (OXT) controls appetite, promotes diet-induced energy expenditure, and may protect against obesity. Furthermore, the oxytocin system controls ovarian follicle luteinization and steroidogenesis as well as adrenal steroidogenesis, which if impaired might lead to anovulation and hyperandrogenism, signs found in women with polycystic ovarian syndrome (PCOS). PCOS is a common complex endocrine disorder of reproductive-age women, and it often presents with impaired glucose metabolism, insulin resistance (IR), and type 2 diabetes (T2D). The oxytocin receptor gene (*OXTR*) may confer a risk for PCOS, conceivably through dysregulation of metabolism, ovarian follicle maturation, and ovarian and adrenal steroidogenesis. Therefore, we aimed to investigate whether *OXTR* variants confer risk for PCOS.

SUBJECTS AND METHODS: In 212 Italian subjects with T2D and PCOS, we have analyzed 22 single nucleotide polymorphisms (SNPs) within the *OXTR* gene for linkage to and/or linkage disequilibrium (LD, i.e., association) with PCOS. We tested whether the significant risk variants were independent or part of an LD block.

RESULTS: We found 5 independent variants significantly linked to/in LD with PCOS within the peninsular families.

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

Key Words:
Oxytocin, OXT, Oxytocin receptor, *OXTR*, Gene, Polycystic ovary syndrome, PCOS, Cortisol, Hypothalamic-pituitary-adrenal axis, HPA-axis, Metabolic, Insulin resistance, IR, Obesity, Type 2 diabetes, T2D, Families, Familial, Peninsular, Italy, Italian, Parametric analysis, Linkage disequilibrium, Association, Single nucleotide polymorphisms, SNP, Risk, Variant, Hyperandrogenism, Irregular menses, Subfertility, Folliculogenesis, Fat metabolism, Ethnic group, Appetite, Energy expenditure, Estrous cycle length, Ovarian, Follicle luteinization, Maturation, Metabolism, Adrenal, Steroidogenesis, Anovulation, Hyperandrogenism, Endocrine, Disorder, Women, Reproductive age, Impaired glucose metabolism.

Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrine disease in women of reproductive age[1] with a worldwide prevalence of 5% to 10%[2]. It most commonly presents with oligomenorrhea (occurring in 70%-80% of women with PCOS) and failure to conceive[3], which accounts for 90%-95% of women visiting fertility clinics for anovulation[4]. Hyperandrogenism is a defining feature, manifesting clinically with male-pattern baldness, acne, or hirsutism[5] and biochemically with increased free androgen indexes[6].

As a complex heterogeneous disorder, PCOS has multifactorial pathogenesis including environmental and genetic factors[7]. Sedentary lifestyle and obesity contribute to manifestation and exacerbation of PCOS[8]. PCOS is correlated with metabolic derangements[9]; importantly, impaired glucose metabolism, insulin resistance[10], and type 2 diabetes (T2D) are associated features[11]. PCOS also confers increased cardiovascular morbidity and mortality[12]. In particular, insulin resistance is considered an important factor for hyperandrogenic anovulation in both lean and obese women with PCOS[13]. Of interest, the peptide oxytocin

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(OXT), which plays a role in sexuality, labor, and lactation[14], controls appetite, promotes diet-induced energy expenditure[15,16], and may protect against obesity[17]. OXT serum levels are low in infertile women with PCOS and in PCOS rat models[18,19]. Administration of OXT improves food intake and fat mass in PCOS rat models[19] and lipid and insulin profiles in high-fat diet mouse models[20].

OXT mediates its effects through the oxytocin receptor (OXTR), which is widely expressed in the human body, including the brain, and importantly the hypothalamus, ovary, adipose tissue, liver, and pancreas[21,22]. The oxytocin system, among several functions, including anti-stress and pair-bonding enhancement, controls estrous cycle length, ovarian follicle luteinization and steroidogenesis, and adrenal steroidogenesis[21]; the latter two, if impaired in women, might lead to anovulation and hyperandrogenism of PCOS. Further, *OXTR*-deficient mice develop late-onset obesity[23]. In humans, *OXTR* polymorphisms are associated with increased glucose and insulin levels and susceptibility to T2D[24,25]. Recent data also suggest that OXT *via OXTR* helps regulate obesity, insulin levels, and glucose metabolism[18] through regulation of appetite and energy homeostasis[16], indicating that the *OXTR* gene variants may confer risk for PCOS, conceivably through metabolic beyond ovarian dysregulation. Therefore, we aimed to investigate whether *OXTR* variants are in linkage to and/or linkage disequilibrium (LD, i.e., association) with PCOS in Italian families.

Subjects and Methods

We previously recruited 212 Italian subjects originally ascertained for T2D and subsequentially phenotyped for PCOS according to the PCOS Rotterdam diagnostic criteria (i.e., presence of at least two of these three characteristics: chronic anovulation or oligomenorrhea, clinical or biochemical hyperandrogenism, and/or polycystic ovaries)[26]. All subjects descended from 3 generational Italian families. Individuals were enrolled following the Helsinki declaration guidelines and provided written informed consent. The Bios Ethical Committee approved this study (Prot.PR/Mg/Cg/311708). We amplified 22 single nucleotide polymorphisms (SNPs) within the *OXTR* gene using microarray and excluded Mendelian and genotyping errors using PLINK[27].

In Silico Analysis

We ran *in silico* prediction tools for the potential transcription factor binding alteration (SNPnexus[29], SNP2TFBS[30], RegulomeDB[31]), regulatory potential (RegulomeDB[31]), miRNA binding (mirSNP[32]), and splicing (SNP function prediction[33]).

Statistical Analysis

Via Pseudomarker[28], we tested the SNPs for parametric linkage to and/or LD with PCOS according to the following models: dominant with complete penetrance (D1), dominant with incomplete penetrance (D2), recessive with complete penetrance (R1) and recessive with incomplete penetrance (R2). We considered $p < 0.05$ statistically significant. Variants were tested for being part of LD blocks (correlation coefficient ≥ 0.8) according to the LD matrix of the Tuscany Italian population derived from the 1000 Genomes Project (https://www.internationalgenome.org/data-portal/population/TSI) or were labelled as "independent".

Results

Out of 22 *OXTR*-risk variants tested, 5 independent variants were significantly ($p < 0.05$) linked to/in LD with PCOS (Table I, Figure 1). Specifically, three intronic variants (rs11706648, rs60345038, and rs237900) were linked to PCOS, and one intronic variant (rs35498753) and one synonymous variant (rs237902) were both linked and associated with PCOS. All variants are novel and have not been previously associated with PCOS or any PCOS-related phenotype (i.e., obesity, insulin resistance, hyperglycemia, oligomenorrhea, hyperandrogenism, male-pattern baldness, acne, hirsutism, infertility, anovulation, and irregular menses).

No transcription factor binding was predicted to be altered by the intronic risk alleles. Three of the variants we found to confer risk for PCOS (rs60345038, rs35498753, and rs237900) intersected with repressed chromatin state (i.e., negative *OXTR* gene expression) in the ovaries.

Discussion

In this study, we reported 5 novel *OXTR* variants significantly linked and/or associated with the risk of developing PCOS in multigenerational Italian families. To our knowledge, no previous study has implicated the *OXTR* gene in predisposing to PCOS or one of its three principal features (i.e., chronic anovulation or oligomenorrhea, hy-

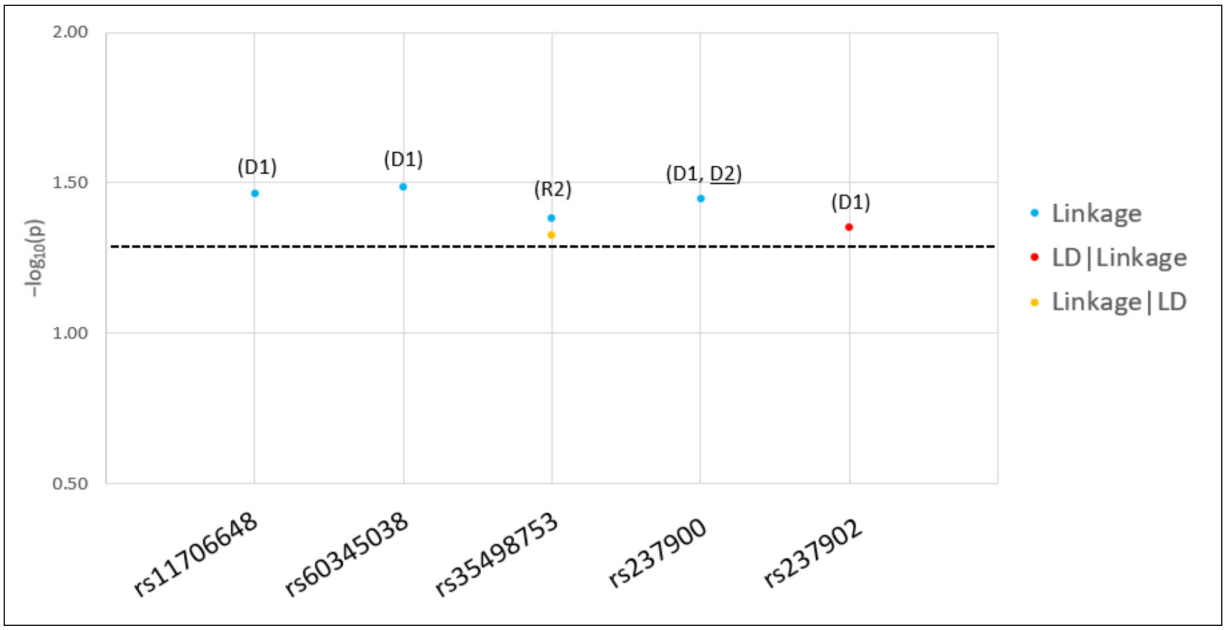

Figure 1. Parametric analysis results of polycystic ovarian syndrome (PCOS) OXTR-risk single nucleotide polymorphisms (SNPs). For each *OXTR*-risk SNP in PCOS, we present the −log10(*p*) as a function of each significant ($p < 0.05$) test statistic [(linkage, linkage disequilibrium (LD) linkage, linkage LD)] and per inheritance model. D1: dominant, complete penetrance, D2: dominant, incomplete penetrance, R2: recessive, incomplete penetrance.

perandrogenism, polycystic ovaries)[26]. We therefore consider *OXTR* as a novel PCOS-risk gene. However, as demonstrated in previous studies[24,25], risk variants in the *OXTR* gene pose increased risk for insulin resistance, T2D, and obesity. The latter metabolic abnormalities lie at the heart of PCOS pathogenesis[13]. Our bioinformatics analyses of the PCOS-risk variants were inconclusive; we did not identify any transcription factor binding impairment mediated by the intronic risk alleles. However, 3 of the risk variants in our study (rs60345038, rs35498753, and rs237900), by intersecting with repressed chromatin state in the ovaries, might confer negative *OXTR* gene expression[31]. Interestingly, this is consistent with the *OXTR* gene-downregulation reported in single-cell transcriptomic analyses of oocytes derived from PCOS patients[34].

Our study has potential therapeutic implications since OXT-administration improves the metabolic profile of PCOS-rat models[19]. Similar metabolic results and potentially resumption of ovulation and cycles regularity could be elicited with the administration of an *OXTR*-agonist in animal models and perhaps human subjects.

Conclusions

This is the first study to report *OXTR* as a novel risk gene in PCOS. Functional and replication studies are needed. However, the reset of the

Table I. Polycystic Ovarian Syndrome (PCOS) OXTR-Risk Single Nucleotide Polymorphisms (SNPs).

Model[1]	SNP	Position	Ref	Alt	Risk Allele	Consequence	LD block	Reported in PCOS?
D1	rs11706648	8754861	A	C	C	Intronic	Independent	Novel
D1	rs60345038	8760830	C	T	T	Intronic	Independent	Novel
R2	rs35498753	8763680	T	G	T	Intronic	NA	Novel
D1, D2	rs237900	8767010	G	A	G	Intronic	Independent	Novel
D1	rs237902	8767498	G	A	G	Synonymous	NA	Novel

[1]Models: D1: dominant, complete penetrance, D2: dominant, incomplete penetrance, R2: recessive, incomplete penetrance.

OXTR action *via* the administration of an *OXTR* agonist has the potential to positively impact prevention and/or improvement of PCOS-related metabolic and cardiovascular morbidity and infertility.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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

Informed Consent
Individuals provided written informed consent prior to participation.

Authors' Contributions
M.A. helped with manuscript drafting and in silico analysis. N.H. drafted the manuscript and helped with literature search. R.W. critically helped in data interpretation and critical revision of the manuscript. C.G. conceived and performed the study and critically revised the manuscript.

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

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