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REVIEW ARTICLE

Genetic and environmental aspect of polycystic ovary syndrome

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ABSTRACT. Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome determined in most patients by the association of two main factors: hyperandrogenism and insulin resistance. These characters are probably independent of each other and seem to be inherited by several different mechanisms. In some patients homozygous gene alteration has been found but in most patients PCOS seems to be determined by the association of gene polymorphisms that are common in the general population but alone are unable to determine phenotypic consequences. Alteration of genes that regulate the initial steps of ovarian steroidogenesis is probably the main causal factor of hyperandrogenism. Insulin resistance may be the result of many different gene alterations including insulin receptor substrate (IRS)-1 and 2, calpain-10 and peroxisome proliferator-activated receptor γ (PPARγ). Some polymorphisms may be protective against other gene alterations. Insulin sensitivity is also modified by socioeconomic and cultural factors that influence quantity and quality of food and energy expenditure. However, even eating behavior and weight response to food intake may be under genetic regulation. Different combinations of multiple gene polymorphisms and of environmental factors explain the heterogeneity of PCOS.

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INTRODUCTION

Almost 70 yr have passed since Stein and Leventhal described the syndrome (1) that we now call polycystic ovary syndrome (PCOS), but our understanding of its causal factors is still incomplete (2, 3). The main problems have probably been the heterogeneity of the syndrome and the lack of agreement on its essential characteristics. In the past, some endocrine (increased LH or LH/FSH ratio) or clinical factors (chronic anovulation, menstrual irregularities, obesity) or a particular ovarian morphology (polycystic ovaries) were considered essential for the development of the syndrome but it is now clear that they may or may not be present (4).

However, in the last decade, evidence has been accumulating that hyperandrogenism (5) and insulin resistance (6) are indeed very important for the development of the PCOS and most Authors believe that the search for causal determinants of PCOS should stem from these factors. More recently, the importance of mild genetic alterations (already suspected in the past) has been demonstrated by many studies (7) while a few researchers have started to explore the relationships between environmental influences, genetic characteristics and the development of the syndrome (8).

The objective of this review is to summarize some of the new findings that have improved our understanding of the possible causes of PCOS. We will first present the evidence that genetic factors are important in the pathogenesis of PCOS and then we will concentrate on the possible genetic and environmental factors that may determine insulin resistance and hyperandrogenism in PCOS. Finally, we will try to understand how genetic and environmental influences may interplay in the development of PCOS.

Key-words: PCOS, hyperandrogenism, insulin resistance, obesity.

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ARE INSULIN RESISTANCE AND HYPERANDROGENISM TWO INDEPENDENT COMPONENTS OF PCOS?

Before evaluating the possible genetic and environmental causes of PCOS, a preliminary question has to be discussed: are hyperandrogenism and insulin resistance independent factors or does one depend on the other?
It is clear that if one factor is determining the other, the search for the causal factors should interest only the primary one.
Many studies have been dedicated to this problem and a detailed discussion of this matter is not the aim of this review. Actually, many Authors believe that, although hyperandrogenism may worsen insulin resistance (9, 10), and at the opposite end hyperinsulinemia increases androgen secretion (11, 12), in PCOS these two factors are independently present (13).
In fact, in PCOS women insulin resistance persists if:

1. ovaries are removed surgically (14);
2. ovarian androgen secretion is suppressed by long-acting GnRH agonists (14) or by other androgen-suppressing agents (15).

On the contrary:

1. most obese women are not hyperandrogenic or have very mild hyperandrogenism, in spite of having insulin resistance (16);
2. treatment of PCOS with insulin-sensitizing agents determines only small reductions of androgens (17).

In Figure 1, the effects of troglitazone, a powerful insulin-sensitizing agent, are presented (18). In PCOS, troglitazone, at different doses, reduced serum insulin levels up to 60% but serum androgen secretion decreased by only 10-15%.
These data suggest that:

1. insulin and hyperandrogenism are two separate components of PCOS;
2. both components are needed for the development of the syndrome.

Not all Authors agree on these conclusions.
In fact, it is possible that androgen-induced changes of insulin resistance (or, on the contrary, insulin-induced changes of enzymes involved in steroidogenesis) are not reversible or require a very long time to be reversed. In alternative, both insulin resistance and hyperandrogenism could be the consequence of another factor. For example, it has been shown that in some animals (rhesus monkeys and sheep), fetal androgen exposure determines a syndrome that is similar to PCOS and that includes both hyperandrogenism and insulin resistance (19). Finally, insulin resistance has not been found in many women with PCOS (20-30%) (6, 13), and all serum androgens are normal in some women who are diagnosed as having PCOS (13).
Stemming from these observations, Abbott et al. (20) have suggested that PCOS may be the consequence of a genetic form of ovarian hyperandrogenism appearing early in life that predisposes to insulin resistance. While it is a reasonable hypothesis, several studies have indicated that, in many women with PCOS, insulin resistance is also inherited (see below). On the other hand, the syndrome is so heterogeneous that different mechanisms may be involved in the pathogenesis of PCOS.

EVIDENCE FOR THE IMPORTANCE OF GENETIC FACTORS IN THE GENESIS OF PCOS

It has been known for many years that in some families PCOS is genetically transmitted (21, 22) and in most studies an autosomal dominant mode of inheritance has been reported. In fact, Carey et al. (23) suggested a dominant mode of inheritance with a 90% of penetrance while Cooper et al. (24) found a dominant inheritance but a reduced penetrance of the gene. More recently, Govind et al. (25) observed an autosomal dominant pattern of inher-

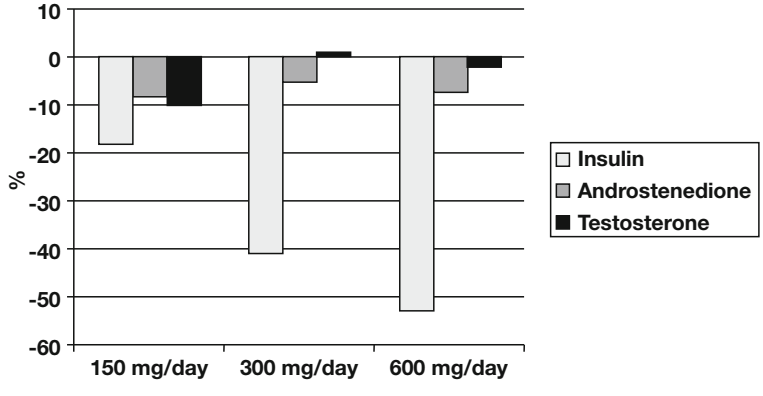

Fig. 1 - *Effect of different doses of troglitazone on insulin and androgen serum levels in women with PCOS. Modified from (18).*

itance, but also reported a wide spectrum of the clinical phenotype in 29 PCOS families.
However, it seems unlikely that such a common disease can be explained by the disorder of a single dominant gene (26). In fact, some larger studies of relatives did not find a well-defined mode of inheritance. Studying 132 PCOS women from Norway and their first and second grade relatives, Lund et al. (27) did not observe a clear inheritance pattern. Azziz et al. (28) found that 35% of mothers and 40% of sisters of 195 women with PCOS reported a clinical history compatible with PCOS. In a successive study, these Authors (29) found hormonal evidence of PCOS in 24% of the mothers and in 32% of the sisters of the women with PCOS. In both studies, no precise mode of inheritance of the syndrome was observed. Similar data have been reported by Legro et al. (30), who studied 307 sisters of women with PCOS. A summary of the familial studies of PCOS women is presented in Table 1.
A different approach has been conducted by studying female twins. The largest of these studies was performed in Australia by Jahanfar et al. (31) and showed that PCOS has a complex, not autosomal dominant, inheritance pattern. These Authors suggested that polygenic factors and influence of environmental factors determine the mode of inheritance of PCOS.
It is the most probable explanation of the complex mode of inheritance of PCOS. In fact, while in some families a single dominant gene may transmit the syndrome, in most cases a polygenic transmission, influenced by environmental factors, is probable. On the other hand, these findings are consistent with the hypothesis that, in most patients, two different factors, hyperandrogenism and insulin resistance, are essential for the development of the syndrome.
A simple possibility is that two different genes, one for the character of insulin resistance, the other for the character of hyperandrogenism, are both needed for the development of PCOS. However, as we will show, in most women with PCOS, the transmission of the syndrome is more complex and requires different gene alterations.

Table 1 - *Familial studies of polycystic ovary syndrome (PCOS).*

Author (ref.)	Studied patients	Mode of inheritance
Givens (21)	3 kindred	X-linked dominant
Hague (22)	50 PCOS women and first degree relatives	No clear inheritance
Cooper (23)	18 PCOS women and first degree relatives	Autosomal dominant with reduced penetrance
Carey (24)	10 PCOS women and first degree relatives	Autosomal dominant
Govind (25)	29 PCOS women and first degree relatives	Autosomal dominant
Lund (27)	132 PCOS women and first and second degree relatives	No clear inheritance
Azziz (28, 29)	195 PCOS women and first and second degree relatives	No clear inheritance
Legro (30)	336 PCOS women and first degree relatives	No clear inheritance

SEARCHING FOR PCOS GENES

The hyperandrogenic genes

Women with PCOS present increased androgen secretion from both adrenals and ovaries (13). However, because adrenal hyperandrogenism seems to be secondary to the ovarian alteration and to hyperinsulinemia (32), most studies have concentrated on ovarian hyperandrogenism (33, 34). Some years ago, Gilling-Smith et al. (35) showed that isolated theca cells of women with PCOS produce excessive quantities of androstenedione. These studies have been confirmed and extended by other groups that have found increased testosterone production from cultures of theca cells of women with PCOS (36, 37). While initially it was believed that an increase of 17α-hydroxylase or 17, 20-lyase could explain the ovarian increased androgen production (5, 37), more recent studies have shown that these enzymes are generally unaffected and that the most common alteration regards an initial step of the steroid production (38).
Studies of other genes, that encode the enzymes responsible for the various steps of steroidogenesis, have given discordant results. In fact, although Franks et al. (33) have reported an alteration of a gene that encodes 11α-hydroxylase, in other studies no polymorphism of this gene was found (39).
More recently Wood et al. (40) have started to analyze the gene expression of theca cells in PCOS. After analyzing 39,000 genes, they found that there are differences in the expression of 104 genes between normal women and women with PCOS. Of course, it is very difficult to imagine that in PCOS so many genes are primarily altered. More probably, an alteration of some regulatory genes determines a secondary modification of other genes. Consistently with this hypothesis, most alterations regarded signal transduction pathway and genes involved in retinoic acid metabolism and signaling (40).

Interestingly, other gene polymorphisms that determine variants of the androgen receptor (41) and of the sex hormone binding globulin (42) have been reported. In the study of Hickey et al. (41) the polymorphism of the androgen receptor was transmitted as an X-linked character, a finding that may recall Givens' old data (21) about some families with PCOS where the familial transmission was X-linked.
In conclusion, most evidence actually supports the hypothesis that PCOS women have an ovarian hyperandrogenism determined by a polymorphism of genes that regulate signal transduction pathway at the level of theca cells. However, it is also possible that different genetic mechanisms are operating and that in some families a clinical syndrome very similar to PCOS (i.e. clinical hyperandrogenism associated with insulin resistance) is determined by polymorphism of genes that regulate peripheral androgen activity or the serum sex hormone binding globulin capacity.
The gene alterations that may determine hyperandrogenism in women with PCOS are summarized in Table 2.

The insulin resistance gene

Many studies have been dedicated to the search for the insulin-resistant gene in PCOS. While several defects have been observed, no agreement exists on the possible genetic alteration.
Some years ago, Dunaif et al. (43) reported that abnormal serine phosphorylation of the insulin receptor [maybe because of altered protein kinase C (PKC), a serine threonine kinase] was present in their PCOS women. However, in other studies no such alteration has been found (44) and, in a large unpublished study, only one patient with PCOS showed this kind of alteration (Stanczyk, personal communication).
Some years later, Franks' English group reported that the insulin gene variable number tandem repeat (VNTR gene) is altered in PCOS (45, 46) [as in Type II diabetes (47)] and that this gene is exclusively paternally transmitted. However, other groups did not find any alteration of VNTR gene in PCOS (48).

Table 2 - *Gene alterations that may determine hyperandrogenism in polycystic ovary syndrome.*

Probably common	Uncommon or disputed
• Theca cell signal transduction	• 11α-hydroxylase
• Theca cell retinoic acid	• 17α-hydroxylase
	• 17,20-lyase
	• Androgen receptor variant
	• SHBG variant

More recently, many other gene alterations have been implicated in the pathogenesis of insulin resistance in PCOS such as polymorphisms of tyrosine kinase domain of the insulin receptor gene (49), of insulin receptor substrate (IRS) proteins (IRS-1 and IRS-2) (50, 51) and of tumor necrosis factor (TNF) receptor 2 gene (52).
However, the association of some of these polymorphisms with PCOS has not been confirmed by other studies (53).
Several groups have focused their attention on possible polymorphisms of the genes of two factors that seem very important for the pathogenesis of insulin resistance: calpain-10 and peroxisome proliferator-activated receptor γ (PPARγ).
Calpain-10 (54) is a protease that is encoded by a gene on chromosome 2q37 (55) and whose polymorphisms have been linked to Type 2 diabetes, mostly in Mexican-Americans (56), but also in Europeans (57). While the biologic function of the calpain-10 is unclear, it has recently been reported that polymorphisms of this gene are associated to insulin resistance (58). Studies of the same polymorphisms of calpain-10 that have been found in Type 2 diabetes did not show any significant association with PCOS (59). A Spanish group has recently reported that a different (SNP-44) polymorphism of the calpain-10 gene is frequently found in women with PCOS (60). However, the same polymorphism was not associated to PCOS in an English study (61).
The second factor, PPARγ, is a nuclear transcriptional factor that is important in adipose tissue differentiation and metabolism (62). Because thiazolidinediones, a class of drugs that improve insulin sensitivity, act by a mechanism that involves the activation of PPARγ (63), it has been suggested that this factor is very important in determining insulin resistance (64). In PCOS, it has been shown that some polymorphisms are more common and are associated to higher body weight and leptin levels (65, 66). However, a particular polymorphism (Pro12Ala) of PPARγ improves insulin sensitivity (65) and may protect against insulin resistance induced by obesity. The same polymorphism may reduce the effect of IRS-1 (Gly972Arg) polymorphism and therefore determine a milder degree of insulin resistance (67).
It is important to observe that some of these polymorphisms are common in the general population, or at least in some particular populations, but alone are not able to determine insulin resistance (68). However, the combination of several polymorphisms that impair insulin resistance may determine insulin resistance (68).

In conclusion:

1. mutations in insulin signaling molecules are very common in the general population;
2. these mutations are homozygous in rare cases while in most cases they are heterozygous and determine mild or very mild insulin resistance with modest or absent phenotypic expression;
3. combinations of different mild heterozygous defects may determine insulin resistance;
4. it is unclear what the most common gene defects that determine insulin resistance in women with PCOS are.

The gene alterations that may determine insulin resistance in women with PCOS are summarized in Table 3.

POSSIBLE ENVIRONMENTAL CAUSES OF PCOS

The pathogenesis of obesity in PCOS

For a long time, the possibility that PCOS has environmental causes has been suspected (69). In particular, the importance of factors that may increase body weight has been studied by many Authors. In fact, it is theoretically possible that some hyperandrogenic women develop PCOS if they become obese because of excessive food intake or decreased energy expenditure. In our experience (70) and in that of most Authors (8), the only long-lasting improvement of PCOS is obtained in the women (not many) that are able to permanently reduce their body weight by decreasing their food intake.

On the other hand, PCOS is common in all studied populations (71-73) but presents a large heterogeneity. In particular, the prevalence of obesity is quite variable and it regards not only the patients of a single population (13) but also the different ethnic populations. Several years ago, comparing three different populations with PCOS, we observed that the Japanese population had a low prevalence of obesity (73). More recent studies have found differences between the Caucasian population and other ethnic populations. For example, Mexican Americans are more obese and more insulin-resistant than white Caucasians (74). We have also found that women with PCOS from Pennsylvania are more obese than women with PCOS from Sicily (75).

It is difficult to say whether these differences between patients and/or populations with PCOS depend on genetic characteristics or on socioeconomic and cultural factors that may condition factors such as quantity and quality of food intake or energy expenditure. In fact, as previously reported, it has been demonstrated that a combination of different genetic patterns may modify the phenotypic appearance of PCOS, including the appearance and the degree of obesity and insulin resistance (67). Moreover, many studies are now showing that genetic factors are very important in the genesis of obesity (76, 77).

However, the importance of environmental factors should not be overlooked because it is well known that cultural and socioeconomic factors are very important in determining the incidence of obesity in a society.

Several studies have been dedicated to evaluating the quantity and the quality of food intake of women with PCOS. All these studies share a common problem because they explore the present status but do not give any information regarding past food intake. Moreover, some genetic traits may affect body weight just by affecting eating behavior (78). However, despite these important limitations, these studies may be useful to understand the relative importance of genetic vs environmental factors.

Comparing the food intake of women with PCOS with that of normal women, Taylor et al. (79) observed that normal weight women with PCOS eat less than normal women of similar body weight. This finding suggests that obesity in PCOS is determined by genetic factors and should be present in most patients but that some women maintain a normal body weight reducing their food intake. Of course, more studies are needed to support this conclusion and, while this mechanism may be important in particular socioeconomic contexts, it seems unlikely that it may explain differences in body weight in all populations.

We have recently compared (75, 80) the food intake of two populations of PCOS (one from Sicily,

Table 3 - *Gene alterations that may determine insulin resistance in polycystic ovary syndrome.*

Probably common	Uncommon or disputed
• IRS-1	• Calpain-10
• IRS-2	• PPP1R3
• PPARγ	• VNTR
	• Serine threonine kinase that phosphorylates insulin receptor
	• Tyrosine kinase domain of the insulin receptor
	• TNF receptor 2

IRS: insulin receptor substrate;
PPARγ: peroxisome proliferator-activated receptor γ;
TNF: tumor necrosis factor.

Italy, and the other from Pennsylvania, USA) that presented differences in body weight. We did not observe any difference in total food intake between the two populations and also energy expenditure did not seem to be different. Interestingly, the quality of eaten fats between the two populations was different because Pennsylvania women eat more saturated fats than Sicilian women. It is difficult to say whether these differences in quality of food intake are sufficient to determine the differences in body weight but several data suggest that indeed they may influence the severity of insulin resistance (81).
It has also been shown that different genetic patterns may influence the effect of the quality of food on body weight. For example, subjects with Ala54ThrFABP2 gene polymorphism present increased fatty acids absorption and become more obese and more insulin-resistant when consuming a high-fat diet (82). Similarly, subjects with Pro12 allele of PPARγ 2 become more easily obese when they eat food rich in saturated fats (82).
It is clear that the limits that separate genetic obesity and environmental obesity in PCOS are still unclear and many more studies are needed to understand the relative importance of the two factors in the pathogenesis of the syndrome.
Anyway, some tentative conclusions could be suggested:

1. in many PCOS women, obesity is genetically determined by the same factor that determines insulin resistance;
2. reduced food intake or increased energy expenditure may prevent the appearance of obesity in women with PCOS;
3. quality of food intake may be as important as quantity in determining obesity in PCOS;
4. genetic factors are important in determining body weight response to food intake.

The role of fetal androgen exposure and birth weight

While food intake is the most important environmental factor that may condition the appearance of PCOS, other possible environmental factors have been suggested. As we have previously discussed, prenatal androgenization of female rhesus monkeys determine, in 30-40% of the animals, the postpuberal appearance of ovarian dysfunction which is similar to human PCOS (19). It is therefore possible that the environmental presence of substances that determine androgenization of the fetus may determine in some patients the appearance of PCOS. It remains to be determined if this model is valid for human PCOS. In fact, in most PCOS patients there is no evidence for androgenization before puberty and this model may be valid only in a few patients [similarly to what happens in patients with adrenal enzymatic deficiencies (32)].
It has also been reported that insulin resistance and ovarian hyperandrogenism may be the long-term consequence of low birth weight (83). Again it does not seem to be a very common mechanism in the pathogenesis of PCOS.

CONCLUSIONS

All data that we have presented suggest that PCOS is a genetic syndrome that in most patients may be determined by the simultaneous presence of at least two genetic alterations, one that produces hyperandrogenism and the other that determines insulin resistance. The combination of several gene polymorphisms may be needed to determine each of these characters. Many of these polymorphisms are common in the general population but alone are not able to determine a clinically evident syndrome. The possibility that different gene combinations determine the same syndrome probably largely explains the heterogeneity of PCOS. Socioeconomic and cultural factors, mostly involving the quality and the quantity of food intake and the energy expenditure, influence the severity of insulin resistance, therefore adding still more variability to the phenotypic presentation of PCOS.

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