The mineralocorticoid receptor gene (NR3C2) is linked to and associated with polycystic ovarian syndrome in Italian families

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Abstract. – OBJECTIVE: Polycystic ovarian syndrome (PCOS) is a complex heterogeneous disorder characterized by hyperandrogenism, irregular menses, and subfertility and often accompanied by other related comorbid disorders such as insulin resistance, obesity, and type 2 diabetes. Several genetic risk factors predispose to PCOS, but most are still unknown. Up to 30% of women with PCOS may have hyperaldosteronism. Blood pressure and the ratio of blood levels of aldosterone to renin are higher in women with PCOS compared to healthy controls, even if still in the normal range; and the aldosterone antagonist spironolactone has been used as therapy for PCOS, mainly due to its antiandrogenic activity. Thus, we aimed to investigate the potential pathogenetic role of the mineralocorticoid receptor gene (NR3C2) as the encoded NR3C2 product binds aldosterone and plays a role in folliculogenesis, fat metabolism, and insulin resistance.

SUBJECTS AND METHODS: Within 212 Italian families with T2D and phenotyped for PCOS, we analyzed 91 single nucleotide polymorphisms in the NR3C2 gene. We tested the NR3C2 variants for linkage and linkage disequilibrium to the PCOS phenotype by using parametric analysis.

RESULTS: We found 18 novel risk variants significantly linked to and/or associated with the risk of PCOS.

CONCLUSIONS: We are the first to report NR3C2 as a risk gene in PCOS. However, our findings need to be replicated in other ethnic groups in order to reach more solid conclusions.

Key Words: Nuclear receptor subfamily 3 group C member 2, NR3C2, Mineralocorticoid receptor, MR, Polycystic ovary syndrome, PCOS, Cortisol, Hypothalamic-pituitary-adrenal axis, HPA-axis, Metabolic, Insulin resistance, Obesity, Type 2 diabetes, Families, Italian, Parametric analysis, Linkage disequilibrium, Association, Single nucleotide polymorphisms, Risk, Variant, Hyperandrogenism, Irregular menses, Subfertility, Folliculogenesis, Fat metabolism, Ethnic group, Hyperaldosteronism, Blood pressure, Ratio of aldosterone to renin, Aldosterone antagonist, Spironolactone, Antiandrogenic activity, Glucocorticoid.

Introduction

Polycystic ovarian syndrome (PCOS) is a complex heterogeneous disorder affecting 5% to 20% of women of reproductive age worldwide. It is characterized by hyperandrogenism and irregular menses, often accompanied by anovulation and polycystic ovaries, and increased risk for infertility, type 2 diabetes (T2D), hypertension, depression, anxiety, and insomnia. Women with PCOS often have insulin resistance and often are overweight and overweight conditions that pose an augmented burden for cardiovascular disease and mortality.

Genetic and environmental factors predispose to PCOS, although most genetic risk factors are still unknown. Genome-wide association studies (GWAS) have identified many chromosomal loci related to PCOS. The genes involved in the pathogenesis of PCOS with the highest predictable impact seem to be genes encoding for enzymes involved in ovarian and adrenal steroidogenesis, such as CYP11A, CYP17, and CYP19 (aromatase), or genes encoding for hormones involved in folliculogenesis, such as the...
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anti-mullerian hormone. Also, genes involved in insulin signaling are associated with PCOS, such as the insulin receptor and insulin receptor substrate-1 (IRS-1). Furthermore, genes related to inflammation may play a role in the pathogenesis of PCOS; in this regard, interleukin-6 (IL-6) variant is associated with PCOS.

As PCOS is associated with insulin resistance, hypertension, cardiovascular disease, mortality, and depression, the mineralocorticoid receptor [MR, also known as nuclear receptor subfamily 3 group C member 2 (NR3C2)], which is involved in fat metabolism, insulin resistance, hypertension and cardiovascular disease, and whose activation protects, especially females, from human depression, might contribute to PCOS. NR3C2, encoded by the NR3C2 gene, is an almost ubiquitous receptor, similar to the glucocorticoid receptor, and regulates sodium intake and sympathetic efflux to kidney, vessels, and heart. Of note, the NR3C2 agonist fludrocortisone inhibits the hypothalamic-pituitary-adrenal axis. Furthermore, up to 30% of women with PCOS may have hyperaldosteronism; in fact, blood pressure and the ratio of blood levels of aldosterone to renin were found to be higher than in healthy controls, even if within the normal range. More than 50% of women with PCOS have increased cortisolemia. NR3C2 binds aldosterone and glucocorticoids, and the NR3C2 antagonist spironolactone has been widely used as therapy for PCOS, but primarily because of its antiandrogenic activity. Although NR3C2 also clearly participates in folliculogenesis in bovines and humans, its potential contribution to PCOS and/or anovulation is not known. We hypothesized that NR3C2 gene variants might increase the risk for PCOS. Thus, we aimed to investigate the role of NR3C2 in Italian families with T2D phenotyped for the presence or absence of PCOS.

Subjects and Methods

We have investigated collectively 212 original Italian peninsular families with enriched history of T2D and with T2D and diagnosed according to the PCOS 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). Families were Italian from at least three generations. Subjects with uncertain paternity or identical twins were excluded. The study was performed following the Helsinki declaration guidelines. Subjects participating in the study provided written informed consent. The data used were fully deidentified and the Bios Ethical Committee approved the study. We genotyped 91 single nucleotide polymorphisms (SNPs) within the NR3C2 gene using microarray. PLINK tool (available at: https://zzz.bwh.harvard.edu/plink/download.shtml) was used to exclude Mendelian and genotyping errors.

Statistical Analysis

We tested the SNPs via Pseudomarker for parametric linkage to and/or linkage disequilibrium (LD) 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). We considered the cut off $p < 0.05$ as statistically significant.

In Silico Analysis

We performed in silico functional analyses that predicted the potential role of risk variants on transcription factor binding, splicing, and regulatory potential (SNPnexus, SNP2TFBS, RegulomeDB, and mirSNP).

Results

We identified a total of 18 SNPs significantly linked to LD with PCOS ($p < 0.05$) across different models (Table I, Figure 1). Sixteen risk variants are intronic, one variant (rs2070951) is located in the 5'-UTR region, and variant (rs34347300) is a 1 base pair intronic deletion. None of the risk variants have been previously reported with any PCOS-related phenotype (e.g., irregular menses, anovulation, infertility, oligomenorrhea, obesity, insulin resistance, T2D, hyperandrogenism, hirsutism). The rs114608157 SNP was the most significantly associated variant with PCOS (i.e., in both LD and LD+linkage),
under the completely penetrant dominant model (D1) \((p < 0.0002)\).

*In silico* analysis found that the PCOS-risk variant (rs55741361) disrupted the binding of atonal bHLH transcription factor 1 (ATOH1), which is involved in neuronal differentiation\(^8\). All risk variants (except rs28594566 and rs12647354) intersected with a strongly transcribed chromatin state in the ovary\(^46\).
Discussion

The mineralocorticoid receptor is ubiquitously expressed and plays various physiologic and mechanistic roles\(^4^9\). Derangements of the mineralocorticoid receptor’s function are mostly associated with cardiovascular and/or metabolic disease\(^4^9,5^0\). However, NR3C2 mediated pathogenic effects in PCOS might be multifaceted given that up to 30% of women with PCOS may have hyperaldosteronism, that blood pressure and the aldosterone to renin ratio appear higher – even if in the normal range – compared to healthy controls\(^3^5\), that more than 50% have increased cortisolemia\(^3^6\), and that NR3C2 is known to bind aldosterone and glucocorticoids\(^3^7\). Also, the NR3C2 antagonist spironolactone, already used as an antihypertensive therapeutic in PCOS, further highlights the diversified various roles that NR3C2 might play in PCOS pathogenesis\(^3^8\). In a previous analysis, we found NR3C2 as a novel risk gene for T2D, major depressive disorder (MDD), and T2D-MDD comorbidity (unpublished results). In this study, we report for the first time the NR3C2 gene as a novel risk gene for PCOS. We identified 18 risk variants in NR3C2 significantly linked to and/or associated with the risk of developing PCOS in Italian females. The same risk alleles of the PCOS-risk variants (rs6823544, rs28594566, rs114608157, rs55741361, rs17581115, rs79064097, rs3846316, rs72655267, rs4087963, rs80260186, and rs2070951) detected in our current study were previously found in a prior analysis of ours to be significantly linked to and associated with the risk of T2D, MDD, and/or T2D-MDD comorbidity (unpublished results) and the non-risk allele of the variant rs11725509 was previously associated with fat deposition. PCOS, obesity, MDD, and T2D are all related phenotypes that intersect in several pathophysiologic pathways and multimodal predispositions\(^3^,5^,6,1^1\).

NR3C2 gene clearly appears to be a pleiotropic gene with mental-metabolic effects. Most risk variants reported in our study are closely positioned, indicating perhaps the presence of a NR3C2 regulatory-specific risk domain. Of note, all NR3C2 detected PCOS-risk variants, excluding rs28594566 and rs12647354, might regulate ovarian gene transcription as they were found by \textit{in silico} analysis to intersect with a strongly transcribed chromatin state in the ovary\(^4^6\). Interestingly, NR3C2-risk variants in our current study are more strongly associated with PCOS than MDD or T2D, indicating that either PCOS could be the principal driving phenotype or that PCOS complex heterogeneous mental-endocrine-metabolic unified disruption better reveals the power of analysis of pleotropic comorbid genes.

Furthermore, the implication of NR3C2 in PCOS in addition to T2D and MDD could be explained by several mechanisms such as inflammation and insulin resistance; traits shared in all three disorders are known to be implicated in NR3C2 dysfunction\(^2^4,5^2\). Interestingly, according to our previous studies, the rs55741361 comorbid PCOS-risk and MDD-T2D-risk variant (unpublished data) affected the binding of a transcription factor (ATOH1), known to be involved in neuronal differentiation\(^4^8\). Thus, an additional potential pathogenetic comorbid mechanism implies neuronal differentiation as several neurotrophins [e.g., nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF)] are directly implicated in PCOS\(^5^3\), T2D\(^5^4\) and depression\(^5^5\). Potential effects on neuronal differentiation might contribute to mental-metabolic co-pathogenesis\(^5^6,5^7\). This described mental-metabolic-reproductive comorbidity risk might imply, at least in part, a neuro-endocrine nature of PCOS pathogenesis.

Conclusions

We are the first to report NR3C2 as a risk gene for PCOS. However, our findings need to be replicated in other ethnic groups and \textit{in vitro} studies should be performed to elucidate the pathogenic effects of identified PCOS-risk variants.

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. Ethics approval was obtained from the Bios Ethical Committee.

Informed Consent

Patients provided written informed consent prior to participation.
Authors’ Contribution
M.A. helped with manuscript drafting and in silico analysis. M.P. drafted the manuscript and helped with the literature search and data interpretation. 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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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.

References
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42) Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Mallier J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559-575.


