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## Chapter

# Interplay between Oxidative Stress and Chronic Inflammation in PCOS: The Role of Genetic Variability in PCOS Risk and Treatment Responses

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## Abstract

PCOS is often accompanied by insulin resistance, which is associated with the pathogenesis of the syndrome and increases the risk of developing the metabolic syndrome, type 2 diabetes, and cardiovascular complications. All these processes are characterized by chronic inflammation, which may be associated with an increased formation of reactive oxygen species and activation of inflammatory pathways that may further aggravate the function of pancreatic beta-cells. It has been shown that PCOS treatment improves metabolic indexes, while at the same time lowering inflammatory indicators. This chapter summarizes the latest findings about the role of oxidative stress and chronic inflammation in pathogenesis of PCOS. It also provides information on genetic variability in these pathways that may lead to interindividual differences in the risk for PCOS-related metabolic complications. Furthermore, genetic variability in these pathways may influence response to different treatment options in PCOS patients.

**Keywords:** PCOS, insulin resistance, chronic inflammation, oxidative stress, reactive oxygen species, inflammatory pathways, metabolic syndrome

## 1. Introduction

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in women in the reproductive period as it affects between 4 and 12% of the female population. Nowadays, it is known that both genetic and environmental factors play a role in PCOS development and its numerous clinical manifestations. The syndrome is characterized by hyperandrogenism, ovarian dysfunction and polycystic ovaries, insulin resistance (IR), and chronic inflammation, all of which in the long term also affects the post-reproductive period. It is associated with the increased risk for infertility, depression, cardiovascular diseases (CVD), and endometrial and even breast cancer [1].

PCOS develops, when ovaries produce an excessive amount of male sex hormones, androgens, in particular, testosterone and androstenedione. This occurs due to two main reasons. The first one is irregular pulsative secretion of luteinizing hormone (LH) from the adenohypophysis, which then increases the production of androgens. The second mechanism is more complex, and it involves IR with associated increased concentration of serum insulin. Insulin stimulates pathways that activate ovarian androgen production while at the same time lowering serum sex hormone-binding globulin (SHBG) concentrations. Patients also intrinsically have higher serum levels of gonadotropin-releasing hormone, which increases the ratio between the LH and follicle-stimulating hormone (FSH). Higher ratio leads to increased androgen production, slower follicle maturation, and decreased binding of sex hormones on SHBG [2].

The choice of treatment is influenced by the desired goals, which in most cases are the treatment for infertility and obesity and the reduction of symptoms of hyperandrogenism. Clomiphene and letrozole are first-line medications for infertility. If patients do not desire pregnancy, hormonal contraceptives are recommended for irregular menses and dermatologic manifestations. Metformin is the first-line treatment for metabolic manifestations. Glucagon-like peptide 1 (GLP-1)-based therapies and glitazones can be used in most severe cardio-metabolic PCOS phenotypes that are resistant to first-line treatments [3, 4].

For quite some time, the subject of research has also been the involvement of chronic inflammation and oxidative stress (OS) in the pathogenesis of PCOS. Several studies have already confirmed that patients have elevated levels of OS and inflammatory mediators and, on the other hand, have decreased antioxidant capacity. There is also an unanswered question of the extent to which OS and inflammation are causally related.

Research has already confirmed that genetic changes in key antioxidant enzymes and inflammatory mediators can affect the individual's defense ability against OS and their predisposition to inflammation. Nowadays, molecular genetic analysis is increasingly being used in clinical practice as it is a widely available method, easily accessible and inexpensive. It also enables identification of groups of patients with an increased risk of developing various diseases and offers a patient-adapted treatment, thereby representing one of the pillars of personalized medicine.

## **2. Chronic inflammation in PCOS**

Inflammation is arguably involved to some degree in the underlying causes of many chronic diseases. It is well known that different types of inflammatory cytokines and chemokines are involved in female reproductive processes, including ovulation, follicular development, fertilization, implantation, and pregnancy [5]. While the exact etiology of PCOS still remains unknown, the amount of evidence in support of an important role of chronic low-grade inflammation in this process is increasing. Chronic proinflammatory state in women with this syndrome is also likely to be associated with other clinical manifestations and complications of PCOS, including IR and CVD.

When studying the relationship between PCOS and chronic inflammation, it is impossible to completely disregard the influence of increased body mass index (BMI) in patients with increased inflammation markers. Similar to PCOS, low-grade chronic inflammation is also present in metabolic syndrome (MS) [6]. This proinflammatory state may be an important part of the pathogenesis of both

syndromes and one of the reasons why they often overlap. The relationship between them is mutual: PCOS women have a higher prevalence of MS, and alternatively, women with MS commonly present the reproductive and endocrine traits of PCOS. The visceral adipose tissue plays a key role in this overlap. It is more abundant and metabolically active in both syndromes, which results in an increased turnover of free fatty acids and an excessive secretion of several molecules, some of which are inflammatory markers [7]. There is still not enough evidence to conclude whether the proinflammatory state is intrinsic to PCOS, or it is rather only a consequence of higher amounts of dysfunctional adipose tissue in those patients. In favor of the latter theory are many studies which show that the higher the BMI in PCOS women, the greater the inflammatory state. However, the fact that PCOS women with lower BMI have more inflammation than healthy controls with higher BMI indicates that chronic low-grade inflammation may not be dependent on increased body mass only [8].

We can find the same degree of uncertainty in the relationship between chronic inflammation and excessive androgen secretion in PCOS. It is unclear whether androgen excess in PCOS promotes proinflammatory state or conversely whether the inflammatory molecules stimulate ovarian androgen production and hyperandrogenemia. Androgens influence the expression of different proteins and enzymes in adipocytes and cause their hypertrophy. Also, hyperandrogenism in PCOS increases mononuclear cell sensitivity to ingested glucose and promotes activation of mononuclear cells. This leads to another important concept in PCOS: diet-induced inflammation. Studies have shown that glucose ingested in vivo, as well as glucose exposure in vitro, stimulates an inflammatory response by promoting the mononuclear cells to release tumor necrosis factor alpha (TNF $\alpha$ ) and interleukin 6 (IL-6) [9]. Furthermore, infiltration of ovarian tissue by macrophages and increased concentrations of TNF $\alpha$  and IL-6 in the follicular fluid (FF) have been previously demonstrated in women with PCOS. It is possible to suspect that mononuclear cells recruited into the polycystic ovaries cause a local inflammatory response that stimulates CYP17, the ovarian steroidogenic enzyme, responsible for androgen production. This establishes the vicious cycle, which indicates that inflammation alone can promote androgen secretion, and this androgen excess can stimulate inflammation through adipocyte hypertrophy and increased mononuclear cell sensitivity to ingested glucose [9].

One of the most often used inflammatory markers is C-reactive protein (CRP). It is primarily synthesized by the liver as an acute-phase protein. There is now growing evidence that CRP has important roles in inflammatory pathways and host responses to infection including the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, and the production of cytokines, particularly IL-6 and TNF $\alpha$  [10]. The first study which demonstrated elevation of serum CRP concentrations in women with PCOS was carried out by Kelly and associates in 2001. They compared 17 women with PCOS and 15 healthy women and concluded that women with PCOS had significantly increased CRP concentrations relative to women with normal menstrual rhythm and normal androgen levels. They were also one of the first to propose low-grade chronic inflammation as a novel mechanism contributing to increased risk of CVD and type 2 diabetes in these women [11]. These findings paved the way for numerous further studies. The first big meta-analysis of all these studies was conducted in 2011 and included 3.648 women in total, and among them, 2.359 had PCOS and 1.289 were healthy controls. Mean serum CRP levels were 95% higher in women with PCOS than controls. Elevated circulating CRP levels in PCOS women were independent of obesity because the finding persisted after excluding all the studies with mismatches in the frequency of obesity or body mass

between groups from the meta-analysis [8]. Some studies examined the effects of different treatment options on CRP levels. Metformin was shown to significantly lower the CRP levels in different treatment protocols [12, 13]. Recent studies also noticed that other PCOS management options, like statins or even increased physical activity only, significantly decreased CRP levels in women with PCOS [14, 15].

When considering other traditional inflammatory markers, females with PCOS were reported to have significantly higher levels of serum monocytes, lymphocytes, eosinophilic granulocytes, TNF $\alpha$ , and IL-6 than controls. Besides, in PCOS, ovarian tissue has more macrophages and lymphocytes than controls. Lymphocytes and macrophages secrete inflammatory cytokines like TNF $\alpha$  and IL-6, which in turn activate more lymphocytes and macrophages to enhance further cytokine secretion [9]. Especially TNF $\alpha$  seems to play a significant role in various clinical manifestations of PCOS. It is one of the most well-known inflammatory factors and has strong scientific evidence to be an important mediator in processes such as obesity, IR, and androgen expression. As a multifunctional hormone-like polypeptide, it has a wide variety of physiological roles including many directly connected with ovaries, such as regulation of ovarian function and exerting an influence on proliferation, differentiation, follicular maturation, steroidogenesis, and apoptosis [16]. A meta-analysis, published in 2016, which examined results from 29 studies with a total of 1960 women (1046 PCOS patients and 914 controls), concluded that TNF $\alpha$  levels in women with PCOS were significantly higher than healthy controls and that high-serum TNF $\alpha$  concentration was related to IR and androgen excess but not to the BMI [17]. TNF $\alpha$  is overexpressed in adipose tissue, so the source of excess circulating TNF $\alpha$  in PCOS is mostly adipose tissue in the obese but remains unknown in lean women with this disorder [18]. To even further emphasize the important role of TNF $\alpha$  in PCOS, its concentration is not elevated on a systemic level only but also in FF. Changes in FF levels of TNF $\alpha$  are associated with poor-quality oocytes in women undergoing in vitro fertilization, which then leads to a reduction in rates of fertilization, embryonic development, and pregnancy outcome [16]. Among other mechanisms, TNF $\alpha$  production is also induced by another proinflammatory cytokine interleukin 18 (IL-18), which was also reported to be increased in PCOS. It is produced by macrophages and induces cell-mediated immunity. Studies suggest that its levels are higher even in lean PCOS patients and are correlated with IR and CVD risk [19, 20].

TNF $\alpha$  promotes the synthesis of two other important cytokines, IL-6 and interleukin 8 (IL-8). IL-8 is mainly synthesized in macrophages and monocytes and is a significant immune response modulator. However, there are more studies regarding the role of IL-6 in PCOS, which has been closely associated with IR and CVD. Obesity was reported to be correlated with elevated IL-6 levels. In contrast, IL-6 levels decreased in PCOS patients after reducing their level of IR and body mass. Although some studies reported significant elevations in IL-6 levels in women with PCOS compared with controls, these findings were not confirmed in similar studies, with some studies even reporting decreased IL-6 levels in patients. However, a meta-analysis published in 2016, which included 922 PCOS patients and 696 controls, suggested that IL-6 levels were higher in women with PCOS than BMI-matched controls and that a high-serum IL-6 concentration was related to IR and androgen levels, but not to the BMI [21]. In a research article published in *Mediators of Inflammation* in 2011, authors concluded that IL-6 production and serum levels are related to an altered immune response in PCOS women with IR. They divided 44 young girls with PCOS aged 15–23 into non-IR and IR groups

based on homeostatic model assessment (HOMA) findings. They were weight- and age-matched with healthy young girls, and they measured a variety of different inflammatory markers. The biggest difference between groups was in significantly higher IL-6 levels in IR PCOS patients. Moreover, in the PCOS group, lipopolysaccharide-activated monocytes secreted significantly higher levels of IL-6 [22].

Several studies investigated two other important cytokines, interleukin 10 (IL-10) and interleukin 1 (IL-1) family in PCOS patients. The former is an important anti-inflammatory cytokine with multiple effects in immunoregulation and inflammation. In a study including 61 PCOS patients and 80 healthy controls, IL-10 levels were significantly lower among PCOS patients [23]. On the other hand, IL-1 family is a group of 11 cytokines that are primarily associated with innate immunity and are closely linked to a proinflammatory response. The IL-1 gene cluster on chromosome 2 contains three related genes *IL-1 $\alpha$* , *IL-1 $\beta$* , and *IL-1RN*, encoding the proinflammatory cytokines IL-1 $\alpha$  and IL-1 $\beta$  and their endogenous receptor antagonist. Previous PCOS research has been focused mainly on IL-1 $\beta$  since many studies suggested its crucial role in inflammatory-linked mechanisms in the ovaries. It probably intervenes in prostaglandin production, mainly by its activity on cyclooxygenase-2 synthesis. It also stimulates the production of other inflammatory cytokines, such as IL-6 and interleukin 12 (IL-12) [24].

At the beginning of this century, Jurg Tschopp introduced the concept of the inflammasome, and since then, inflammasomes have become an important topic of immunological studies. The inflammasome is a macromolecular cytoplasm complex that senses many different signals for cell damage on the molecular level and initiates the inflammatory response. It promotes the maturation and secretion of proinflammatory cytokines IL-1 $\beta$ , IL-18, and interferon gamma (IFN $\gamma$ ) and activates nuclear factor  $\kappa$ B (NF- $\kappa$ B). There are many different types of inflammasomes, but NLRP3 inflammasome is most commonly associated with obesity and IR. It consists of three subunits: NLRP3, the adaptor protein apoptosis-associated speck-like protein (ASC), and caspase-1. It initiates an inflammatory form of cell death and triggers the release of proinflammatory cytokine IL-1 $\beta$  through the mechanism of procaspase-1 cleavage. However, the role of NLRP3 inflammasome in the development of PCOS remains largely unknown [25]. In a study published in 2017, the NLRP3 and ASC mRNA levels, caspase-1 activation, and IL-1 $\beta$  production were unregulated in PCOS patients, and those levels significantly improved after treatment with dimethylbiguanide [26]. NLRP3 inflammasome may present an important overlapping pathway between OS and chronic inflammation in PCOS, which will be discussed in further detail in Chapter 4. In contrast with other previously described inflammatory markers, there have been no published studies regarding different genetic polymorphisms in NLRP3 inflammasome and PCOS at the time of writing. However, there is a lot of evidence that gain-of-function polymorphisms in the NLRP3 inflammasome play an important role in rheumatoid arthritis and Crohn's disease [27].

There has been a lot of progress in the pharmacogenetics of PCOS in recent years. Its main goal is to find associations between genetic polymorphisms and the course of the disease and response to treatment. In **Table 1**, we summarized main studies which pointed out positive associations between different polymorphisms in genes related to previously described inflammatory markers and PCOS risk or clinical manifestations. At the time of writing, we could not find any study which would explore the relationship between polymorphisms in those genes and treatment response.

Gene	Variants	Predicted effect	Reference
TNF $\alpha$	rs1799964 -1031 T > C	<i>Susceptibility</i> The TT genotype was more frequent in controls ( $p = 0.0002$ ) and TC genotype in patients ( $p = 0.0003$ ) <i>Clinical manifestations</i> TC genotype was associated with lower BMI ( $p = 0.03$ ). TT genotype was associated with early onset and hyperandrogenism ( $p < 0.05$ )	[28]
		<i>Susceptibility</i> The frequency distribution of TT, TC, and CC genotypes differed between PCOS and control group ( $p = 0.0003$ )	[29]
		<i>Susceptibility</i> A significant difference in allele transmission was found in families with proband for PCOS ( $p = 0.0013$ )	[30]
	rs1799724 -857 C > T	<i>Susceptibility</i> T allele showed a protective role against PCOS ( $p = 0.0032$ )	[31]
	rs4645843 6213 C > T	<i>Susceptibility</i> Genotype and allele distribution differed significantly between PCOS patients and controls ( $p = 0.03$ and $0.024$ , respectively) <i>Clinical manifestations</i> Polymorphism was significantly associated with serum testosterone levels ( $p = 0.01$ ), HOMA-IR ( $p = 0.034$ ), and BMI ( $p < 0.05$ ).	[32]
IL-6	rs1800795 -174 G > C	<i>Susceptibility</i> Genotype and allele distribution differed significantly between PCOS patients and controls. The G allele frequency was significantly higher in PCOS patients than controls (all $p$ values $< 0.05$ )	[33]
		<i>Susceptibility</i> Polymorphism was associated with decreased PCOS susceptibility in the overall population under the allelic model (G vs. C, $p = 0.005$ ), the homozygous model (GG vs. CC, $p = 0.001$ ), heterozygous model (GG vs. CG, $p = 0.036$ ), and the dominant model (GC+CC vs. GG, $p = 0.020$ )	[34]
		<i>Susceptibility</i> G allele was more frequent in patients with hyperandrogenism both when only homozygous and when homozygous and heterozygous G allele carriers were considered ( $p < 0.05$ for all analyses)	[35]
		<i>Susceptibility</i> The genotype as well as the polymorphic G allele distribution differed between PCOS patients and controls (both $p < 0.001$ )	[36]
		<i>Clinical manifestations</i> A relationship was detected between hirsutism, FSH, LH, total testosterone, HDL-cholesterol and triglyceride levels, and CG+GG genotypes. Furthermore, an association was found between IL-6 levels and CC genotype in the obese PCOS patients ( $p < 0.05$ for all analyses)	[37]
	rs1800797 -597 A > G	<i>Susceptibility</i> G allele was more frequent in patients with hyperandrogenism both when only homozygous and when homozygous and heterozygous G allele carriers were considered ( $p < 0.05$ for all analyses)	[35]

Gene	Variants	Predicted effect	Reference
IL-10	rs1800896 -819 T > C	<i>Susceptibility</i>	[23]
		The frequency of TT genotype was significantly increased ( $p < 0.05$ ) in PCOS group	
		<i>Clinical manifestations</i>	[38]
		CT and TT genotypes were associated with lower total cholesterol and triglyceride levels in PCOS patients ( $p < 0.05$ )	
	rs1800871 -1082 A > G	<i>Susceptibility</i>	[23]
		G allele was significantly increased among PCOS patients ( $p < 0.01$ ), while A allele was significantly increased ( $p < 0.001$ ) in controls	
		<i>Clinical manifestations</i>	[38]
		GA and AA genotypes were associated with lower total cholesterol and triglyceride levels ( $p < 0.05$ )	
	rs1800872 -592 C > A	<i>Susceptibility</i>	[39]
AA genotype carriers had increased risk of PCOS ( $p = 0.001$ )			
	<i>Clinical manifestations</i>	[38]	
	CA genotype was associated with lower total cholesterol and triglyceride levels ( $p < 0.05$ )		
IL-1 $\beta$	rs16944 -511 T > C	<i>Susceptibility</i>	[40]
		Both TT genotype frequency and T allele frequency were significantly higher in PCOS patients than controls (both $p < 0.01$ )	
		<i>Susceptibility</i>	[24]
		CC genotype frequency was significantly higher in PCOS patients than controls ( $p < 0.001$ )	
		<i>Clinical manifestations</i>	[41]
		T allele showed significant association with several metabolic features associated with PCOS ( $p < 0.05$ for all analyses)	
		<i>Clinical manifestations</i>	[42]
		Both TT genotype frequency and T allele frequency were significantly higher in obese PCOS patients than nonobese patients ( $p < 0.05$ for all analyses)	
IL-18	rs187238 -137 C > G	<i>Clinical manifestations</i>	[43]
		C allele frequencies were significantly higher in PCOS patients with IR than PCOS patients without IR ( $p = 0.048$ )	
		<i>Susceptibility</i>	[44]
		CC and GC genotypes were associated with increased risk of developing PCOS ( $p < 0.05$ )	
		<i>Clinical manifestations</i>	[45]
	PCOS patients with GG genotype had a significantly increased risk of impaired glucose regulation compared to C allele carriers ( $p < 0.05$ )		
IL-1 $\alpha$	rs1800587 -889 C > T	<i>Susceptibility</i>	[46]
		The distribution of genotype frequencies was statistically different in women with PCOS compared to controls ( $p = 0.04$ )	
	<i>Clinical manifestations</i>		
	The serum level of FSH and subsequent LH/FSH ratio correlated with the polymorphism within the PCOS group ( $p = 0.005$ and $0.01$ , respectively)		

**Table 1.**

The associations between genetic polymorphisms in genes related to inflammatory markers and PCOS risk or clinical manifestations.

### **3. Oxidative stress in PCOS**

OS has been a highly researched topic in the last two decades, due to the discovery that imbalance between oxidants and antioxidants results in an abnormal redox state of cells. This state is involved in the development of many diseases, such as diabetes, cancer, atherosclerosis, depression, PCOS, and a few neurological diseases. OS reflects an imbalance between production and scavenging of reactive oxygen (ROS) and nitrogen species (RNS). The ROS include superoxide radical, hydrogen peroxide, and hydroxyl radical, and the RNS include NO and its metabolites. Some ROS can also act as cellular messengers. The peroxides and free radicals are unstable and highly reactive and can damage different cell components. The most worrying long-term effects are caused by the damage to the DNA [47, 48].

OS is considered as a potential inducing factor in the PCOS pathogenesis. In most studies, PCOS patients present with higher levels of OS than controls. However, results often vary, mainly due to the employment of different markers and evaluation of the same marker in different sources and even with different investigation methods. Moreover, OS is not necessarily associated only with PCOS pathogenesis, since many clinical manifestations of PCOS, like hyperandrogenism, obesity, and IR, may be a contributing factor in the development of the local and systemic OS, which may then reciprocally worsen those metabolic abnormalities [49].

Levels of molecular markers that could reflect the systemic OS, such as oxidized low-density lipoprotein, malondialdehyde, thiobarbituric reactive substances, and advanced oxidation protein products, were significantly increased in obese people as compared with controls. On the other hand, markers that could reflect antioxidant activity such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) were significantly decreased in obese as compared to controls. However, obesity is probably only a contributing factor to OS in PCOS. When obese patients are ruled out based on BMI, nonobese women with PCOS still have higher OS markers than healthy controls [47].

One other important contributing factor to OS in PCOS women is IR. The mechanism behind this is that hyperglycemia and higher levels of free fatty acids lead to ROS production. On the other hand, OS can be an important factor in the development of IR. There is a well-known phenomenon called OS-induced IR. The full mechanism behind it is still unknown, but it was reported in multiple studies that exposure to OS inhibits insulin-stimulated glucose uptake, glycogen, and protein synthesis. Increased OS activates various protein kinases, which induce serine or threonine phosphorylation on insulin receptor substrate (IRS) instead of normal tyrosine phosphorylation. This reduces the ability of the IRS to combine with the insulin receptor and suppresses its activation of downstream phosphatidylinositol 3-kinase. Furthermore, the wrong phosphorylation could also induce the degradation of IRS. Insulin signaling pathways could also be activated by OS through Jun N-terminal kinase/stress-activated protein kinase signaling pathway and inflammatory signaling pathway (I $\kappa$ B kinase/NF- $\kappa$ B), leading to IR via post-insulin receptor defect [47].

Antioxidants, on the other hand, are a class of molecules that can reduce the destructive effects of free radicals. In general, we can divide antioxidants into two groups: enzymatic like (SOD, catalase (CAT), GPx, glutathione reductase, paraoxonase 1 (PON-1)) and nonenzymatic (glutathione, alfa-tocopherol, ascorbate, and beta-carotene). These antioxidants have already been reported to have an important role in the female reproductive system and the pathogenesis of female infertility [50].

In general, chemical substances used for evaluation of OS status can be divided into chemical components modified by ROS, ROS scavenging enzymes or antioxidative chemicals, and transcription factors regulating ROS production. Many studies tried to evaluate OS markers in PCOS women, but due to small study groups, results often varied. A meta-analysis of these markers was published in 2013. It encompassed 63 different studies and included 4933 PCOS patients and 3671 controls. It showed that the serum concentrations of several promoters and by-products of OS were significantly increased in patients with PCOS compared with control women. Homocysteine concentrations were 23% higher in PCOS patients than control women according to this meta-analysis. Homocysteine induces OS by promoting ROS production, and its high serum level makes a person more prone to endothelial cell injury. A 47% increase was noticed in malondialdehyde, an end-product of lipid peroxidation and a useful and frequently used marker of OS. On the other hand, some circulating antioxidant markers were decreased in PCOS. Glutathione, which plays a key protective role against OS, was decreased by 50%. A similar decrease was noticed in the activity of PON-1, which is an antioxidant enzyme that prevents the oxidation of lipoproteins and hydrolyzes atherogenic products of oxidative lipid modification such as phospholipid peroxides and cholesterol ester hydroperoxides. Contrary to what was expected, a meta-analysis found an increase in the SOD activity in PCOS patients. An increase in that potent protective enzyme, scavenging superoxide anion radical, may be interpreted as a compensatory mechanism in response to the increased production of other oxidant molecules. This meta-analysis did not find significant differences among patients with PCOS and control women in NO levels, GPx activity, and total antioxidant capacity, although many individual studies reported significant differences [49]. A similar increase in some antioxidative enzymes has already been described in few other studies. A study published in 2018 noticed a significant increase in CAT and SOD activity, and their interpretation of those results focused on an inner stress-counterbalanced effect [51].

Since a large proportion of studies indicated an important imbalance in the oxidative status of patients with PCOS, there have been a few attempts to treat patients with antioxidants. A single-blind randomized control trial involving 200 patients with PCOS was published in 2018. Patients were randomized into intervention and control groups, and baseline serum levels of OS markers, antioxidant enzymes, vitamins, and minerals were determined. Antioxidant supplementation and placebo were given to the intervention and control groups, respectively. All patients had ovulation induction with clomiphene and were followed up for 6 months. There was statistically significant difference in the serum levels of OS marker, antioxidant enzymes, vitamins, and minerals between the two groups. Not only did the antioxidant supplementation significantly improve oxidative status in those patients, but it also significantly affected pregnancy rate [52]. A recent study has also shown promising results with the mitochondria-targeted antioxidant for PCOS IR in animal model [53].

Researches have already confirmed that genetic changes in key antioxidant enzymes and inflammatory mediators can affect the individual's defense ability against OS and their predisposition to inflammation. In **Table 2**, we summarized the main studies which pointed out positive associations between different polymorphisms in genes related to previously described oxidative markers and antioxidant enzymes and PCOS risk or clinical manifestations. At the time of the writing, we could not find any study that explored the relationship between polymorphisms in those genes and treatment response.

Gene	Variants	Predicted effect	Reference
PON-1	rs705379 -108 C > T	<i>Susceptibility</i> Genotype distribution differed significantly between PCOS patients and controls ( $p < 0.05$ )	[54]
		<i>Susceptibility</i> Association with increased risk of PCOS was observed in three genetic models: allelic comparison, homozygote comparison, and recessive comparison ( $p < 0.05$ for all analyses)	[55]
		<i>Susceptibility</i> Results show a significant association between PCOS and this polymorphism (for T vs. C: $p = 0.012$ , for TT vs. CC: $p = 0.005$ , for TT vs. TC+CC: $p = 0.01$ )	[56]
		<i>Susceptibility</i> The TT genotype was more frequent in PCOS patients than in controls ( $p < 0.01$ ) <i>Clinical manifestations</i> Free androgen index levels were higher in patients with TT genotype ( $p < 0.05$ )	[57]
rs854560	163 T > A	<i>Susceptibility</i> Genotype and allele frequency distributions differed significantly between lean controls and lean PCOS women ( $p < 0.05$ ). This polymorphism reduced the risk of PCOS in lean but not in obese Indian women ( $p < 0.05$ ) <i>Clinical manifestations</i> This polymorphism influenced glucose metabolism, lipid parameters, and hyperandrogenemia in the study group ( $p < 0.05$ for all analyses)	[58]
		<i>Susceptibility</i> The association between polymorphism and a decreased risk of PCOS was found in dominant model ( $p < 0.05$ )	[55]
rs662	575 A > G	<i>Susceptibility</i> GG genotype and G allele frequencies were higher in patients with PCOS than in control women ( $p < 0.05$ ) <i>Clinical manifestations</i> Compared with patients with AA genotype, patients with GG or AG genotype had significantly higher waist circumference and fasting insulin and triglyceride levels, patients with GG genotype had significantly higher waist-to-hip ratio, and patients with AG genotype had significantly higher HOMA index ( $p < 0.05$ for all analyses)	[59]
		<i>Susceptibility</i> Women with AG or GG genotypes had a 2.5-fold increased risk of PCOS compared to AA genotype ( $p = 0.03$ )	[60]
		<i>Susceptibility</i> Polymorphism was significantly associated with PCOS (for G allele vs. A allele: $p = 0.02$ , for GG+AG vs. AA: $p = 0.043$ )	[56]
		<i>Susceptibility</i> GG genotype and G allele frequencies were higher in PCOS than in controls ( $p < 0.05$ for all analyses) <i>Clinical manifestations</i> Testosterone, free androgen index, and dehydroepiandrosterone sulfate levels were higher in patients with GG than in patients with the wild or the heterozygous genotype group ( $p < 0.05$ for all analyses)	[57]

Gene	Variants	Predicted effect	Reference
GSTM1	deletion	<i>Clinical manifestations</i> Adolescent girls with PCOS with GSTM1-null genotype (no active alleles present) presented significantly lower testosterone concentrations than those with the GSTM1-active genotype ( $p < 0.05$ )	[61]
SOD2	rs4880 47 T > C	<i>Susceptibility</i> The prevalence of the C allele was significantly greater in PCOS patients than in controls ( $p < 0.05$ ) <i>Clinical manifestations</i> Patients carrying the C allele had significantly higher serum LH levels and ratio of LH to FSH than patients with the TT genotype ( $p < 0.05$ for all analyses)	[62]

**Table 2.**  
 Associations between genetic polymorphisms in antioxidant genes and PCOS risk or clinical manifestations.

#### 4. Interplay between oxidative stress and chronic inflammation

OS and inflammation are closely related pathophysiological processes, one of which can be easily induced by another. They usually occur simultaneously, and because of that, it is hard to distinguish them completely in pathological conditions.

On the one hand, inflammatory processes can induce OS through different pathways. Phagocytic cells like neutrophils and macrophages produce large amounts of ROS and RNS to destroy invading agents. During pathological inflammatory conditions, there may be an exaggerated generation of these reactive species. Some of those diffuse out of their phagocytic cells and can induce localized OS. The similar process happens in nonphagocytic cells, which can also produce reactive species in response to proinflammatory cytokines [63]. Recent studies have also shown that activation of multiple Toll-like receptors produces unbalance between proinflammatory and anti-inflammatory cytokines, and this leads to the insurgence of OS [64]. Moreover, the proinflammatory cytokine IL-6 has been found to produce ROS through increased expression of nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) in non-small cell lung cancer. Similarly, the NOX4 overexpression can then enhance IL-6 production, and that forms a positive reciprocal feedback loop between these two mediators of inflammation and OS [63].

On the other hand, OS has been shown to induce inflammation. Growth factors and chemokines produced by inflammatory cells induce the overexpression of several transcription factors, such as NF- $\kappa$ B, signal transducer and activator of transcription 3 (STAT3), activating protein-1 (AP-1), and hypoxia-inducible factor-1 (HIF-1). They also activate redox-sensitive signal transduction pathways such as c-Jun N-terminal kinase and p38 mitogen-activated protein kinase (MAPK) [65, 66]. OS also plays an important role in the activation of the NLRP3 inflammasome, which promotes the maturation and secretion of proinflammatory cytokines IL-1 $\beta$ , IL-18, and IFN $\gamma$  and activates NF- $\kappa$ B. NLRP3 inflammasome can be activated in multiple ways by OS. Besides activation by transcription factors, NLRP3 can be activated by ROS released from the damaged mitochondria and oxidized mitochondrial DNA during apoptosis. In conditions of OS, the ROS causes the thioredoxin-interacting protein, an inhibitor of endogenous antioxidant thioredoxin, to dissociate from thioredoxin and to bind with NLRP3 leading to additional activation of NLRP3 inflammasome [63]. Another possible mechanism for NLRP3 inflammasome activation is through ROS-induced DNA base

modifications. Moreover, the OS-induced oxidation of the extracellular redox potential of plasma cysteine and its disulfide cysteine has been shown to trigger monocyte adhesion to vascular endothelial cells, activate NF- $\kappa$ B, and increase the expression of proinflammatory cytokine IL-1 $\beta$  [63]. OS also induces heat shock proteins, which in turn stimulate the production of proinflammatory cytokines and expression of the adhesion molecules like E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1 [66].

All these pathways seem to connect OS and inflammation into a self-perpetuating cycle. In pathological conditions where OS is the primary abnormality, inflammation will eventually develop and will further accentuate OS. Conversely, when inflammation is the main abnormality, OS will develop and will increase the damage from inflammation. Therefore identification of the primary abnormality could be extremely important in many conditions because its treatment could resolve additional problems from secondary OS or inflammation [63].

When it comes to PCOS, there is not yet enough evidence to confirm that either OS or inflammation is the main abnormality. However, the majority of studies agree that both phenomena at least play an important role in the clinical manifestations of that syndrome. One clinical manifestation that has been studied for its role in both OS and inflammation is obesity. Adipocytes have been identified as a source of proinflammatory cytokines which can then stimulate the production of ROS and RNS by macrophages and monocytes. One possible trigger for adipose tissue production of those cytokines is hypoxia. Adipose tissue hypoxia may underlie the dysregulated production of adipocytokines. In obese patients, adipose tissue also has the secretory capacity of angiotensin II, which stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. NADPH oxidase comprises the major route for ROS production in adipocytes. The second clinical manifestation that has already been connected with both OS and inflammation is IR. OS has been recently recognized as one of the key mechanisms in IR. The hypothesis that inflammation is causally linked to IR is supported by clinical evidence of correlations between inflammatory markers and measures of IR and also by biochemical evidence indicating that proinflammatory markers can interfere with insulin action by directly inhibiting insulin receptors [67]. IR then plays a key role in hyperandrogenemia, likely due to compensatory hyperinsulinemia. Insulin is reported to stimulate ovarian androgen secretion directly alone and/or through augment LH-stimulated androgen secretion. Studies show that excessive activation of androgen receptor may provoke systemic OS. In vitro, OS was also reported to enhance the activities of ovarian steroidogenesis enzymes [47, 68]. Considering all previously discussed pathways, it seems that all the described changes—*inflammation, OS, obesity, IR, and hyperandrogenism*—interact among themselves and amplify each other in PCOS patients [69].

## **5. Conclusions**

The majority of studies confirm that PCOS patients have elevated levels of OS and inflammatory mediators and that OS and inflammation may play an important role in the pathogenesis of the syndrome and its clinical manifestations. There are also several possible pathways how those two processes could form positive reciprocal feedback between each other and also between clinical manifestations of PCOS, such as obesity, IR, and hyperandrogenism.

Given the importance of OS and inflammation in PCOS, many researchers have tried to find associations between different polymorphisms in genes related to these

pathways and PCOS risk, clinical manifestations, or treatment response. Evidence shows that different genetic polymorphisms in those pathways have an important role in susceptibility for the development of PCOS and that they greatly influence its clinical manifestations. There is still a lack of studies that would evaluate how those polymorphisms affect individual treatment response. Large pharmacogenetic studies would improve our understanding of PCOS pathogenesis, and they could identify polymorphisms potentially used as a predictive biomarker for evaluating the risk for developing PCOS and for predicting treatment response in an individual patient.

## Abbreviations

AP-1	activating protein-1
ASC	apoptosis-associated speck-like protein
BMI	body mass index
CAT	catalase
CRP	C-reactive protein
CVD	cardiovascular disease
FF	follicular fluid
FSH	follicle-stimulating hormone
GLP-1	glucagon-like peptide 1
GPx	glutathione peroxidase
HIF-1	hypoxia-inducible factor-1
HOMA	homeostatic model assessment
IFN $\gamma$	interferon gamma
IL-1	interleukin 1
IL-6	interleukin 6
IL-8	interleukin 8
IL-10	interleukin 10
IL-12	interleukin 12
IL-18	interleukin 18
IR	insulin resistance
IRS	insulin receptor substrate
LH	luteinizing hormone
MAPK	mitogen-activated protein kinase
MS	metabolic syndrome
NF- $\kappa$ B	nuclear factor $\kappa$ B
NO	nitric oxide
NOX4	nicotinamide adenine dinucleotide phosphate oxidase 4
OS	oxidative stress
PCOS	polycystic ovary syndrome
PON-1	paraoxonase 1
RNS	reactive nitrogen species
ROS	reactive oxygen species
SHBG	sex hormone-binding globulin
SOD	superoxide dismutase
STAT3	signal transducer and activator of transcription 3
TNF $\alpha$	tumor necrosis factor alpha

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