The Molecular-Genetic Basis of Functional Hyperandrogenism and the Polycystic Ovary Syndrome

Article *in* Endocrine Reviews · May 2005

DOI: 10.1210/er.2004-0004 · Source: PubMed

3 authors, including:

Manuel Luque-Ramírez
Hospital Universitario Ramón y Cajal & Universidad de Alcalá
171 PUBLICATIONS **5,511** CITATIONS

0163-769X/05/$20.00/0
Printed in U.S.A.

Endocrine Reviews 26(2):251–282

doi: 10.1210/er.2004-0004

The Molecular-Genetic Basis of Functional Hyperandrogenism and the Polycystic Ovary Syndrome

Héctor F. Escobar-Morreale, Manuel Luque-Ramírez, and José L. San Millán

Departments of Endocrinology (H.F.E.-M., M.L.-R.) and Molecular Genetics (J.L.S.M.), Hospital Ramón y Cajal, Madrid, Spain E-28034

The genetic mechanisms underlying functional hyperandrogenism and the polycystic ovary syndrome (PCOS) remain largely unknown. Given the large number of genetic variants found in association with these disorders, the emerging picture is that of a complex multigenic trait in which environmental influences play an important role in the expression of the hyperandrogenic phenotype.

Among others, genomic variants in genes related to the regulation of androgen biosynthesis and function, insulin resistance, and the metabolic syndrome, and proinflammatory genotypes may be involved in the genetic predisposition to functional hyperandrogenism and PCOS.

The elucidation of the molecular genetic basis of these disorders has been burdened by the heterogeneity in the diagnostic criteria used to define PCOS, the limited sample size of the studies conducted to date, and the lack of precision in the identification of ethnic and environmental factors that trigger the development of hyperandrogenic disorders. Progress in this area requires adequately sized multicenter collaborative studies after standardization of the diagnostic criteria used to classify hyperandrogenic patients, in whom modifying environmental factors such as ethnicity, diet, and lifestyle are identified with precision.

In addition to classic molecular genetic techniques such as linkage analysis in the form of a whole-genome scan and large case-control studies, promising genomic and proteomic approaches will be paramount to our understanding of the pathogenesis of functional hyperandrogenism and PCOS, allowing a more precise prevention, diagnosis, and treatment of these prevalent disorders. (*Endocrine Reviews* 26: 251–282, 2005)

First Published Online November 23, 2004

Abbreviations: AR, Androgen receptor; CRP, C-reactive protein; CYP, cytochrome P450; gp130, gp130 subunit of IL-6 receptor; HSD, hydroxysteroid dehydrogenase; INS, insulin gene; INSR, insulin receptor gene; IRS, insulin receptor substrate; LHβ, β-subunit of LH; PAI-1, plasminogen activator inhibitor-1; PCOS, polycystic ovary syndrome; PON1, paraoxonase; PPAR-γ2, peroxisome proliferator-activated receptor-γ2; SORBS1, human homolog for the sorbin and SH3-domain-containing 1 gene; SNP, single nucleotide polymorphism; SRD5A, steroid 5α-reductase; TNFR2, type 2 TNF receptor; VNTR, variable number of tandem repeats.

Endocrine Reviews **is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

I. Introduction

POLYCYSTIC OVARY SYNDROME (PCOS) is one of the most common endocrinopathies in women of childbearing age (1). PCOS is characterized by increased ovarian and adrenal androgen secretion (2); hyperandrogenic symptoms such as hirsutism, acne, and/or alopecia; menstrual irregularity; and, in a significant proportion of patients, insulin resistance (3).

The presence of male sexual secondary characteristics in women has been recognized from ancient times, but it was not until 1921 when Achard and Thyers (4) reported the association of hyperandrogenic symptoms with abnormalities in glucose metabolism, highlighting the presence of polycystic ovaries in some of their patients. However, only after the description of seven cases of amenorrhea and bilateral polycystic ovaries by Stein and Leventhal in 1935 (5) was PCOS considered a separate entity that interested clinicians and researchers worldwide.

Although for many years the interest in PCOS has been

focused on the cutaneous and reproductive manifestations of this disorder, the recent evidence suggests that metabolic and cardiovascular risk factors cluster in these patients (6–8). This evidence has renewed research efforts on hyperandrogenism and PCOS, including those directed toward the identification of the genetic and environmental factors involved in the pathogenesis of these prevalent conditions.

At present, there is no consensus on the criteria for the diagnosis of PCOS (Table 1). Most clinicians and researchers from the United States and from southern Europe use the criteria derived from the conference held at the National Institute of Child Health and Human Development (NICHD) in 1990: clinical and/or biochemical hyperandrogenism, menstrual dysfunction, and exclusion of specific etiologies (9). According to these criteria, the presence of polycystic ovaries on ultrasound examination is not needed for the diagnosis. On the contrary, most specialists from the other European countries, Asia, and Oceania rely mostly on the presence of polycystic ovaries on ultrasound examination for this diagnosis, whereas menstrual dysfunction is not required (10). A recent consensus workshop held in The Netherlands in 2003 under the auspices of the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine proposed a revision of the criteria for the diagnosis of PCOS, of which two of the following three would be needed: 1) oligo- and/or anovulation; 2) clinical and/or biochemical signs of hyperandrogenism; and 3) polycystic ovaries, together with the exclusion of other etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors, or Cushing's syndrome (11, 12).

However, the term PCOS as used in the literature is not specific and has been applied to a wide range of hyperandrogenic women, including women with or without menstrual dysfunction or polycystic ovaries. For that reason, in the present review, we will use the more general term functional hyperandrogenism to define these patients presenting with androgen excess, including those diagnosed with PCOS according to any of the current definitions of the syndrome, in whom a specific etiology such as congenital adrenal hyperplasia or androgen-secreting tumors cannot be identified, and, whenever possible, we will describe the particular characteristics of the patients included in the different studies. Of note, we will not include idiopathic hirsutism in this definition, because this infrequent disorder, in which hyperandrogenemia, menstrual dysfunction, and ultrasonographic polycystic ovaries are not present, appears to be a separate entity in which increased skin activity of 5α-reductase (resulting in increased conversion of testosterone into the more potent androgen dihydrotestosterone), androgen receptor (AR) polymorphisms, and altered local androgen metabolism contribute to the development of hirsutism (13).

Functional hyperandrogenism and PCOS cluster in first-degree relatives of patients (14) and are inherited together with insulin resistance and metabolic disorders (15, 16). During the past decades, the inheritance of these disorders has been the subject of intense research (17–19), but many questions remain unanswered.

The pattern of inheritance is still unknown. Initially, an autosomal dominant model, including premature balding as the male phenotype, was proposed (20, 21), but later studies did not confirm this hypothesis. The heterogeneity of the populations, the large number of candidate genes studied to date (22), and the difficulty inherent to identifying the molecular genetic mechanism leading to a complex metabolic disorder such as PCOS, in which environmental factors play a major role (23, 24), provide an explanation for the fact that the molecular genetic basis of functional hyperandrogenism and PCOS remains largely unknown despite significant efforts.

The purpose of this review is to provide a systematic evaluation of the studies conducted to date in functional hyperandrogenism and PCOS and to suggest priorities and new strategies that may contribute to understanding the pathogenesis of these disorders.

II. Evidence Suggesting a Genetic Origin for Functional Hyperandrogenism and PCOS

A. Familial aggregation (Table 2)

The familial aggregation of PCOS, hyperandrogenemia, and associated metabolic abnormalities suggests a genetic origin for functional hyperandrogenism and PCOS. Back in 1968, Cooper *et al.* (25) studied the families of 18 Caucasian women with polycystic ovaries and clinical and biochemical traits associated with PCOS, and the families of 18 paired control women. The incidence of oligomenorrhea and polycystic ovaries was increased in first-degree relatives of PCOS patients compared with controls, and males in these families had increased hairiness according to a questionnaire, suggesting an autosomal dominant pattern of inheritance (25). Givens and colleagues (26–28) published a series of family-based studies in patients presenting with hirsutism, oligomenorrhea, and increased ovarian size, and they found familial aggregation of hyperandrogenic symptoms (hirsutism and oligomenorrhea) and of metabolic disorders (diabetes mellitus, dyslipidemia, arterial hypertension, and athero-

TABLE 1. Diagnostic criteria for the diagnosis of PCOS

NICHD criteria (9)	Ultrasonographic criteria (10)	Rotterdam criteria (11, 12)
1) Oligoovulation 2) Clinical and/or biochemical hyperandrogenism	1) Ultrasonographic polycystic ovaries 2) Clinical and/or biochemical hyperandrogenism	1) Oligo- and/or anovulation 2) Clinical and/or biochemical hyperandrogenism 3) Polycystic ovaries
Exclusion of secondary etiologies such as congenital adrenal hyperplasia, androgen-secreting tumors, and hyperprolactinemia.		

Criteria 1 and 2 must be present for the diagnosis of PCOS according to NICHD and ultrasonographic criteria. The Rotterdam criteria require the presence of two of the three individual criteria. All definitions require exclusion of secondary etiologies.

TABLE 2. Studies of familial aggregation in functional hyperandrogenism and PCOS

Authors[a]	Phenotype in first-degree relatives	Suggested inheritance
Cooper *et al.* (25)	Women: oligomenorrhea and PCO Men: increased hairiness	Autosomal dominant with variable penetrance
Wilroy *et al.* (26), Givens (27, 28)	Women: hyperandrogenism and metabolic disorders Men: oligospermia and LH hypersecretion	X-linked
Ferriman and Purdie (29)	Women: infertility, oligomenorrhea, hirsutism	Not determined
Hague *et al.* (30)	Women: PCO	Not determined
Lunde *et al.* (31)	Women: hyperandrogenic symptoms Men: premature baldness and increased hairiness	Autosomal dominant
Carey *et al.* (20)	Women: PCO Men: premature baldness	Monogenic
Jahanfar *et al.* (40, 41)	Twin studies: fasting insulin, androstanediol glucuronide, lipid profile	Polygenic
Norman *et al.* (36)	Men: premature baldness, hypertriglyceridemia, and hyperinsulinemia	Not determined
Legro *et al.* (33, 34, 39)	Women: PCOS (NICHD), hyperandrogenemia, insulin resistance Men: increased DHEA-S	Monogenic
Azziz *et al.* (14), Kahsar-Miller *et al.* (32)	Women: PCOS (NICHD)	Not determined
Mao *et al.* (37)	Men: premature baldness	Not determined
Yildiz *et al.* (35)	Women: PCOS (NICHD) and insulin resistance Men: insulin resistance	Not determined

[a] Authors are cited in chronological order. PCO, Polycystic ovaries on ultrasound examination; DHEA-S, dehydroepiandrosterone sulfate.

sclerosis). These authors suggested both a maternal and paternal pattern of inheritance, in which the latter showed higher penetrance and expression (26–28). Of note, in some of these male subjects, oligospermia and increased LH secretion were found pointing to an X-linked pattern of inheritance.

Ferriman and Purdie (29) studied first-degree relatives of hirsute women presenting with or without enlarged ovaries in gynecography. Compared with a control group, patients had an increased prevalence of oligomenorrhea and infertility, and the prevalence of hirsutism was increased in their female first-degree relatives. Hague *et al.* (30) studied the families of 61 patients with ultrasonographic polycystic ovaries and hyperandrogenic symptoms and found that 67% of the mothers and 87% of the sisters of probands were affected. Lunde *et al.* (31) studied the families of 132 Norwegian women with polycystic ovaries previously treated by ovarian wedge resection, who also had two or more of the following symptoms: menstrual irregularity, hirsutism, infertility, and/or obesity. A control group of 71 women and their families was used for comparison (31). Clinical manifestations of hyperandrogenism were found in 31.4% of the female relatives of the patients compared with only 3.2% of the female relatives of the controls (31). Among male relatives, premature balding and increased hairiness were found in 19.7% of the relatives of the patients, but only in 6.5% of the relatives of the controls (31). Azziz and colleagues (14, 32), using NICHD criteria for the diagnosis of PCOS, found that PCOS was present in 35% of the mothers and 40% of the sisters of PCOS patients.

In addition to hyperandrogenism, insulin resistance clusters in the families of hyperandrogenic women. Legro *et al.* (33), also using also NICHD criteria for the definition of PCOS, found that 22% of the sisters of patients actually had PCOS, whereas a further 24% of the sisters of these patients presented with hyperandrogenemia and regular menstrual cycles. This bimodal pattern suggested a monogenic defect for hyperandrogenism (33). Additional studies from Legro *et al.* (34) recently demonstrated that in these families, insulin resistance is associated with hyperandrogenemia rather than with menstrual dysfunction. This suggests that insulin resistance and hyperandrogenemia share the same pathogenic mechanisms, whereas the presence of menstrual dysfunction needed for the diagnosis of PCOS may be only a matter of degree. Therefore, of PCOS sisters, those with PCOS and those presenting with hyperandrogenemia and regular menstrual cycles may actually have functional hyperandrogenism.

The finding of an increased prevalence of insulin resistance in families of PCOS patients has been replicated in the Turkish population. Yildiz *et al.* (35) recently studied 102 relatives of 52 PCOS patients defined by NICHD criteria and found that, compared with different population-based control groups matched for sex, age, and pre- or postmenopausal status with the relatives of PCOS patients, insulin resistance and disorders of carbohydrate metabolism were more frequent in the mothers, sisters, and brothers of PCOS patients, and that the mothers and sisters of PCOS women had increased serum androgen levels compared with the controls.

B. Male phenotype

The lack of a clearly defined male phenotype in families of PCOS patients has burdened the progress in the search for the genetic origin of functional hyperandrogenism and PCOS. Although initial studies suggested that male pattern premature balding was the male equivalent for PCOS (20, 29, 31, 36, 37), these findings have not been universally confirmed (38). More recently, increased serum dehydroepiandrosterone sulfate concentrations in the brothers of PCOS patients described above (39) and insulin resistance in the fathers and brothers of PCOS women (35) have been proposed as the male phenotype in PCOS families. However, there was a considerable overlap between male PCOS relatives and controls in serum dehydroepiandrosterone sulfate

levels and indexes of insulin resistance, and additional studies are needed to establish the actual usefulness of these abnormalities as markers of the syndrome in male relatives of PCOS patients.

C. Twin studies

There are very few data regarding PCOS in twins. Jahanfar *et al.* (40) studied 34 (19 monozygotic and 15 dizygotic) twin pairs, in which PCOS diagnosis was based on ultrasonographic and biochemical findings. Although 11 pairs were discordant for the presence of polycystic ovaries, model-fitting analysis suggested that fasting insulin level, serum androstanediol glucuronide, and body mass index were significantly influenced by genetic factors (40). A later report of this study suggested a genetic origin for an unfavorable lipid profile, especially for increased circulating concentrations of lipoprotein (a), but only in twin pairs concordant for polycystic ovaries (41). These data point to a genetic component in the metabolic abnormalities associated with PCOS but suggest a polygenic etiology in which environmental influences play a significant role.

D. Environmental and other confounding factors

Familial aggregation of hyperandrogenism and PCOS strongly suggest a genetic origin for these disorders, but another possibility is that clustering of PCOS within families results from nongenetic inheritance related to certain environmental factors that are present in the affected families, and not in the families of unaffected women.

As exemplified in Fig. 1, insults during pregnancy may induce intrauterine growth retardation, which has been proposed to induce a thrifty phenotype in small for gestational

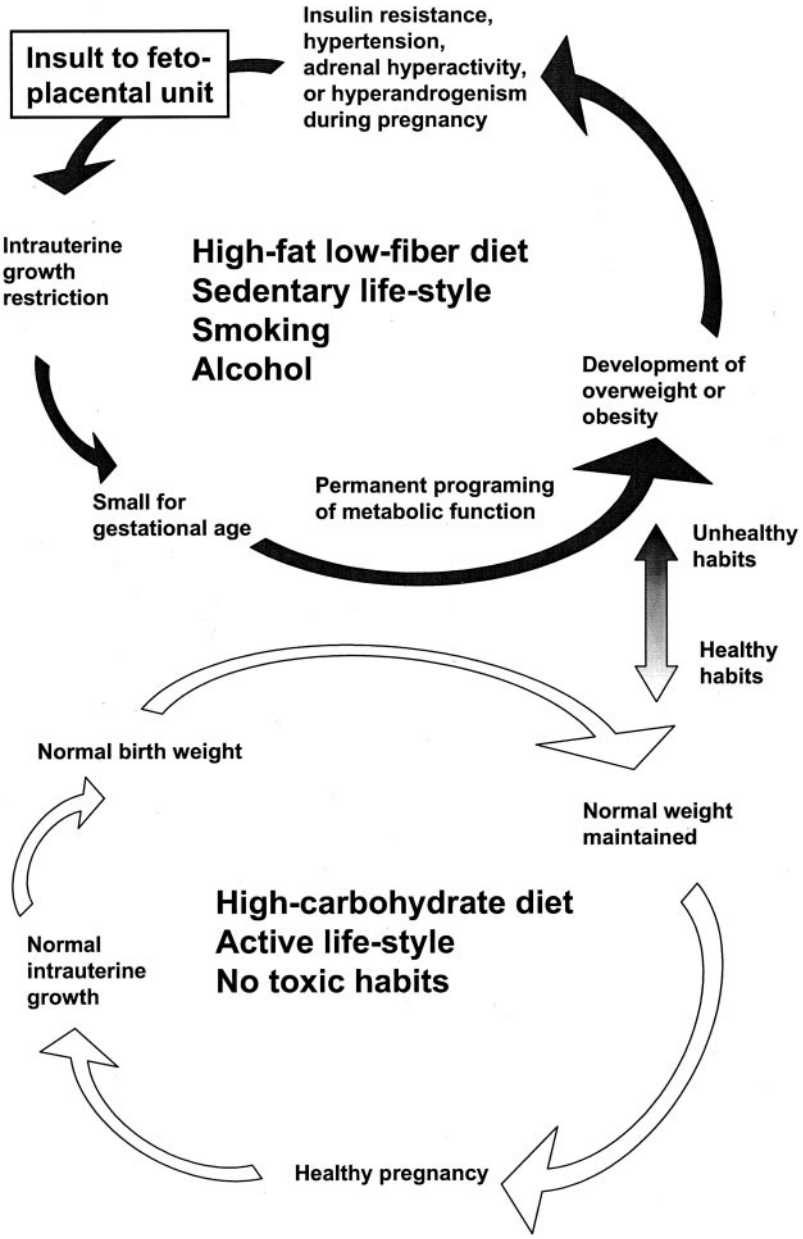

FIG. 1. Intrauterine growth retardation and insulin resistance as an example of nongenetic inheritance, markedly influenced by environmental factors. Insults during pregnancy may result in intrauterine growth retardation, inducing a thrifty phenotype in small for gestational age babies. These women are predisposed to suffer from insulin resistance and may develop hypertension, glucose intolerance, adrenal axis hyperactivity with relative cortisol excess, functional hyperandrogenism, and PCOS later in life, especially when these individuals are exposed to environmental factors such as sedentary lifestyle and a diet rich in saturated fat. These environmental factors may also cluster in certain families because exercising and diet are heavily influenced by parental habits. The metabolic abnormalities associated with the thrifty phenotype can induce further insult to the pregnancies of small for gestational age women, and the defect might be transmitted to another generation without the participation of any genetic abnormality. However, if small for gestational age babies maintain healthy habits, insulin resistance and its consequences might be avoided and, at least in theory, their fetuses will not be exposed to an unfavorable metabolic milieu during pregnancy, preventing nongenetic inheritance of these conditions.

age babies. These babies are predisposed to suffer from insulin resistance, which may result in hypertension, glucose intolerance, adrenal axis hyperactivity with relative cortisol excess, functional hyperandrogenism, and PCOS (42) later in life, especially when these individuals are exposed to environmental factors such as a sedentary lifestyle and a diet rich in saturated fat. These environmental factors may cluster in certain families because exercising and dieting are heavily influenced by parental habits.

The metabolic abnormalities associated with the thrifty phenotype can induce additional insult to the pregnancies of small for gestational age women, and the defect might be transmitted to another generation without the participation of any genetic abnormality. Therefore, the presence of more than one small for gestational age baby in a family may result from exposure of the mothers and their fetuses to the same unfavorable environmental conditions, rather than being related to genetic factors.

Yet it is important to highlight, as shown in Fig. 1, that if small for gestational age babies have healthy habits, insulin resistance and its consequences might be ameliorated, and, at least in theory, their fetuses will not be exposed to an unfavorable metabolic milieu during pregnancy, preventing nongenetic inheritance of these conditions. Nevertheless, intrauterine growth retardation may also be influenced by genetic variants, and the most probable scenario is that of an interaction between predisposing genetic abnormalities with unfavorable environmental conditions. Of note, even the influence of intrauterine growth restriction on the development of PCOS is debatable (43–45), considering that the presence of polycystic ovaries also has been related to above-average birth weight in babies born from obese, androgenized mothers (43–45).

There are very few data on the influence of environmental factors on the development of hyperandrogenism and PCOS, other than the triggering role for obesity on the development of these disorders (46). Although the prevalence of PCOS is similar in different countries, ethnic factors influence the clinical manifestations of the syndrome. In Caucasian premenopausal women, the reported prevalences of PCOS defined by NICHD criteria ranged from 4.7% in Alabama (47) to 6.5% in Spain (48) and 6.8% in the Greek island of Lesbos (49). Moreover, the prevalence in African-American women from Alabama (3.4%) was comparable with that in Caucasian women (47).

However, ethnicity influences the manifestations of PCOS. Carmina *et al.* (50) compared the clinical histories, physical examinations, ovarian morphology by ultrasound, insulin sensitivity, serum gonadotropin, and steroid profiles in 25 Japanese, 25 Italian, and 25 Hispanic-American women with PCOS. The three groups were homogeneous in terms of serum testosterone and adrenal androgen concentrations, insulin resistance, and polycystic ovaries, but Japanese women were less obese and did not present with hirsutism (50).

Dunaif *et al.* (51) found that insulin resistance is higher in Hispanic-Caribbean women with PCOS defined by NICHD criteria compared with Caucasian patients and with control women paired for age, weight, body composition, and ethnicity. Williamson *et al.* (52) recently reported a higher incidence of obesity, dyslipidemia, and infertility, as well as a higher degree of insulin resistance in Maori and Pacific Islander PCOS patients, defined by ultrasonography and clinical symptoms, compared with European patients. In the United Kingdom, the prevalence of polycystic ovaries and type 2 diabetes is increased in Indian subcontinent Asian women (53). Compared with Caucasian women, PCOS patients of southeastern Asian origin presented with oligomenorrhea and were diagnosed at younger ages; hirsutism, acne, acanthosis nigricans, and subfertility were more prevalent, and patients were more insulin resistant (54).

These ethnic differences in the clinical presentation of PCOS may be related to environmental factors such as diet, exercise, and lifestyle. Of note, even the content of polyunsaturated or monounsaturated fatty acids in the diet may influence the metabolic manifestations of the syndrome (55). Thus, because of differences in the environmental factors triggering the development of PCOS, the genes contributing to PCOS patients may also be different, depending on the population studied. These considerations may also apply to the discrepant results of previous studies regarding the association of genomic variants with PCOS.

III. Classic Techniques Used in Molecular Genetic Studies

Before reviewing the molecular genetic studies conducted to date in functional hyperandrogenism and PCOS, we will provide a brief description of the techniques usually used for molecular genetic studies, focusing on the particular advantages and disadvantages of each technique.

A. Linkage analysis

Linkage analysis is a family-based molecular genetic technique that has permitted the identification of the genetic abnormalities leading to many monogenic Mendelian disorders (56). However, most complex metabolic disorders result from the interaction between multiple genes and environmental factors, making their identification much more difficult than in disorders with a Mendelian pattern of inheritance (57–63).

In linkage analysis, markers frequently located at intergenic regions of DNA are studied in multiple members of affected families. The cosegregation of alleles with a disease phenotype is studied, and the linkage of the disease with any marker focuses the search for genomic abnormalities to the DNA regions close to the polymorphic marker segregating with the disorder.

Linkage analysis is based on the meiotic recombination that occurs during ovogenesis and spermatogenesis. The closer two genomic markers are, the more seldom these markers are separated during meiotic recombination. The probability of recombination between two markers is an estimation of the distance between them, and is expressed in centimorgans (cM). Two markers are defined to be 1 cM apart if they become separated by recombination in 1% of meioses. One centimorgan is equivalent on average to 10^6 bp of physical distance. The markers usually studied in linkage studies

include microsatellites and single nucleotide polymorphisms (SNPs).

Classical or parametric linkage analysis requires the specification of a model for the disease or trait *a priori*, in terms of allelic frequencies, penetrance, and pattern of inheritance, and the result is expressed by the LOD score (64). The LOD score is the base 10 logarithm of the odds that two markers (one being the disease-causing or disease-associated genomic variant) are truly linked, divided by the odds that the observed set of data may result from chance if the markers are unlinked. Usually, significant positive proof of genetic linkage requires a LOD score of 3.0 or greater, corresponding to odds favoring linkage of at least 1000:1 at a given specified recombination frequency. Because the pattern of inheritance of functional hyperandrogenism and PCOS is complex and uncertain, nonparametric or model-free linkage analysis is required to ensure the power to detect linkage (22). In nonparametric linkage analysis, multiple DNA markers are obtained from siblings and, if possible, from their parents, and allele sharing between relatives is investigated (64). Allele sharing is defined as identity-by-state (having the same DNA sequence) or as identity-by-descent (when two alleles come from the same ancestral allele) (64). Nonparametric linkage scores use a similarity statistic defined by the average of the possibilities that relatives are identity-by-state (64). However, the number of meioses assessed, disease penetrance, knowledge of the marker coupling phase, and other relevant variables must be considered before reaching a clinically significant conclusion (65).

There are several factors that may limit the usefulness of linkage studies including, among others, the uncertainty about the pattern of inheritance, a variable penetrance of the genetic defect, the delay in the debut of the disease, possible errors in clinical diagnosis, gene to gene interactions, interaction with environmental factors, and the limited resolution of these techniques. The sample size needed to detect a particular effect varies inversely with the square of the effect (62), and therefore genes that contribute modestly to the phenotype may be missed because of the extremely large sample size needed to demonstrate the association. This is especially important given that PCOS affects women in reproductive age and is associated with reduced fertility, limiting the pedigrees and the generations studied to two (mothers and daughters) in most of the linkage analyses performed to date, resulting in small sample sizes within families (66, 67).

The resolution of linkage analysis may be improved using tests such as affected sibling-pair identity by descent analysis or the transmission disequilibrium test (68), allowing the detection of genes of modest effects that might be otherwise missed by linkage analysis. The transmission disequilibrium test involves genotyping the parents of the proband and the proband, and it determines whether or not the parent heterozygous for the putative allele transmits it more often to their affected children than other allele(s). These tests are frequently used to confirm the linkage already established but may also be useful when used without previous evidence for linkage (69).

Linkage with a polymorphic marker only serves to focus the search of the genomic abnormality associated with the disorder to an area of approximately 1–2 cM, and locating the precise genetic defect may involve studying as many as 30–100 genes in the linked area. After obtaining linkage with a genomic region, the search of the particular genomic abnormality involved can be accomplished by fine-mapping of that region using additional markers, and by a candidate gene approach searching for variants in biologically appealing genes located there. This approach has been facilitated by the growing number of genes identified all across the genome, but frequently the demonstration that a gene has a causative role in a disease requires direct sequencing of the whole gene in affected and nonaffected individuals (70).

B. Case-control studies

These studies look for specific genetic markers or alleles that are more frequent in affected individuals (cases) compared with unaffected subjects from the same population (controls). These studies are focused on association of a genetic variant with a disease in a population and not on the mode of inheritance of a trait (71, 72). Association studies are preferable for finding "susceptibility" loci, low-risk alleles that are often found in relatively high frequencies in the general population, and do not result in robust signals in family-based studies (73). A positive association indicates an increased risk for the disease in subjects carrying the at-risk allele, which may have a causative role in the disorder or be in linkage disequilibrium with the gene actually causing the disease. Population stratification is a major problem in case-control studies, because the higher prevalence of particular alleles in different ethnic groups might bias the results and lead to false-positive associations. Moreover, case-control studies are especially prone to type II errors, that is, ruling out an association that is actually present in the population because of the small sample sizes often used in these studies.

In summary, linkage studies and case-control studies are both valid approaches for the study of the genetics of functional hyperandrogenism and PCOS, provided that the sample sizes of these studies are sufficiently large, the diagnostic criteria used to define affected and nonaffected subjects are clearly defined, and both ethnicity and differences in environmental factors are carefully considered.

IV. Studies in Pediatric and Adolescent Hyperandrogenism

Functional hyperandrogenism and hyperinsulinism may be detected early in life in affected women, even before pubertal development. As stated above, the presence of low birth weight in small for gestational age newborns, resulting from an unfavorable environment during intrauterine life and/or to defective maternal nutrition, has been related to the development of insulin resistance (74), disorders of glucose tolerance (75), hypertension, and cardiovascular disease (76–79) later in life.

The association of low birth weight with the development of hyperinsulinism and type 2 diabetes mellitus has been confirmed in different populations (80, 81), and these children present with an accelerated growth and increased body mass index during adolescence.

Although environmental factors may be an important contributor to the restriction of fetal growth in small for gestational age newborns, genetic factors may also be involved in this association (82). According to this hypothesis, affected fetuses might suffer insulin resistance because of the influence of several unknown genes or genomic variants. Insulin resistance would lead to restriction of intrauterine growth and abnormal fetal vascular development, ultimately leading during adult life to increased vascular resistance and endothelial dysfunction. The compensatory increase in circulating insulin concentrations would contribute to the abdominal deposition of fat, and inflammatory cytokines and other mediators secreted by abdominal adipocytes would perpetuate insulin resistance and endothelial dysfunction, leading to abnormal glucose tolerance, atherosclerosis, and cardiovascular disease (82).

However, the analysis of the influence of genomic variants on the association of birth weight and insulin resistance has yielded conflicting results. Dunger *et al.* (83) analyzed a variable number of tandem repeats (VNTR) polymorphism in the insulin gene (*INS*) at 11p15.5. This VNTR polymorphism consists of a repeated sequence of 14 to 15 bp (ACAGGGGTGTGGGG), and is located at −596 bp of the start of transcription site (84). Among Caucasians, these alleles have been typed as class I (small, with 28–44 repeats, frequency approximately 70%), class II (intermediate, rare), and class III (large, with 138–159 repeats, frequency approximately 30%). The VNTR polymorphism in the *INS* promoter regulates the transcriptional rate of the gene (85) and probably that of the gene encoding IGF-II (84–89). Newborns homozygous for class III alleles, which are those associated with increased transcription of the *INS*, presented with increased head circumference, length, and weight at birth as compared with babies homozygous for class I alleles, possibly favoring newborn survival. On the contrary, data from the same researchers suggested that the maternally inherited 16,189 mitochondrial DNA variant, which increases the risk for type 2 diabetes mellitus, was associated with restrained fetal growth, thereby favoring maternal survival by decreasing birth-related morbidity and mortality (90). Therefore, other genetic and environmental factors are possibly involved in the relationship between birth weight and the development of insulin resistance and type 2 diabetes mellitus in adulthood.

Given the association of insulin resistance with functional hyperandrogenism, Ibáñez *et al.* (91) have proposed that low birth weight is related to the development of premature pubarche, and of functional hyperandrogenism during adolescence, insulin resistance being the underlying disorder common to both conditions. Retrospective analysis of a cohort of patients presenting with premature pubarche showed, especially in those girls who developed hyperinsulinemia and functional ovarian hyperandrogenism at adolescence, a lower birth weight compared with a control group, suggesting that low birth weight, premature pubarche, and functional hyperandrogenism are different manifestations of a unique disorder originating during prenatal life (92–95), but becoming clinically apparent peripubertally (42).

Additional analysis of this cohort showed that adolescent girls who had anovulation and higher androgen levels were those with lower birth weight (96) and also confirmed the association of adrenal androgen excess, low birth weight, premature pubarche, and hyperinsulinemia (97).

In conceptual agreement, an exaggerated adrenal response to adrenocorticotropin stimulation, similar to that described in women with functional hyperandrogenism and PCOS, has been found in girls with premature pubarche, suggesting that this disorder can be considered an early manifestation of the syndrome (98). However, the relationship of low birth weight with hyperandrogenism and PCOS has not been universally confirmed. Jaquet *et al.* (99) compared a large population of young women born with intrauterine growth retardation with age-matched healthy controls, and although low birth weight was associated with insulin resistance, androgen levels were similar in both groups of women. Moreover, birth weight had no influence on the presence of hyperandrogenic symptoms suggestive of PCOS in a large cohort of Finnish women recently reported (100).

Given the association of functional hyperandrogenism and insulin resistance in children and adolescents, several genomic variants related to insulin resistance and the metabolic syndrome have been studied in these patients, mostly by the group of Ibánez in Spain and the group of Witchel in Pittsburgh.

The VNTR locus at the *INS* has been studied in girls with a history of premature pubarche (101). Although class I and class III alleles were equally distributed in patients and controls, patients carrying class I alleles presented with lower birth weight and lower insulin sensitivity compared with patients homozygous for class III alleles (101).

The common $Gly^{972}Arg$ variant in the gene encoding the insulin receptor substrate 1 (*IRS-1*), which has been shown to influence insulin resistance and glucose tolerance in adult PCOS patients (102, 103), has also been studied in adolescents (104). The frequencies of heterozygosity for the Gly972 allele were 31% among girls with a history of premature pubarche, 40% among girls with hyperinsulinemic ovarian hyperandrogenism, and only 19% among healthy control subjects. Carriers of Gly972 alleles presented with decreased sex hormone-binding levels (104).

The human homolog for the sorbin and SH3-domain-containing-1 gene (*SORBS1*), which encodes for an important signaling molecule in insulin-stimulated glucose uptake in the mouse, might play a role in human disorders with insulin resistance. The Ala228 allele of the $Thr^{228}Ala$ polymorphism of *SORBS1* is a protective factor for both obesity and diabetes (105). However, alleles of the $Thr^{228}Ala$ polymorphism of *SORBS1* were equally distributed in a multiethnic group of healthy adolescents compared with those presenting with premature pubarche and/or functional hyperandrogenism (106).

The Arg64 allele of the $Trp^{64}Arg$ polymorphism in the β_3-adrenergic receptor gene is associated with abdominal obesity and resistance to insulin and may contribute to the early onset of type 2 diabetes mellitus (107). However, Witchel *et al.* (108) found no differences in the distribution of $Trp^{64}Arg$ alleles in the girls presenting with premature pubarche and/or functional hyperandrogenism described above compared with healthy adults, and neither was an

TABLE 3. Candidate genes involved in androgen biosynthesis, transport, action, and their regulation, in functional hyperandrogenism (FH) and PCOS

Gene	Variant/locus	Design	Subjects	Phenotypic trait	Association
CYP17					
Carey *et al.* (125)	−34T/C	FBS/case-control	PCOS/MPB	PCOS	Yes
Gharani *et al.* (129)	−34T/C	Case-control	PCOS	PCOS/increased T levels	No
Techatraisak *et al.* (131)	−34T/C	Case-control	PCOS	PCOS/increased T levels	No
Liovic *et al.* (133)	−34T/C	Case-control	PCOS	PCOS	No
Urbanek *et al.* (132)	D10S192	FBS (TDT)	PCOS	PCOS	No
Diamanti-Kandarakis *et al.* (128)	−34T/C	Case-control	PCOS	Increased T levels	Yes
Marszalek *et al.* (130)	−34T/C	Case-control	PCOS	PCOS and hormone profile	No
SF1, DAX-1, StAR protein					
Urbanek *et al.* (132)	D8S1821 (StAR)	FBS (TDT)	PCOS	PCOS	No
Calvo *et al.* (144)	Mutation scanning (SF-1, DAX-1, StAR protein)	Case-control	Hirsutism	Hyperandrogenism	No
CYP11B2					
Zhao *et al.* (207)	−344T/C	Case-control	PCOS	PCOS	Yes
CYP21					
Hague *et al.* (148)	HLA: ↑ DRW6 and ↓ DR7	Case-control	PCOS/CAH	PCOS	Yes
Witchel *et al.* (112, 113)	Heterozygosity for *CYP21* mutations	Case-control	PP/FH	Hyperandrogenic symptoms in children and adolescents	Yes
Escobar-Morreale *et al.* (147)	Heterozygosity for *CYP21* mutations	Case-control	FH	Origin of androgen excess	No
HSD3B2					
Chang *et al.* (155)	Mutation scanning	Case series	PP/hirsutism	↑ 17-Hydroxypregnenolone	No
Nayak *et al.* (111)	Case-control	Case-control	PP, FH (adolescents)	PP, FH	No
Urbanek *et al.* (132)	D1S514	FBS (TDT)	PCOS	PCOS	No
HSD17B					
Moghrabi *et al.* (159)	*HSD17B3* G289A	Case-control	PCOS	PCOS	No
Urbanek *et al.* (132)	*HSD17B1*, *HSD17B2*, *HSD17B3* (D9S1809)	FBS (TDT)	PCOS	PCOS	No
CYP19					
Gharani *et al.* (140)	*CYP19*(ttta)n/D15S103	FBS/case-control	PCOS	PCOS	No
Urbanek *et al.* (132)	*CYP19*	FBS (TDT)	PCOS	PCOS	No
LHβ					
Rajkhowa *et al.* (169)	$Trp^{8}Arg$ and $Ile^{15}Thr$	Case-control	PCOS	Increased in obese PCOS	Yes
Liao *et al.* (174)	G1502A	Case-control	PCOS	PCOS	No
Tapanainen *et al.* (170)	$Trp^{8}Arg$ and $Ile^{15}Thr$	Case-control	PCOS	Reduced in obese PCOS	Yes
Ramanujam *et al.* (172)	$Trp^{8}Arg$ and $Ile^{15}Thr$	Case-control	Menstrual disorders	Menstrual disorders	Yes
Elter *et al.* (173)	$Trp^{8}Arg$ and $Ile^{15}Thr$	Case-control	PCOS	PCOS	No
Takahashi *et al.* (175)	−894C/T, −1018G/C, −1036C/A, −1098C/T and −1423C/T	Case-control	Ovulatory disorders	Ovulatory disorders	Yes
FSHβ					
Tong *et al.* (176)	TAT/TAC in codon 76	Case-control	PCOS	Obesity and PCOS	Yes
FSH receptor					
Urbanek *et al.* (132)	D2S1352	FBS (TDT)	PCOS	PCOS	No
Tong *et al.* (177)	$Thr^{307}Ala/Ser^{680}Asn$	Case-control	PCOS	PCOS	No
Takakura *et al.* (178)	Exons 6, 7, 9, and 10	Case-control	PCOS	PCOS	No
GnRH receptor					
Cohen *et al.* (179)	Mutation scanning	Case series	PCOS	PCOS	No
Dopamine receptor					
Legro *et al.* (181)	*Msc*I polymorphism	Case-control	PCOS	PCOS	Yes
Kahsar-Miller *et al.* (182)	*Msc*I polymorphism	Case-control	PCOS	PCOS	No
SHBG					
Urbanek *et al.* (132)	D17S1353	FBS (TDT)	PCOS	PCOS	No
Hogeveen *et al.* (184)	P156L	Case-series	PCOS, hirsutism, and ovarian failure	PCOS, hirsutism, and ovarian failure	Yes
Xita *et al.* (185)	(TAAAA)n	Case-control	PCOS	PCOS and SHBG levels	Yes
Cousin *et al.* (186)	(TAAAA)n	Case series	Hirsutism	SHBG levels	Yes
	$Asp^{327}Asn$	Case series	Hirsutism	SHBG levels	Yes
Glucocorticoid receptor					
Witchel and Smith (188)	Mutation scanning, N363S	Case-control	PP, FH (adolescents)	PP, FH	No
Kahsar-Miller *et al.* (189)	N363S	Case-control	PCOS	PCOS/adrenal androgens	No
Calvo *et al.* (187)	Mutation scanning	Case series	Adrenal FH	Adrenal androgen excess	No

TABLE 3. *Continued*

Gene	Variant/locus	Design	Subjects	Phenotypic trait	Association
AR					
Legro *et al.* (198)	(CAG)n	Case-control	Hyperandrogenism	Hirsutism	Yes
Sawaya and Shalita (200)	(CAG)n	Case-control	Hirsutism, acne	Clinical hyperandrogenism	Yes
Vottero *et al.* (202)	X-inactivation	Case-control	Hirsutism	Idiopathic hirsutism	Yes
Urbanek *et al.* (132)	*AR*	FBS (TDT)	PCOS	PCOS	No
Mifsud *et al.* (201)	(CAG)n	Case-control	PCOS	PCOS and normal T levels	Yes
Calvo *et al.* (204)	(CAG)n/X-inactivation	Case-control	Hirsutism	Idiopathic hirsutism	No
Hickey *et al.* (203)	(CAG)n/X-inactivation	Case-control	PCOS	PCOS and T levels	Yes
Ibáñez *et al.* (116)	(CAG)n	Case-control	PP	PP and ovarian hyperandrogenism	Yes
UDP-glucuronyltransferase 2B15					
Tomboc and Witchel (117)	D85Y	Case-control	PP, FH (adolescents)	PP, FH	No

Authors are cited in chronological order. CAH, Congenital adrenal hyperplasia; FBS, family-based study; MPB, male premature baldness; PP, premature pubarche; T, total testosterone; TDT, transmission disequilibrium test; X-inactivation, skewed X chromosome inactivation.

influence of this polymorphism on the body mass index of these girls. Similarly, the study of the common $Pro^{12}Ala$ polymorphism in the gene encoding the peroxisome proliferator-activated receptor-γ2 (*PPAR-γ2*), which influences insulin sensitivity in Caucasians (109), has been studied in children with premature pubarche and in adolescent hyperandrogenic girls, but no association was found with any of the *PPAR-γ2 alleles* and hyperandrogenism (110).

In addition to genes related to insulin resistance, those encoding for the steroidogenic enzymes involved in androgen biosynthesis and the genes encoding several molecules involved in androgen metabolism and action have been considered candidate genes to explain premature pubarche and adolescent hyperandrogenism.

Witchel and colleagues from Pittsburgh conducted a series of studies focused on the steroidogenic enzymes involved in androgen synthesis, which are shared by the adrenal and the ovaries. These authors have found that children with premature pubarche and adolescent girls with hyperandrogenism were heterozygous for mutations in *CYP21*, the gene encoding for 21-hydroxylase, and in the gene encoding 3β-hydroxysteroid-dehydrogenase (*HSD3B2*), more frequently than nonhyperandrogenic controls (111–113). In these patients, *CYP17* mutations were ruled out as the cause of androgen excess (114). However, because congenital adrenal hyperplasia is an autosomal recessive disease requiring mutations in both alleles–in homozygosity or double heterozygosity–of *CYP21* or of *HSD3B2*, it is unclear how carrying only one defective allele of these genes in these clinically symptomatic heterozygotes contributes to the hyperandrogenic phenotype (113). Moreover, defective *CYP21* and *HSD3B2* alleles were found only in a minority of these patients, and the authors concluded that other genomic variants were also involved in the pathogenesis of these disorders (111–113).

To further explore this possibility, Witchel *et al.* (115) evaluated the prevalence of the common variants and mutations in *CYP21*, *HSD3B2*, *IRS-1*, β_3-adrenergic receptor gene described above, and the glucocorticoid receptor gene within their series of children with premature pubarche and adolescent hyperandrogenism. They found that the prevalence of these variants and mutations was clearly increased when compared with 15 healthy controls, suggesting that the occurrence of multiple sequence variants in these genes might contribute to the development of hyperandrogenism.

Finally, the AR and uridine diphosphate-glucuronyltransferase 2B15, an enzyme involved in androgen inactivation, have been considered candidate genes for premature pubarche and adolescent hyperandrogenism. The study of the AR gene CAG repeat polymorphism suggested that shorter AR gene CAG numbers, indicative of increased androgen sensitivity, were associated with premature pubarche (116), whereas the allele frequencies of the D85Y polymorphism in uridine diphosphate-glucuronyltransferase 2B15 gene were similar in patients and in controls (117).

In summary, studies conducted to date in girls with premature pubarche and adolescent hyperandrogenism suggest a polygenic etiology for these disorders, which can be considered predictors of functional hyperandrogenism and PCOS later in life.

V. Studies in Hyperandrogenic Adults

Most if not all the studies conducted to date to elucidate the genetics of functional hyperandrogenism and PCOS have used a candidate gene approach, because no genome-wide scan has been conducted comparable with those done for other complex metabolic disorders, such as type 2 diabetes mellitus. Although many genes have been considered candidates to explain PCOS inheritance, most studies have included genes related to androgen biosynthesis and action and their regulation, genes involved in insulin resistance and associated disorders, and lately genes involved in chronic inflammation and atherosclerosis.

A. Genes involved in androgen biosynthesis, transport, and action, and their regulation (Table 3)

1. CYP17. The limiting step in androgen biosynthesis in the ovary and adrenal gland is an enzyme termed P450c17α, which possesses both 17α-hydroxylase and 17,20-lyase activities. Therefore, P450c17α may catalyze the conversion of pregnenolone and progesterone into 17-hydroxypreg-

nenolone and 17-hydroxyprogesterone, respectively, and of these steroids into dehydroepiandrosterone and androstenedione, although in humans only the conversion of Δ^5 steroids has been demonstrated (118).

Rosenfield *et al.* (2, 119, 120) proposed several years ago that women with functional hyperandrogenism and PCOS had an exaggerated adrenal and ovarian responsiveness, and that increased activity of P450c17α was responsible for the enhanced androgen synthesis and secretion. They proposed the increase in serum 17-hydroxyprogesterone in response to the GnRH analog nafarelin as the marker of functional ovarian hyperandrogenism (119). Studies using adrenal stimulation with ACTH and ovarian stimulation and suppression with the long-acting GnRH triptorelin also suggested that most hyperandrogenic women have increased P450c17α activity in the adrenal and ovary (121–123), and that the increased adrenal P450c17α activity was not influenced by ovarian function (122). Moreover, P450c17α expression and activity are increased in ovarian theca cells from PCOS women, defined by NICHD criteria, compared with those from nonhyperandrogenic controls (124).

Given these findings, the gene encoding the P450c17α enzyme, *CYP17*, was considered a candidate gene in early studies. *CYP17* is located in chromosome 10q24.3 (125, 126), and its promoter contains a T/C SNP at −34 bp from the start of transcription site that might modulate enzyme activity. Some studies suggested that this polymorphism was associated with the presence of polycystic ovaries on ultrasound (125), and PCOS patients homozygous for C alleles of this polymorphism presented with increased serum testosterone levels (127, 128). Other studies failed to confirm these observations, suggesting that this base change is a polymorphism without functional consequences for the development of polycystic ovaries and hyperandrogenism (129–131). Also, no evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the *CYP17* locus in a family-based study that included mostly families of European ancestry from the United States (132). Moreover, no abnormalities were found after single-strand conformational polymorphism analysis of the entire coding region of *CYP17* in a small sample of PCOS patients with or without exaggerated 17-hydroxyprogesterone response to a GnRH analog (133), ruling out *CYP17* as a major candidate gene for the pathogenesis of PCOS and functional hyperandrogenism. However, posttranscriptional hyperphosphorylation of the serine residues of P450c17α by a defective serine kinase might increase the 17,20-lyase activity of this enzyme, contributing to hyperandrogenism (118, 134). Confirmation of this hypothesis is still pending (135).

2. CYP11A. Ovarian theca cells from women with PCOS defined by NICHD criteria overexpress all the steroidogenic enzymes involved in androgen biosynthesis (136, 137), and these cells secrete increased amounts of progesterone, 17-hydroxyprogesterone, testosterone, and androstenedione compared with theca cells from nonhyperandrogenic women (136, 138).

The initial step in adrenal and ovarian steroidogenesis is the conversion of cholesterol into progesterone, which is catalyzed by the cholesterol side chain cleavage enzyme. The *CYP11A* gene, located at 15q24, encodes the cholesterol side chain cleavage enzyme and has therefore been considered a candidate gene for functional hyperandrogenism and PCOS (139). It has been proposed that a VNTR polymorphism, consisting in repeats of a (tttta)n pentanucleotide at −528 bp from the ATG start of translation site in the *CYP11A* promoter, plays a role in the pathogenesis of PCOS (140). Evidence for linkage with the *CYP11A* locus was found in 20 pedigrees presenting with PCOS, based mostly on the presence of polycystic ovaries or male pattern premature balding, and the absence of the more common four-repeats allele (this VNTR appears with four, six, eight, and nine repeats in Caucasians) was associated with hirsute PCOS patients and with higher serum testosterone levels (140). Diamanti-Kandarakis *et al.* (141), using NICHD criteria for the definition of PCOS, confirmed its association with absence of four-repeat alleles in Greek patients. In women from the United States, nine-repeat alleles were more frequent in PCOS patients, defined by oligomenorrhea and polycystic ovaries, and four- and six-repeat alleles were more frequent in controls, but these allelic differences did not influence *CYP11A* expression in theca cells (142).

Other studies failed to demonstrate linkage with the *CYP11A* locus in PCOS patients defined by NICHD criteria (132) or association of *CYP11A* VNTR alleles with functional hyperandrogenism (143). Moreover, in a small sample of hirsute hyperandrogenic patients from Spain (144), no consistent genomic abnormalities have been found in the entire *CYP11A* coding region, nor in the genes encoding the steroidogenic acute regulatory protein, steroidogenic factor-1, and dosage-sensitive sex reversal-adrenal hypoplasia gene on the X chromosome gene-1 (DAX-1), which are also involved in the first step of steroidogenesis and its regulation. The family-based study cited above failed also to demonstrate linkage or association with the steroidogenic acute regulatory protein (132). Finally, Gaasenbeek *et al.* (145) recently failed to confirm any influence of *CYP11A* VNTR alleles on polycystic ovaries and on serum testosterone levels in a series of experiments involving a large number of subjects, concluding that the existence of associations between *CYP11A* promoter variation and androgen-related phenotypes had been substantially overestimated in previous studies, including their own preliminary report cited above (140).

3. CYP21. An exaggerated serum 17-hydroxyprogesterone response to ACTH stimulation is a common finding in women with PCOS or functional hyperandrogenism (121, 146). This finding prompted several groups to study *CYP21*, which encodes the 21-hydroxylase enzyme catalyzing the conversion of 17-hydroxyprogesterone into 11-deoxycortisol; increased serum 17-hydroxyprogesterone levels are the biochemical marker for 21-hydroxylase deficiency. This type of congenital adrenal hyperplasia is an autosomal recessive disease resulting from homozygosity or double heterozygosity for missense or nonsense mutations in *CYP21* and is characterized by hyperandrogenism with or without mineralocorticoid deficiency.

However, whether or not heterozygous *CYP21* mutations influence functional hyperandrogenism and PCOS is still matter of debate. As stated above, girls presenting with pre-

mature adrenarche and adolescent hyperandrogenism carry *CYP21* mutations more frequently than nonhyperandrogenic controls (112). Clinically symptomatic heterozygotes for *CYP21* mutations present with a phenotype that resembles that of PCOS (113), but it is unclear whether all carriers of *CYP21* mutations have an increased risk of having PCOS. Moreover, in hyperandrogenic women carrying *CYP21* mutations there is no clear concordance between the *CYP21* genotype and the functional origin of androgen excess (147).

Finally, the presence of polycystic ovaries in ultrasound scans has been associated with increased frequency of DRW6 and decreased DR7 human leukocyte antigen haplotypes, whereas 21-hydroxylase deficiency is associated with the Bw47, B14, or DR1 haplotypes (148). Moreover, in families of patients with 21-hydroxylase deficiency, polycystic ovaries segregate independently from adrenal dysfunction (149).

4. HSD3B2. This enzyme catalyzes the conversion of Δ^5 steroids into Δ^4 steroids in the adrenal and in the ovary. Mutations in *HSD3B2* result in a rare form of classic congenital adrenal hyperplasia, causing various degrees of salt wasting in both sexes and incomplete masculinization of the external genitalia in genetic males because both adrenal and ovarian steroidogenesis are severely impaired (150). Biochemically, this disorder is characterized by a marked increase in serum 17-hydroxypregnenolone and dehydroepiandrosterone concentrations (150).

Mild increases in these steroid precursors, and in the ratio of Δ^5 to Δ^4 steroids, are not infrequent in hyperandrogenic patients, and hyperandrogenic patients presenting with increased 17-hydroxypregnenolone concentrations and/or increased Δ^5 to Δ^4 ratios after adrenal stimulation with cosyntropin were initially considered to have a nonclassic form of HSD3B2 deficiency (151–153). However, these mild increases in serum 17-hydroxypregnenolone concentrations and in the Δ^5 to Δ^4 ratios were interpreted by others as an exaggerated adrenal response to cosyntropin stimulation without a genetic origin (154). Molecular analysis of *HSD3B2* of hyperandrogenic patients presenting with increased Δ^5 steroids and increased Δ^5 to Δ^4 ratios revealed no abnormalities (155, 156), ruling out the existence of a nonclassic form of HSD3B2 deficiency in these women. In conceptual agreement, a marker close to the HSD3B2 locus was not in linkage or association with PCOS, defined by NICHD criteria, in the American family-based study cited above (132).

5. 17β-Hydroxysteroid dehydrogenases. 17β-Hydroxysteroid dehydrogenase type III is also known as 17-ketosteroid reductase. This enzyme catalyzes the conversion of androstenedione into testosterone in the testis. Up to 20 mutations in *HSD17B3* have been identified as the cause of male pseudohermaphroditism because of testosterone deficiency (157). Pang *et al.* (158) hypothesized that genetic females with ovarian 17-ketosteroid reductase deficiency would probably have a normal female phenotype at birth and normal pubertal breast development but would present at puberty with virilization and menstrual disorders because of the sudden increase of the ovarian secretion of androstenedione. However, no mutations have been identified in *HSD17B3* in Caucasian and African-American women presenting with PCOS defined by NICHD criteria (159). Also, no evidence for linkage or association with the type 1, type 2, and type 3 17β-hydroxysteroid dehydrogenase loci was found in the American family-based study cited previously (132).

6. Aromatase. This enzyme, encoded by *CYP19*, is responsible for the conversion of C19 steroids (androgens) into C18 steroids (estrogens). Aromatase activity may be decreased in granulosa cells and follicles from women with PCOS, and the possible androgen excess resulting from this decreased activity might contribute to abnormal follicle development (160, 161). Although mutations in *CYP19* have been associated with multicystic ovaries in a case report, the clinical picture was that of hypergonadotropic hypogonadism (162). Moreover, no evidence for linkage of *CYP19* with PCOS was found in studies conducted in the United Kingdom (140) and in the United States (132).

7. 11β-Hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase. These enzymes regulate the inactivation of cortisol into cortisone at the tissue level. 11β-Hydroxysteroid dehydrogenase type 1 converts cortisol into cortisone directly, and endoluminal hexose-6-phosphate dehydrogenase regenerates reduced nicotinamide adenine dinucleotide phosphate in the endoplasmic reticulum, thereby influencing the directionality of 11β-hydroxysteroid dehydrogenase type 1 activity. Mutations in the genes encoding 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase have been recently shown to cause cortisone reductase deficiency (163), and because the phenotypic features of affected women resemble those of PCOS, these genes should be considered candidates to explain the pathogenesis of PCOS. However, this possibility has not been explored in any series of patients with functional hyperandrogenism and PCOS to date.

8. Gonadotropins. In as many as 40% of PCOS patients, LH hypersecretion is present (164). The gene encoding the β-subunit of LH (LHβ), which is responsible for LH specificity, has been explored in PCOS and hyperandrogenic women. Initially, two point mutations, $Trp^{8}Arg$ and $Ile^{15}Thr$, were identified as the cause of an immunologically abnormal LHβ molecule (165). These mutations induce structural changes in the mutant LHβ molecule (166) and have a 15% prevalence worldwide, although with significant differences depending on the population studied (167). The resulting LHβ molecule has an increased *in vitro* activity and a decreased *in vivo* half-life compared with the wild-type molecule (168).

This LHβ variant has been associated with increased serum testosterone, estrogen, and SHBG levels in healthy women, but not in PCOS patients (169). Furthermore, the prevalence of this LHβ variant is reduced in obese women with PCOS, suggesting a protective role against the development of hyperandrogenic symptoms in these women and a possible usefulness in determining the risk for PCOS in obese women (170, 171). However, no association with PCOS, hyperandrogenism, or serum androgen or estrogen concentrations has been found in other studies (172, 173).

Other variants in the LHβ gene might influence hyperandrogenism and PCOS. A $Gly^{102}Ser$ mutation in exon 3 has been related to menstrual disorders in Chinese women (172,

174). Finally, in Japanese patients with ovulatory disorders, several SNPs in the promoter of the LHβ gene (−894C/T, −1018G/C, −1036C/A, −1098C/T, and −1423C/T) are more frequent than in normal ovulatory women (175).

Polymorphisms in the FSH β-subunit gene have also been reported to influence PCOS. In Chinese women, homozygosity for a thymine-cytosine substitution in exon 3 (codon 76, TAT to TAC) has been found more frequently in PCOS patients, as defined by oligomenorrhea and polycystic ovaries, compared with nonhyperandrogenic women, especially in obese patients, and correlating with higher serum androgen concentrations (176). In these women, two SNPs in the gene encoding the FSH receptor, Thr^{307}Ala and Ser^{680}Asn, showed similar allele distribution among PCOS patients and controls (177). Moreover, the search for mutations in exons 6, 7, 9, and 10 of the FSH receptor gene, which have been shown previously to inactivate the receptor, yielded negative results in Japanese patients with oligomenorrhea and polycystic ovaries (178). This locus was not in linkage or association with PCOS in a family-based study conducted in the United States (132). Finally, mutations in the gene encoding the GnRH receptor have not been found in PCOS patients, defined by NICHD criteria (179).

9. Dopamine receptor. Given that dopamine is involved in the hypothalamic control of gonadotropin secretion, the association between polymorphisms in the dopamine receptor and disorders of ovulation and fertility has been studied. Legro *et al.* (180) reported that SNPs in exons 5 and 6 of the gene encoding the type 2 dopamine receptor influenced serum gonadotropin and prolactin levels, as well as parity and prevalence of miscarriage in women of Hispanic ancestry. In these women, a genomic variant in the type 3 dopamine receptor was associated with hyperandrogenic chronic anovulation and resistance to clomiphene citrate (181), but this association was not confirmed in a large series of non-Hispanic women (182).

10. SHBG. SHBG regulates the access of testosterone and estradiol to target tissues. Decreased SHBG concentrations are characteristic of hyperandrogenic women, contributing to increased tissue androgen availability (183). Despite the fact that evidence for linkage or association was not found between a marker close to the *SHBG* locus and PCOS in a family-based study conducted in the United States (132), a missense Pro^{156}Leu mutation in the SHBG gene, resulting in abnormal glycosylation and secretion of a SHGB mutant that retains binding capacity (184), has been detected in a women presenting with severe hyperandrogenism during pregnancy. However, it is seldom found in unselected hyperandrogenic women (184).

Recently, an association between a (TAAAA)n polymorphism in the promoter of the SHBG gene and PCOS has been reported (185). Longer alleles (more than eight repeats) were frequent in Greek PCOS patients, defined by NICHD criteria, whereas nonhyperandrogenic women presented with a higher frequency of shorter alleles (185). Furthermore, in the PCOS group, carriers of the longer allele genotypes had lower SHBG levels than in those with shorter alleles (185). Therefore, this polymorphism might be related to the low SHBG levels characteristic of PCOS, facilitating androgen availability to target tissues. Cousin *et al.* (186) studied the (TAAAA)n polymorphism in hirsute women and found strong disequilibrium linkage with an Asp^{327}Asn SNP in exon 8 of *SHBG*, 327Asn alleles being associated with eight-repeat (TAAAA)n alleles, and resulting in increased serum SHBG levels when compared with subjects homozygous for 327Asp alleles. Moreover, longer (TAAAA)n alleles resulted in decreased serum SHBG levels when compared with six-repeat alleles (186), in conceptual agreement with the results in PCOS women described above (185).

11. Glucocorticoid receptor. Mutations in the glucocorticoid receptor gene result in a compensatory increase in circulating ACTH, resulting in excess secretion of adrenal androgens. Therefore, the glucocorticoid receptor has been studied as a candidate gene for functional hyperandrogenism and PCOS. However, genomic abnormalities and polymorphisms in the glucocorticoid receptor gene do not appear to influence the pathogenesis of functional hyperandrogenism (187, 188) and PCOS (189).

12. Steroid 5α-reductase (SRD5A). This enzyme catalyzes the conversion of testosterone into the more potent androgen dihydrotestosterone. SRD5A1 and SRD5A2 are the two isoforms of the enzyme. Mutations in *SRD5A2* result in male pseudohermaphroditism (190, 191). Because total SRD5A activity is increased in polycystic ovaries (192), the genes encoding the SRD5A isoforms can be considered candidate genes for functional hyperandrogenism and PCOS. To date, no data have been published regarding the possible involvement of molecular genetic variants in these genes in hyperandrogenic disorders.

13. AR. The AR is a member of the superfamily of ligand-activated transcription factors that regulate many biological processes and is encoded by a gene located at Xq11–12 (193, 194). The AR has three functional domains responsible for transactivation, for ligand binding, and for binding to DNA. Exon 1 contains a VNTR polymorphism consisting of (CAG)n repeats, which encodes for a polyglutamine tract in the N-terminal transactivation domain of the AR protein (195).

The number of CAG repeats in the normal population varies between 11 and 31, with 20 repeats being the most frequent (196). The number of CAG repeats is inversely related to the transactivation of the AR and its activity (197). Thus, the decreased number of CAG repeats has been proposed to increase androgen activity at target tissues, favoring hirsutism (198), premature pubarche, and ovarian hyperandrogenism (116) in women, infertility in men (199), as well as androgen-dependent skin disorders in both men and women (200). A decreased number of AR gene CAG repeats has been suggested to explain the normal serum androgen levels found in some women with polycystic ovaries, infertility, and oligomenorrhea, in whom the hyperandrogenic symptoms would result from the intrinsic increase in the AR activity (201).

One of the two X chromosomes in every cell of a woman undergoes inactivation, a process that involves methylation of DNA and occurs in a random fashion. Skewed inactivation

of X chromosome with the larger CAG repeat, favoring expression of the shorter allele, has been proposed to play a role for idiopathic hirsutism and PCOS (202, 203). However, we have shown that both the number of CAG repeats and the prevalence of skewed X chromosome inactivation were equally distributed in hirsute patients with or without hyperandrogenemia and in healthy women (204). Moreover, no evidence for linkage or association with PCOS was found with the *AR* locus in the family-based study conducted in the United States cited above (132).

14. Aldosterone synthetase. The renin-angiotensin system may be hyperactive in women with PCOS (205, 206). Recently, a −344T/C SNP in the promoter of the gene encoding aldosterone synthetase, *CYP11B2*, has been proposed to influence the pathogenesis of PCOS, because C alleles are more frequent in these patients than in healthy controls (207). Interestingly, women homozygous for C alleles presented with increased plasma renin activity and increased serum angiotensin II, aldosterone, and testosterone levels compared with women homozygous for T alleles, suggesting that this polymorphism influences the activity of aldosterone synthetase and also contributes to androgen excess (207).

B. Genes involved in insulin resistance and associated disorders (Table 4)

1. Insulin receptor gene (INSR). The presence of insulin resistance is common in PCOS (3) and is frequent in other hyperandrogenic patients (208–210). Therefore, the genes encoding the INSR and those encoding several molecules involved in postreceptor signaling have been studied in hyperandrogenic patients.

The INSR is a heterotetrameric glycoprotein consisting of two α- and two β-subunits, and it is encoded by a gene located at chromosome 19. A marker relatively close (1 cM) to *INSR*, D19S884, has been reported in association with PCOS, defined by NICHD criteria, using the transmission disequilibrium test in a family-based study (132), and in a case-control study (211), both conducted in the United States. However, this association was not confirmed in PCOS patients from Spain and Italy using a case-control design (212). In conceptual agreement with the latter, the 22 exons of *INSR* have been sequenced in PCOS patients with negative results (213). *INSR* contains several polymorphisms, yet most of them are silent polymorphisms or are located in intronic regions and are present with similar frequencies in patients with polycystic ovaries and hyperandrogenism and in controls (214).

Recently, Siegel *et al.* (215) have observed a C/T SNP at the tyrosine kinase domain of *INSR* associated with PCOS defined by NICHD criteria. This SNP could be a susceptible variant for PCOS, or it could be in linkage disequilibrium with another *INSR* polymorphism; such an association must be confirmed in future studies.

Studying cultured skin fibroblasts and muscle samples from PCOS patients defined by NICHD criteria, Dunaif *et al.* (216) found increased INSR serine phosphorylation, which decreases its protein tyrosine kinase activity, in as many as 50% of the cases. Subsequent studies in these patients confirmed *in vivo* the defect in insulin signaling, using serial skeletal muscle biopsies obtained during euglycemic glucose clamp studies (217). However, the search for mutation in the tyrosine kinase domain of the INSR gene did not show abnormalities other than polymorphisms in exon 17, which were not associated with insulin resistance (218). Therefore, the still unidentified factor responsible for the increased phosphorylation of the serine residues of the INSR appears to be extrinsic to the receptor and might also contribute to the increased serine phosphorylation of P450c17α also found in some PCOS patients (118).

2. IRS-1 and IRS-2. After insulin binding, autophosphorylation of tyrosine residues results in the activation of the INSR, and tyrosine kinase activity phosphorylates intracellular substrates such as IRS-1 and IRS-2 (3). PCOS women present a defect in insulin receptor signaling characterized by a decreased IRS-1-associated phosphatidylinositol 3-kinase activity (217). Two common SNPs in the genes encoding insulin-receptor substrates, $Gly^{972}Arg$ in *IRS-1* and $Gly^{1057}Asp$ in *IRS-2*, are susceptibility genes for type 2 diabetes mellitus (219, 220); they have been studied in PCOS patients, despite the fact that evidence for linkage or association with PCOS was not found with *IRS-1* in a family-based study conducted in the United States (132).

Carriers of Arg972 *IRS-1* alleles presented with increased fasting insulin levels compared with women homozygous for Gly972 alleles, whereas carriers of Asp1057 *IRS-2* alleles presented with increased glucose and insulin levels 2 h after an oral glucose load and had an increased prevalence of glucose intolerance compared with subjects homozygous for Gly1057 alleles (102). However, a subsequent study in a larger series of PCOS patients showed only the effect of the $Gly^{1057}Asp$ polymorphism in *IRS-2* on glucose tolerance, and no effect of the $Gly^{972}Arg$ polymorphism in *IRS-1* (103). Surprisingly, the effect was just the opposite found previously, because the 2-h glucose values were actually increased in subjects homozygous for Gly1057 alleles when compared with carriers of Asp1057 alleles (103). Additional studies are needed to confirm the influence of these polymorphisms on glucose tolerance and insulin resistance in PCOS.

3. INS. The presence of pancreatic β-cell dysfunction in women presenting with PCOS appears to have a genetic origin (221). Therefore, *INS* has been studied in women with PCOS and functional hyperandrogenism. Waterworth *et al.* (222) found that women with menstrual disturbances and/or hirsutism and polycystic ovaries, who were homozygous for class III alleles, were more frequently anovulatory and had increased body mass index and fasting insulin compared with women homozygous for class I alleles. Moreover, class III alleles were associated with symptomatic women (222, 223). Paternal transmission of class III alleles from heterozygous fathers to anovulatory PCOS patients is more frequent than maternal transmission of the allele (222–224), and class III alleles predisposed these patients to both PCOS and type 2 diabetes mellitus. However, later case-control studies in European Caucasian women, conducted outside the United Kingdom, have failed to reproduce these results (225, 226), and the *INS* locus was not associated with PCOS in a linkage study in American PCOS patients (132).

TABLE 4. Genes involved in insulin resistance and associated disorders, in functional hyperandrogenism (FH), and PCOS

Gene	Variant/locus	Design	Subjects	Phenotypic trait	Association
INSR					
Sorbara *et al.* (213)	Mutation scanning	Case series	PCOS	Insulin resistance	No
Conway *et al.* (218)	Mutation scanning	Case series	PCOS	Insulin resistance	No
Urbanek *et al.* (132)	D19S884 and other loci	FBS (TDT)	PCOS	PCOS	Yes
Talbot *et al.* (214)	Mutation scanning	Case-control	PCOS	Insulin resistance	No
Tucci *et al.* (211)	D19S884	Case-control	PCOS	PCOS	Yes
Siegel *et al.* (215)	C10923T	Case-control	PCOS	Lean PCOS patients	Yes
Villuendas *et al.* (212)	D19S884	Case-control	PCOS	PCOS	No
IRS 1 and 2					
Urbanek *et al.* (132)	*IRS1*	FBS (TDT)	PCOS	PCOS	No
El Mkadem *et al.* (102)	$Gly^{972}Arg$ (*IRS-1*)	Case-control	PCOS	↑ Insulin resistance	Yes
	$Gly^{1057}Asp$ (*IRS-2*)	Case-control	PCOS	↑ 2 h Insulin and glucose (OGTT)	Yes
Ehrmann *et al.* (103)	$Gly^{972}Arg$ (*IRS-1*)	Case series	PCOS	Insulin and glucose levels	No
Ibáñez *et al.* (104)	$Gly^{1057}Asp$ (*IRS-2*)	Case series	PCOS	↓ 2 h Glucose (OGTT)	Yes
	$Gly^{972}Arg$ (*IRS-1*)	Case-control	PP	PP, ovarian hyperandrogenism and ↓ SHBG	Yes
INS					
Waterworth *et al.* (222)	*INS* VNTR	FBS/case-control	PCOS/MPB	PCOS and MPB	Yes
Urbanek *et al.* (132)	*INS* VNTR	FBS (TDT)	PCOS	PCOS and T levels	No
Eaves *et al.* (224)	*INS* VNTR	FBS (TDT)	PCOS	PCOS	Yes
Michelmore *et al.* (223)	*INS* VNTR	FBS/case-control	PCOS	Insulin resistance and T levels	Yes
Ibáñez *et al.* (101)	*INS* VNTR	Case-control	PP	Birth weight and insulin sensitivity	Yes
Calvo *et al.* (225)	*INS* VNTR	Case-control	FH	FH	No
Vankova *et al.* (226)	*INS* VNTR	Case-control	PCOS	PCOS, insulin secretion and action	No
IGF system					
Urbanek *et al.* (132)	*IGF-I, IGF-IR, IGFBP-1 and IGFBP-3*	FBS (TDT)	PCOS	PCOS	No
San Millán *et al.* (229)	*IGF-2* (*Apa*I)	Case-control	PCOS	PCOS	Yes
	IGF-IR	Case-control	PCOS	↑ Fasting glucose and insulin resistance	Yes
	IGF-I, IGF-IIR	Case-control	PCOS	PCOS	No
PPAR-γ2					
Urbanek *et al.* (132)	D3S1263	FBS (TDT)	PCOS	PCOS	No
Witchel *et al.* (110)	$Pro^{12}Ala$	Case-control	PP/FH	Weight gain	Yes
Hara *et al.* (245)	$Pro^{12}Ala$	Case series	PCOS	↓ Insulin resistance in PCOS	Yes
Korhonen *et al.* (246)	$Pro^{12}Ala$	Case-control	PCOS	PCOS	Yes
Orio *et al.* (247)	$CAC^{478}CAT$	Case-control	PCOS	PCOS, obesity, and leptin levels	Yes
	$Pro^{12}Ala$	Case-control	PCOS	PCOS	No
San Millán *et al.* (229)	$Pro^{12}Ala$	Case-control	PCOS	PCOS	No
PON1					
San Millán *et al.* (229)	−108 C/T	Case-control	PCOS	PCOS	Yes
	$Leu^{55}Met$	Case-control	PCOS	↑ Insulin resistance and BMI	Yes
	$Gln^{192}Arg$	Case-control	PCOS	PCOS	No
SORBS1					
Witchel *et al.* (106)	$Thr^{228}Ala$	Case-control	PP/FH	PP/FH, obesity	No
San Millán *et al.* (229)	$Thr^{228}Ala$	Case-control	PCOS	↑ BMI	Yes
β_3-Adrenergic receptor					
Witchel *et al.* (108)	$Trp^{64}Arg$	Case-control	PP/FH	PP/FH, obesity	No
Calpain-10					
Ehrmann *et al.* (263)	UCSNP-43, -19, and -63	FBS/case-control	PCOS	PCOS and insulin levels	Yes
Haddad *et al.* (264)	UCSNP-43, -44, -19, and -63	FSB/case-control	PCOS	PCOS and insulin levels	No
Escobar-Morreale *et al.* (265)	UCSNP-43	Case-control	Hirsutism	Hirsutism score	Yes
	UCSNP-44	Case-control	Hirsutism	PCOS, idiopathic hirsutism, FH	No
	UCSNP-45	Case-control	Hirsutism	Idiopathic hirsutism	Yes
González *et al.* (266, 267)	UCSNP-43, -44, -19, and -63	Case-control	PCOS	PCOS	Yes
Glycogen synthetase					
Rakjhowa *et al.* (268)	*Xba*I polymorphism	Case-control	PCOS	PCOS and insulin sensitivity	No
Resistin					
Urbanek *et al.* (269)	−420C/G	FBS (TDT)	PCOS	PCOS, obesity, and insulin resistance	No
Leptin and leptin receptor					
Oksanen *et al.* (270)	Mutation screening of leptin gene, polymorphisms in leptin receptor gene	Case-control	PCOS	PCOS and obesity	No

TABLE 4. *Continued*

Gene	Variant/locus	Design	Subjects	Phenotypic trait	Association
Apolipoprotein E					
Heinonen *et al.* (271)	Alleles E2, E3, and E4	Case-control	PCOS	PCOS	No
PC-1					
San Millán *et al.* (229)	$Lys^{121}Gln$	Case-control	PCOS	PCOS	No
PTP1B					
San Millán *et al.* (229)	981C/T and 1484 insG	Case-control	PCOS	PCOS	No
Adiponectin					
San Millán *et al.* (229)	45T/G and 276G/T	Case-control	PCOS	PCOS	No

Authors are cited in chronological order. BMI, Body mass index; FBS, family-based study; IGF-IR, IGF-I receptor; IGF-IIR, IGF-II receptor; IGFBP, IGF binding protein; MPB, male premature baldness; OGTT, oral glucose tolerance test; PC-1, plasma cell differentiation antigen glycoprotein; PP, premature pubarche; PTP1B, protein tyrosine phosphatase 1B; T, total testosterone; TDT, transmission disequilibrium test.

4. IGF system. IGFs, their receptors, binding proteins, and proteases are important for the normal development of the ovary (227). IGFs stimulate ovarian cellular mitosis and steroidogenesis, inhibit apoptosis, and might be related to the development of functional hyperandrogenism and PCOS (228).

No evidence for linkage with PCOS was found for markers close to the genes encoding IGF-I and IGF-binding proteins 1 and 3 in a family study conducted in the United States (132). San Millán *et al.* (229) recently found an association of PCOS with homozygosity for G alleles of the *Apa*I polymorphism in *IGF-II*, but not with a dinucleotide polymorphism in *IGF-I*, a trinucleotide polymorphism in the *IGF-I receptor*, or with an ACAA-insertion/deletion polymorphism at the 3′ nontranslated region of the *IGF-II receptor*, previously described (230–232). G alleles of the *Apa*I polymorphism in *IGF-II* have been attributed to increased IGF-II mRNA in leukocytes compared with A alleles (233), and possibly result in increased liver IGF-II expression and secretion (234). Given that IGF-II stimulates adrenal (235, 236) and ovarian (237) androgen secretion, the increased frequency of homozygosity for these alleles might contribute to hyperandrogenism in some PCOS patients, provided we assume that G alleles may increase IGF-II expression at the ovary, as reported for other tissues. In the same case-control study involving PCOS patients cited above (229), we found that subjects homozygous for 90-bp alleles of a trinucleotide repeat polymorphism in the gene encoding IGF-I receptor had increased fasting glucose levels and fasting insulin resistance index compared with subjects carrying 93-bp alleles, but no association of any genotype with PCOS.

5. PPAR-γ2. Activation of PPAR-γ by using the insulin sensitizer drugs, thiazolidinediones, has been one of the most important advances for the treatment of type 2 diabetes mellitus in past years. As described above, insulin resistance is a common finding in hyperandrogenic patients, and thiazolidinediones improve insulin sensitivity, hyperandrogenism, and ovulation in women with PCOS (238–243).

The $Pro^{12}Ala$ SNP in *PPAR-γ2* has been studied in women with PCOS, despite the fact that evidence for linkage or association with PCOS was not found for a marker close to the PPAR-γ2 gene, in a family-based study conducted in the United States (132). Ala12 alleles of the PPAR-γ2 gene favor weight gain in obese adults (244) and in obese hyperandrogenic girls and adolescents (110). Also, Ala12 alleles preserve insulin sensitivity in Caucasian men (109) and in Caucasian women presenting with PCOS defined by NICHD criteria (245). Recently, a marginally significant decrease in the frequency of the Ala12 allele has been reported in women with polycystic ovaries from Finland (246), but this result has not been confirmed in a small case-control study of PCOS patients conducted in Spain (229) or in a recently published study in Italian PCOS patients defined by NICHD criteria and ultrasonography (247). In the latter, a silent C to T substitution at position 142 in exon 6 was differentially distributed in PCOS patients and controls, T alleles being more frequent in women with PCOS (247). This silent polymorphism was not in linkage disequilibrium with the $Pro^{12}Ala$ polymorphism, but the possibility of an association with other unknown genomic variant in the PPAR-γ2 gene was not explored (247). Nevertheless, both Ala12 and T142 alleles are relatively uncommon (less than 20%) in normal and hyperandrogenic populations (229, 245–247), and therefore their putative influences on insulin sensitivity and/or hyperandrogenism would be restricted to a small number of PCOS patients.

6. Paraoxonase (PON1). We have recently explored the −108C/T, $Leu^{55}Met$, and $Gln^{192}Arg$ polymorphisms in the gene encoding serum PON1 in PCOS patients defined by NICHD criteria. The PON1 gene is expressed mainly in the liver and encodes for serum PON1, which is an antioxidant high-density lipoprotein-associated enzyme. Liver PON1 mRNA expression is influenced by genetic and environmental factors, and both androgens and proinflammatory mediators decrease liver PON1 expression (248). Interestingly, both androgen excess and proinflammatory genotypes contribute to the pathogenesis of PCOS (249–251).

Homozygosity for T alleles of the −108C/T polymorphism in *PON1* was more frequent in patients compared with nonhyperandrogenic women (229). As expected from the association with PCOS, subjects homozygous for −108T alleles of *PON1* presented with increased hirsutism scores, total testosterone, and free testosterone and androstenedione concentrations compared with carriers of −108C alleles (229). Moreover, in a logistic regression model, homozygosity for −108T alleles of *PON1* was associated with a 7.1 odds ratio (95% confidence interval, 2.1–23.8) of having PCOS (229).

The −108C/T polymorphism is responsible of approximately 23% of PON1-expression levels in some cell systems, in which −108TT constructs showed reduced PON1 expression compared with −108CC constructs (252). We thus speculated that homozygosity for −108T alleles, hyperandrogenism, and proinflammatory genotypes might contribute to reduced PON1 expression, resulting in a higher oxidative stress in these women. The latter has been found in PCOS patients (253).

Oxidative stress may impair insulin action (254). Therefore, reduced serum PON1 activity might contribute to the insulin resistance of PCOS patients. This hypothesis is supported by the finding of reduced serum PON1 activity in other insulin-resistant disorders such as type 2 diabetes mellitus (255, 256) and cardiovascular atherosclerotic disease (257, 258). If confirmed in future studies, the association of homozygosity for −108T alleles of *PON1* with PCOS might contribute to explaining the insulin resistance and the increased risk for atherosclerosis associated with this syndrome (259).

In our study (229), subjects homozygous for Met55 alleles presented with increased body mass index and indexes of insulin resistance compared with carriers of Leu55 alleles, further suggesting the involvement of *PON1* in PCOS, despite the fact that the Leu^{55}Met and Gln^{192}Arg polymorphisms in *PON1* were not associated with PCOS.

7. Human homolog for the sorbin and SH3-domain-containing-1 gene (SORBS1). In addition to the studies in adolescents cited above (106), we have recently studied the Thr^{228}Ala polymorphism in adult PCOS patients. Allele frequencies were similar in PCOS patients and nonhyperandrogenic women, but carriers of Ala228 alleles of *SORBS1* presented with increased body mass index compared with subjects homozygous for 228T alleles (229), in conceptual agreement with a large study conducted in Europe (260).

8. Calpain-10. This enzyme is a cysteine protease that plays a role in insulin secretion and action (261). The 112/121-haplotype combination of University of Chicago single nucleotide polymorphism (UCSNP)-43, UCSNP-19, and UCSNP-63 polymorphisms in the gene encoding calpain-10, located at 2q37.3, has been reported to increase the risk for diabetes (262). Ehrmann *et al.* (263) found no association between this haplotype and any of the phenotypic features of PCOS in Caucasian nondiabetic PCOS patients, defined by NICHD criteria, whereas the 112/121-haplotype was significantly associated with higher insulin levels in response to an oral glucose tolerance test in African-American, nondiabetic PCOS women. Moreover, when considering Caucasian and African-American, nondiabetic PCOS patients as a whole, the 112/121 haplotype was associated with a 2-fold increase in susceptibility to PCOS.

However, the association of *calpain-10* SNPs with PCOS, as defined by polycystic ovaries, hyperandrogenism, and/or anovulation, was not confirmed by Haddad *et al.* (264) in 330 PCOS patients from the United Kingdom. We have studied three common polymorphisms in the calpain-10 gene in 97 Spanish hyperandrogenic patients and 37 controls, including UCSNP-43 (265). C alleles at the UCSNP-45 locus were associated with idiopathic hirsutism, but neither the UCSNP-43 nor the UCSNP-44 was associated with hyperandrogenism or PCOS (265). However, in a different population from the south of Spain, González *et al.* (266, 267) recently reported an association between PCOS and USCNP-44. Additional studies are needed to clarify this issue, especially because the physiological roles of calpain-10 remain mostly unknown.

9. Genes encoding for other molecules related to insulin resistance and associated disorders. Among other genes tested, no association has been reported in PCOS with genomic variants in the genes encoding glycogen synthetase (268), resistin (269), leptin and its receptor (270), apoprotein E (271), or with variants in the genes of plasma cell differentiation antigen glycoprotein, protein tyrosine phosphatase 1B, and adiponectin (229).

C. Proinflammatory genotypes (Table 5)

Chronic inflammation is involved in the development of metabolic syndrome and cardiovascular disease (272, 273), and serum inflammatory markers cluster in patients with cardiovascular disease, suggesting a role in the pathogenesis of atherosclerosis (272, 273).

Inverse correlations have been reported between indexes of insulin sensitivity and inflammatory markers such as circulating levels of TNF-α (274), soluble type 2 TNF receptor (TNFR2) (275), IL-6 (276), C-reactive protein (CRP) (276), and soluble intercellular cell adhesion molecule-1 (276).

Adipose tissue plays a central role in the relationship between cytokines and insulin resistance. The expression of TNF-α and TNFR2 in adipose tissue is increased in obesity (277, 278). TNF-α expression correlates with indexes of insulin resistance and decreases with weight loss in parallel with the improvement in insulin sensitivity (277). Similar results have been reported for IL-6 (279). Moreover, inflammatory cytokines may induce insulin resistance by direct actions on insulin-signaling postreceptor molecules (280) or by inducing central obesity through activation of the hypothalamic-pituitary-adrenal axis (281).

Because obesity and insulin resistance are common findings in hyperandrogenic women (3), chronic inflammation might be involved in the pathogenesis of functional hyperandrogenism and PCOS. In animal models in which polycystic ovaries were induced by neonatal administration of estradiol, the production of TNF-α and IL-6 was increased in ovaries and in peritoneal macrophages (282). The concentrations of IL-12 were decreased, and production of IL-13 and the number of activated lymphocytes were increased in follicular fluid of women with PCOS (283).

The study of serum inflammatory markers in PCOS has resulted in conflicting reports. Increased CRP levels have been reported in PCOS patients defined by NICHD criteria (284, 285). Similarly, increased serum IL-6 (286) and TNF-α (287–289) concentrations have been reported in women with PCOS or functional hyperandrogenism.

However, we have recently reported that obesity, and not PCOS, appears to be the major determinant of the increase in serum CRP and IL-6 in premenopausal women, and this and other inflammatory markers such as serum TNF-α, soluble

TABLE 5. Proinflammatory genotypes, functional hyperandrogenism (FH), and PCOS

Gene	Variant	Design	Subjects	Phenotypic trait	Association
TNF-α					
Milner *et al.* (296)	−308G/A	Case-control	PCOS	PCOS	No
Mao *et al.* (297)	−308G/A	Case-control	PCOS	PCOS	No
Escobar-Morreale *et al.* (288)	−308G/A	Case-control	FH	↑ Serum androgens and 17-hydroxyprogesterone levels	Yes
	−1196C/T, −1125G/C, −1031T/C, −863C/A, −857C/T, −316G/A, −238G/A, and −163G/A	Case-control	FH	FH	No
Korhonen *et al.* (298)	−850C/T	Case-control	PCOS	PCOS	No
TNFRSF1B					
Peral *et al.* (250)	$Met^{196}Arg$	Case-control	PCOS/FH	PCOS/FH	Yes
	1663G/A, 1668T/G, and 1690T/C	Case-control	PCOS/FH	PCOS/FH	No
IL-6					
Villuendas *et al.* (249)	−597G/A and −174G/C	Case-control	FH	FH and adrenal hyperactivity in GG homozygotes	Yes
	−572G/C and 373A(n)T(n)	Case-control	FH	FH	No
Mohlig *et al.* (291)	−174G/C	Case-control	PCOS	↓ Serum androstenedione in −174GC genotype	Yes
gp130					
Escobar-Morreale *et al.* (251)	$Gly^{148}Arg$	Case-control	PCOS/FH	PCOS/FH	Yes
IL-6Rα					
Escobar-Morreale *et al.* (251)	CA-repeat polymorphism	Case-control	PCOS/FH	Obesity	Yes

Authors are cited in chronological order. gp130, 130-kDa IL-6 Signal transducer; IL-6R, IL-6 receptor; *TNFRSF1B*, gene encoding TNFR2.

TNFR2, or soluble intercellular cell adhesion molecule-1 are not increased by PCOS when controlling for confounding factors such as smoking and obesity (290). Similar findings have been published recently by Mohlig *et al.* (291). These results cast doubt upon the usefulness of these serum inflammatory molecules as markers of the inflammatory process associated with hyperandrogenism. On the contrary, another novel inflammatory marker of cardiovascular risk, IL-18, is increased in serum both by obesity and by PCOS, suggesting that this molecule may be a useful marker of inflammation in PCOS patients (292).

Given that proinflammatory genotypes influence obesity, type 2 diabetes mellitus, and insulin resistance-related disorders (273), over the past years our group has studied genomic variants in the genes encoding several inflammatory mediators and their receptors. Some of these variants are associated with functional hyperandrogenism and PCOS.

1. TNF-α. TNF-α induces reproductive changes that closely resemble those found in patients with PCOS and functional hyperandrogenism. TNF-α facilitates the effects of insulin and IGF-I on the ovary in a dose-dependent and additive fashion (293), stimulating proliferation and steroidogenesis in rat theca cells *in vitro* (293, 294). Moreover, TNF-α may be involved in apoptosis and anovulation in the rat ovary (295).

We have recently studied serum TNF-α levels and nine common polymorphisms (−1196C/T, −1125G/C, −1031T/C, −863C/A, −857C/T, −316G/A, −308G/A, −238G/A, and −163G/A) in the TNF-α gene in 60 hyperandrogenic women and 27 healthy controls matched for body mass index (288). As a group, hyperandrogenic patients presented with increased serum TNF-α levels, but this increase was only present in lean patients when compared with lean controls, and not in obese patients (288).

No differences between patients and controls were found in the allele frequencies of any of the polymorphisms studied (288). In conceptual agreement, −308G/A alleles were equally distributed between patients with polycystic ovaries and hyperandrogenic symptoms and controls in other studies (296, 297), and similar results were reported for the −805C/T polymorphism in the TNF-α gene (298). However, when considering patients and controls as a whole in our series, carriers of −308A alleles presented with increased serum androgen and 17-hydroxyprogesterone levels before and after stimulation with the GnRH analog leuprolide (288). Therefore, polymorphisms in the TNF-α gene do not appear to play a major role in the pathogenesis of functional hyperandrogenism and PCOS but might be a modifying factor for phenotypic traits associated with these disorders.

2. TNFR2 gene (TNFRSF1B). TNFR2 mediates most of the metabolic effects of TNF-α (299). We have recently studied serum soluble TNFR2 levels and several polymorphisms in the *TNFRSF1B* in women with functional hyperandrogenism, including PCOS defined by NICHD criteria (250).

TNFRSF1B has been studied in several metabolic disorders. The 1690T/C variant in exon 10 has been described to influence body mass index and insulin resistance (300). The CA-repeat polymorphism in intron 4 and the $Met^{196}Arg$ polymorphism in exon 6, which are in strong linkage disequilibrium, influence serum lipid levels (301–303) and diastolic blood pressure (302); linkage studies suggest that the *TNFRSF1B* locus is associated with hypertension (302) and familial combined hyperlipidemia (301). Moreover, the CA-repeat polymorphism has been recently proposed as a contributing factor to coronary artery disease (304).

In our series, the uncommon 196Arg allele of the $Met^{196}Arg$ (676T/G) polymorphism in exon 6 of *TNFRSF1B* was more frequent in patients with PCOS compared with healthy controls (250). When the study was extended to include Italian subjects, this variant was more frequent not only in PCOS patients but also in women with hyperandrogenic hirsutism and regular

menstrual cycles (250). However, the Met[196]Arg polymorphisms did not influence any phenotypic trait associated with hyperandrogenism, insulin resistance, or obesity when studying patients and controls separately (250).

We also studied three SNPs in the 3′-untranslated region of *TNFRSF1B* in exon 10, 1663G/A, 1668T/G, and 1690T/C, which were not associated with hyperandrogenism (250). Serum soluble TNFR2 levels were not increased in hyperandrogenic women compared with controls, but they were influenced by the interaction between the 1663G/A and 1668T/G variants. We hypothesized that the Met[196]Arg variant in *TNFRSF1B* might contribute to PCOS by modulating TNF-α actions at target tissues.

3. IL-6. Among cytokines, IL-6 circulates in plasma and acts in distant tissues (305). TNF-α stimulates IL-6 secretion by adipocytes, and mounting evidence suggests that IL-6 is also implicated in insulin resistance and associated syndromes (273, 306–308). Although serum IL-6 levels are not increased in women presenting with functional hyperandrogenism and PCOS (249, 290), IL-6 concentrations are increased in peritoneal fluid in clomiphene-resistant, anovulatory PCOS patients, suggesting a role in the pathogenesis of hyperandrogenic disorders (309).

We recently studied four common polymorphisms in the promoter of the IL-6 gene (−597G/A, −572G/C, −373A(n)T(n), and −174G/C) in 85 hyperandrogenic patients and 25 healthy women (249). The −597G/A and −174G/C variants were in strong disequilibrium linkage. When considering the three biallelic SNPs, five haplotypes were found (relative frequencies in *parentheses*): GGG (0.505), AGC (0.377), GGC (0.059), GCG (0.055), and GCC (0.005). The frequency of the GGG haplotype was increased in patients (0.559) compared with controls (0.320), and conversely, the frequency of the AGC haplotype was reduced in patients (0.318) compared with controls (0.580) ($P < 0.02$). Homozygosity and heterozygosity for −597G and −174G alleles were more frequent in controls, and controls carrying these alleles presented with increased serum IL-6, cortisol, 11-deoxycortisol, and 17-hydroxyprogesterone levels, and a tendency toward increased serum total testosterone levels compared with controls homozygous for −597A and −174C alleles. These findings suggest a protective role for the latter against IL-6 excess, adrenal hyperactivity, and hyperandrogenism. The −572G/C and −373A(n)T(n) were not associated with hyperandrogenism or with any androgen-related phenotypic trait (249). In conceptual agreement, Mohlig *et al.* (291) recently reported that the heterozygous −174G/C genotype in PCOS patients was associated with lower serum androstenedione levels.

4. IL-6 receptor. This is a heterodimeric receptor consisting of two membrane-bound glycoproteins: an 80-kDa IL-6 binding unit and a 130-kDa IL-6 signal transducer (IL-6 receptor β or gp130). gp130 is a transducer chain shared by other cytokines and is responsible for signal transduction of the chain-ligand complex through the Janus kinase/signal transducer and activator of the transcription pathway (310).

We have recently studied common polymorphisms in both subunits of the IL-6 receptor in a series of 145 hyperandrogenic women and 45 controls from Spain (251). The uncommon Arg148 allele of the Gly[148]Arg polymorphism in the gp130 gene was more frequent in controls compared with hyperandrogenic patients, and controls carrying Arg148 alleles had lower 11-deoxycortisol and 17-hydroxyprogesterone concentrations, a lower response of androstenedione to 1–24 adrenocorticotropin, and an almost statistically significant decrease in free testosterone levels, suggesting that, as occurred for the IL-6 polymorphisms described above, Arg148 alleles in the gp130 gene have a protective effect against androgen excess and adrenal hyperactivity (251). When considering patients and controls as a whole, a microsatellite CA-repeat polymorphism in the 80-kDa IL-6 binding unit locus was associated with obesity. The frequency of the common 149-bp allele was markedly increased in obese women compared with controls, further supporting the involvement of inflammatory genotypes in obesity and related syndromes (251).

Overall, our studies over the past years suggest that chronic inflammation underlies the pathogenesis of functional hyperandrogenism and PCOS, as has been proposed for other disorders associated with insulin resistance, and that proinflammatory genotypes may be involved.

D. Other candidate genes

1. Follistatin. This protein binds to activin, inhibiting its action both *in vivo* and *in vitro* (311). Activin and follistatin are expressed in multiple tissues, including pituitary, ovary, adrenal, and pancreas (312, 313). In the ovary, activin promotes follicular development and inhibits androgen secretion by theca cells (314). Conversely, transgenic mice overexpressing follistatin present with follicular arrest and infertility (315), characteristics frequently found in hyperandrogenic women.

However, the expression of human inhibin/activin subunit, follistatin, type 2 activin receptor mRNAs, and their encoded proteins in ovarian follicles from PCOS patients suggested that an increase in the availability of activin, relative to inhibin, was actually present in the arrested follicles in PCOS patients (316). Moreover, the location of the mRNAs of the follistatin subunits is not altered in PCOS (317).

Initial molecular genetic studies in humans by Urbanek *et al.* (132) suggested evidence for linkage between the *follistatin* locus and PCOS, as cited above. Subsequent studies by these authors (318) have failed to confirm the involvement of the follistatin gene with PCOS in a large multiethnic study. The coding regions and some introns of the follistatin gene were sequenced, disclosing at least 17 polymorphisms; however, 16 of them were rare, making a significant contribution of these variants to the pathogenesis of hyperandrogenism unlikely (318). Moreover, the only common polymorphism found, located in exon 6 but not translated, was not associated with PCOS when correcting for multiple testing, and the authors concluded that contributions to the etiology of PCOS from the follistatin gene, if any, are probably small (318).

In conceptual agreement, no mutations in the follistatin gene have been found in Chinese PCOS patients defined by menstrual dysfunction, hyperandrogenism, and polycystic ovaries (319), and the only mutation found in our series of patients from Spain, a silent G951A variant, was equally distributed in PCOS patients and in healthy women (320).

2. Thrombophilic factors. Women with PCOS have increased miscarriage rates, as happens in women with inherited thrombophilic conditions. The secretion of plasminogen activator inhibitor-1 (PAI-1) in adipose tissue is enhanced by inflammatory cytokines and by insulin (321–323). In agreement, the increased circulating PAI-1 levels found in PCOS patients decrease after treatment with insulin sensitizers (324). Homozygosity for 4G alleles of the −675 4G/5G polymorphism in the gene encoding PAI-1, which modulates PAI-1 activity, have been reported in association with obesity (325) and PCOS (326). We have found a similar trend in our series on Spanish women, although the difference in allele frequencies did not reach statistical significance, possibly because of small sample size (229).

Other genetic abnormalities associated with thrombophilia have been studied in PCOS. Tsanadis *et al.* (327) recently reported that the prevalence of antithrombin III, protein S and protein C deficiencies, factor V Leiden, prothrombin G20210A factor, and methylene tetrahydrofolate reductase 677C/T mutations is not increased in Greek patients with polycystic ovaries, menstrual dysfunction, and hyperandrogenism when compared with nonhyperandrogenic controls. Similar results have been found for the methylene tetrahydrofolate reductase variant in Italian women (328).

3. Microsomal epoxide hydrolase. Two SNPs, $Tyr^{113}His$ and $His^{139}Arg$, in the gene encoding the detoxifying enzyme microsomal epoxide hydrolase have been studied in women with PCOS, defined by the presence of polycystic ovaries and hyperandrogenic symptoms (329). Although none of the polymorphisms was associated with PCOS, the presence of the His113-Arg139 haplotype was associated with an odds ratio for PCOS of 2.28 and 95% confidence interval of 1.1–4.8 (329). However, it is unclear how changes in the activity of this enzyme might relate to functional hyperandrogenism and PCOS, and therefore, this result should be considered with caution unless confirmed in future studies.

4. Bone morphogenetic proteins. The intraovarian bone morphogenetic protein system is involved in the control of granulosa cell proliferation and cytodifferentiation and plays a role in oocyte development (330). The genes encoding the growth differentiation factor 9 and bone morphogenetic protein 15 have been studied in Japanese women with polycystic ovaries, but no missense mutations have been found in these women (331).

VI. Hyperandrogenism, PCOS, and Survival Advantage

The increasing prevalence of complex metabolic disorders in developed countries raised the possibility that, from an evolutionary perspective, the pathogenetic mechanisms underlying these disorders might have provided survival advantages (332). However, these previously beneficial mechanisms may lead to disease with prolonged life expectancy or when these subjects are exposed to the present lifestyle in Western countries.

As suggested by Witchel *et al.* (333) for congenital adrenal hyperplasia, which is one of the most common inherited disorders with carrier frequencies of approximately 10% in all world populations studied to date and a relatively common cause of hyperandrogenism in children and adults, the presence of hyperandrogenism in women might have provided survival advantage for these women and their children.

The rapid maturation of the reproductive axis found in these subjects, together with the increase in assertive behavior resulting from increased androgen secretion, might be advantageous during times of environmental stress (333–335). Moreover, the relative infertility of these women could increase the interval between pregnancies, decreasing the birth rate and favoring maternal and infant survival (333). In addition to the lower rate of pregnancies secondary to oligoovulation, pregnancies may also occur at an older age, favoring the survival of these women. In agreement, an early beginning of fecundity is associated with higher mortality rates in animal models (335).

Yet survival advantage may also contribute to explaining the association of hyperandrogenism with other disorders related to insulin resistance. Human metabolism may be genetically adapted to the dominant conditions that have predominated for ages: near-continuous physical activity, a diet rich in carbohydrates and proteins yet poor in fat, and long periods of famine or food shortage (336–338). Survival was therefore favored by a combination of thrifty genotypes and phenotypes, in which insulin resistance played a central role (Fig. 2).

Insulin resistance increases glucose availability for brain metabolism. It also increases salt and water retention and sympathic tone and induces endothelial dysfunction, favoring an increase in blood pressure, obviously beneficial when trauma occurs. Similarly, the increased coagulability and decreased fibrinolysis associated with insulin resistance are defensive mechanisms against bleeding. But more important is that insulin resistance favors obesity, protecting against starvation, and obesity contributes to a proinflammatory state through the secretion of several cytokines, contributing to the defense against infection, and possibly to the development of functional hyperandrogenism and PCOS.

When the environmental conditions change and access to food is not restricted, significant trauma and epidemics seldom occur, and life expectancy increases markedly, these defensive mechanisms are no longer beneficial, and the price to pay is atherosclerosis and cardiovascular disease (Fig. 2).

For the reasons outlined above, it is not surprising that the genomic variants associated with obesity, type 2 diabetes mellitus, and other disorders in which insulin resistance plays a major role are frequently associated with functional hyperandrogenism and PCOS. However, genomic variants-associated insulin resistance may contribute to hyperandrogenism only indirectly, by inducing insulin resistance and hyperinsulinemia and/or by direct actions at the adrenal or the ovary. For example, TNF-α induces insulin resistance by interfering with IRS-1-mediated insulin signaling (280) and also has reproductive actions that closely resemble those found in patients with PCOS and functional hyperandrogenism, as summarized above (293, 294).

Therefore, the precise elucidation of the mechanisms underlying these associations requires studies of the functional consequences of the genomic variants associated with hy-

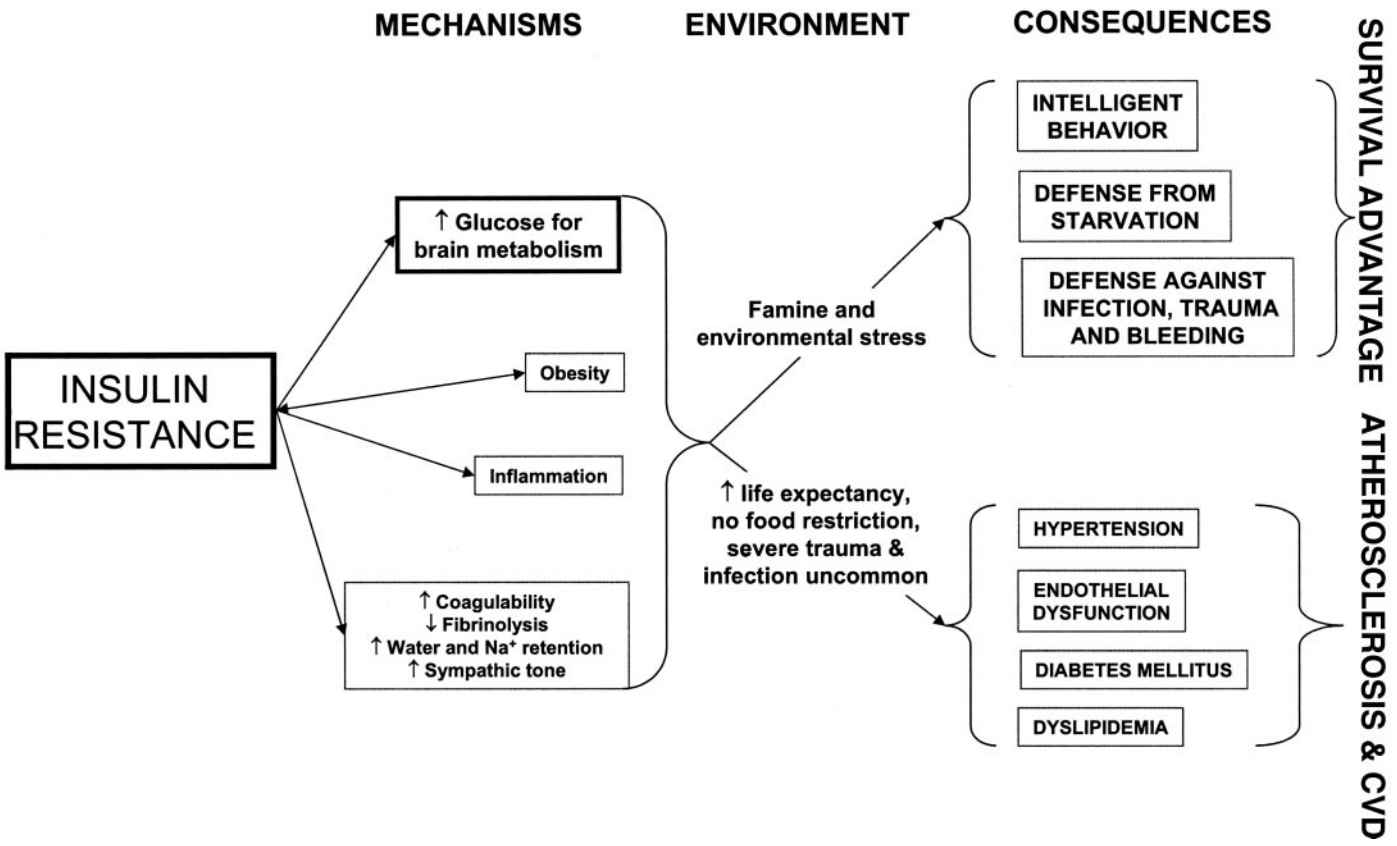

FIG. 2. Insulin resistance, survival advantage, and disease. Insulin resistance and related pathogenic mechanisms may have been selected during evolution because of survival advantage during times of environmental stress. However, with the sudden change in life conditions occurring during the last century in most developed countries, where access to food is not restricted, severe trauma and infection are relatively uncommon, and life expectancy has increased markedly, these mechanisms are no longer beneficial and result in atherosclerosis and cardiovascular disease (332). CVD, Cardiovascular disease.

perandrogenism in different target tissues, especially when some of the genomic variants appear to facilitate insulin resistance and hyperandrogenism, whereas others may protect against these disorders.

At present, the emerging picture for the molecular genetic mechanisms leading to functional hyperandrogenism and PCOS is that of a complex interaction between predisposing and protective genomic variants, and the strong impact of modifying environmental factors including diet, exercise, and lifestyle (Fig. 3).

VII. Explanations for the Lack of Reproducible Association of Hyperandrogenism and PCOS with Molecular Genetic Abnormalities and Genomic Variants

To date, most of the associations between genomic variants and functional hyperandrogenism or PCOS failed to replicate when studied in different populations. An immediate consideration is that most studies conducted to date have been modest with regard to the number of subjects included. A small sample size, leading to a lack of statistical power to detect the modest effects that genomic variants possibly play in the pathogenesis of a complex disorder such as functional hyperandrogenism, might explain the negative results found in many cases when efforts have been made to confirm previous studies. Although at present there are no doubts about the need of studies with large sample sizes, lack of consistency of the reported findings is not limited to hyperandrogenism and is a recurrent problem in the study of many complex metabolic disorders (339). Therefore, other factors might also contribute to the discrepancies observed when studying candidate genes for hyperandrogenism in different populations, summarized below.

A. Ascertainment issues

One of the essential requirements for the effectiveness of molecular genetic studies is a clear definition of the phenotype under study. This has not been the case for functional hyperandrogenism and PCOS. As an example, the linkage studies cited above (140, 222) of *CYP11A* and *INS* in women from the United Kingdom relied mostly on the use of ultrasonography for the diagnosis of PCOS, and this series included "ovulatory" PCOS patients. On the contrary, a linkage study conducted in the United States relied on the NICHD criteria (132), in which chronic anovulation is strictly required for the diagnosis of PCOS (9). Given the considerable differences in the criteria used to define the phenotype, it is unlikely that the genes associated with the disease would be the same in both studies.

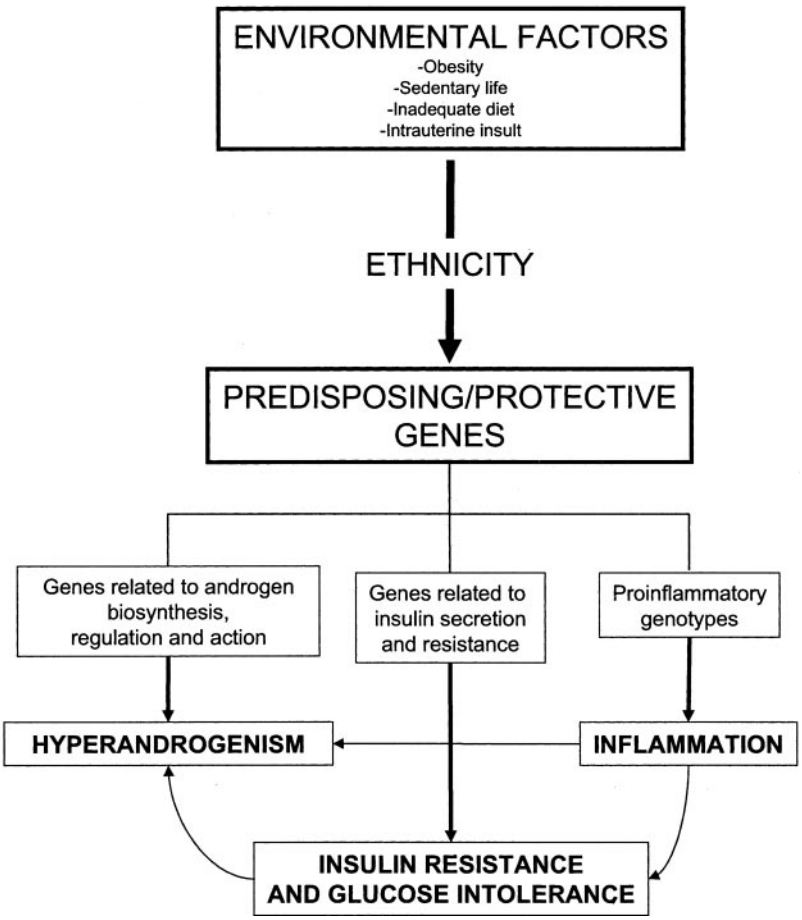

FIG. 3. A unifying hypothesis for the association of hyperandrogenism, inflammation, and insulin resistance. Environmental factors, influenced by ethnicity, act on a delicate balance between predisposing and protective common genetic variants that have been selected during evolution because of previous survival advantage. The genes involved in the pathogenesis of hyperandrogenism may vary depending on the particular and/or ethnic factors that predominate in the different populations studied, providing an explanation for the phenotypic variability of hyperandrogenic disorders.

Moreover, the spectrum of disorders covered by the criteria derived from the Rotterdam Consensus Workshop (11, 12) is even broader than those included with the NICHD definition: PCOS would be diagnosed not only in women presenting with hirsutism, hyperandrogenemia, and anovulation, but also in patients presenting with anovulation and polycystic ovaries but no clinical and/or biochemical evidence of hyperandrogenism. Therefore, these revised criteria are unlikely to be of significant advantage over previous ones with respect to molecular genetic studies. And to further complicate the search for PCOS-related genomic abnormalities in family-based studies, the male PCOS phenotype is still uncertain.

Because of the heterogeneous and syndromic nature of PCOS, it would be more helpful for molecular genetic studies to use criteria oriented toward the identification of specific clinical, reproductive, and metabolic traits associated with hyperandrogenism. This would require an important effort to identify and especially to standardize the methods used to identify these traits in large populations such as those needed for family-based or case-control designs.

Instead of trying to reach a consensus on how to diagnose precisely a disorder that is heterogeneous by definition, the practical issue would be to standardize the definitions and methods used to identify specific traits such as insulin resistance, overweight, visceral adiposity, dyslipidemia, hirsutism, acne, oligoovulation, and many others that characterize or are frequently found in hyperandrogenic women.

Given the apparently multigenic etiology for functional hyperandrogenism and PCOS, this approach would permit a more precise definition of the kind of hyperandrogenic patients included in the different molecular genetic studies. It would facilitate well-sized multicollaborative studies and thereby increase the probability of success in identifying genomic variants and abnormalities related to these particular traits that might be used as diagnostic and/or therapeutic targets in the future. It is hoped that scientific societies that address the study of hyperandrogenism, such as the recently created Androgen Excess Society (http://www.androgenexcesssociety.org), will serve as an adequate forum where these efforts in standardization could be made.

In addition to the problems of defining the PCOS phenotype, sampling issues also should be considered. For family-based studies, a common sampling procedure is to collect from an affected proband with at least one or two affected relatives, instead of the ideal, but rarely performed, procedure of selecting families from the population at random (340). Nonrandom ascertainment makes it more likely that families with multiple PCOS members enter the study than families with no affected relative, resulting in biased heritability estimates (340, 341). Another common source of error is that the affected status in relatives of the proband is typically determined on the basis of his or her report because of constraints in time and resources (340). Moreover, affected subjects may be more likely to be aware of the diagnosis in their relatives than nonaffected subjects (340), or more likely

to misinterpret the symptoms of their relatives (340), such as considering the presence or absence of hirsutism as the equivalent of having or not having PCOS.

B. Involvement of environmental factors

The interactions between genetic and environmental factors are essential for the comprehension of the pathogenesis of common complex disorders (342). The precise knowledge of these interactions requires long-term studies analyzing the impact of different environmental factors in specific subgroups of patients, because controlling all the confounding environmental variables is extremely difficult (342).

Environmental factors such as weight gain may trigger the development of PCOS in predisposed women, as occurs in other complex metabolic disorders in which insulin resistance plays a major role. The identification and precise delimitation of the contribution of the environmental influences triggering the development of functional hyperandrogenism and PCOS may direct the search for the specific proteins and/or genomic variants involved to the metabolic pathways influenced by these environmental factors.

However, the environmental factors contributing to complex metabolic disorders may change depending on the population studied, because diet, exercise, and lifestyle have wide ethnic variations. Therefore, it should not be surprising that the genomic abnormalities contributing to these disorders may also change depending on the environmental conditions (*i.e.*, the genes contributing to PCOS in obese sedentary women from a Western country are probably different from those involved in the PCOS phenotype of lean women from the Mediterranean area or from Asia). Finally, to further complicate the study of complex metabolic disorders, the phenotype changes during the life of the affected subjects as age advances. Therefore, different phenotypic traits may not be present when these women were phenotyped, but became apparent later in life, constituting one more confounding factor that is especially difficult to control in molecular genetic studies.

C. Possible polygenic etiology for functional hyperandrogenism and PCOS

It has been suggested that the phenotypic heterogeneity observed in PCOS patients, even within the same family, could be attributed to the interaction of a small number of genes with one another and with environmental factors (18, 22, 33). Furthermore, given the large number of genomic variants found associated with functional hyperandrogenism and PCOS to date, the emerging picture may be that of a complex metabolic disorder resulting from small predisposing or protecting effects arising from the interaction of multiple genomic variants and several environmental factors.

Even if a more precise definition for hyperandrogenic phenotypes was used, and that has not been the case, the classic requisite of replication of linkage or association of a genomic abnormality with monogenic diseases may not be applicable for complex disorders because the functional consequences of the genomic variant may be only apparent in certain populations or when a particular environmental factor is present (339). However, it is important that internal replication of findings is provided in larger series, because this might reveal false-positive associations reported in preliminary studies when limited sample sizes were studied.

Nowadays, it is technically feasible to genotype large series of individuals for multiple genomic variants, allowing whole-genome scans and high-throughput candidate gene analysis. These approaches will disclose reliable information of the relative contribution of these genomic variants to functional hyperandrogenism and PCOS and will facilitate the study of gene to gene and gene-environment interactions that probably contribute to the development of these prevalent disorders.

Finally, to avoid spurious associations from being considered causative of any disease-associated trait, every effort should be made to demonstrate *in vivo* or *in vitro* a functional consequence of the associated genomic variant that might reasonably account for the contribution of the variant to the disorder or its associated traits.

D. Limitations of the genetic techniques used to date

As discussed previously, the molecular genetic studies regarding PCOS and functional hyperandrogenism conducted to date had important limitations, especially because adequately sized whole-genome scans and large case-control association studies are still lacking, and therefore associations with genomic variants that have small effects on hyperandrogenism might have been missed by these studies.

Another source of confusion is the fact that quality control of genetic data has not been as strict as that applied to other methods used in clinical research or even in routine clinical practice. Genetic data are usually obtained from a single measure, and reliability and reproducibility of these analyses might be a problem, because genotyping errors may severely bias the estimates of genetic studies (339, 343). Although departure from the Hardy-Weinberg equilibrium may be useful for the detection of these genotyping errors (344), and although modern methods incorporate models of typing error (343, 345), efforts in standardization of these techniques should be made in the future (339).

VIII. Future Perspective: Functional Hyperandrogenism and PCOS in the Age of "Omics"

The suffix "omics" is being applied to recent technologies that are exponentially increasing our knowledge of human biology. Perhaps at present the most developed one is genomics.

Genomics aims to map, sequence, and analyze all the genes and their products in the genome, with the final intention of providing the complete and accurate DNA sequence of an organism (56, 346). The number of genes in the human genome is in the order of 35,000, and because of exon shuffling, alternative splicing, and posttranslational modifications, as many as 100,000 different proteins may be encoded in the human genome. Therefore, identification of all the genes and proteins of the human organism and their interactions requires an enormous effort.

Genomics is in constant evolution, and more specific subspecialties are being developed, such as functional genomics. Its aim is the identification of the biological functions of genes and their products, and how they interact with the environment in health and disease (347). To date, functional genomics has contributed to unravel the mechanisms of many diseases (348–350), and the genomic approach might be especially adequate for the study for complex polygenic disorders, given that traditional molecular genetic approaches have not been successful to date (351).

Genomic techniques, such as differential gene expression analyzed by DNA microarrays, allow the identification of genes that are differentially overexpressed or suppressed in patients compared with controls. This approach has the advantage over molecular genetic analyses in that the result integrates the presence of molecular genetic abnormalities with both gene-gene and gene-environment interactions. Therefore, the genes identified can be considered potential candidates to explain the disorder and also potential diagnostic and therapeutic targets. Recently, comparison of gene expression in cultured theca cells from PCOS patients and controls using DNA microarrays identified the genes encoding aldehyde dehydrogenase 6 and retinol dehydrogenase 2 as candidate genes for PCOS (352). These factors play a role in all-*trans*-retinoic acid biosynthesis and the transcription factor GATA6, which increase the expression of 17α-hydroxylase (352), a characteristic of PCOS theca cells (137). In the near future, undergoing studies using DNA microarrays to compare the expression profiles of tissues such as adipose tissue and muscle, which are essential for the development of insulin resistance, will undoubtedly contribute to the identification of candidate genes in hyperandrogenic women.

But given that gene expression and protein concentration and activity are poorly correlated (353), modern techniques have been developed to study the "proteome" of a cell, tissue, or organism. Proteomics allows large-scale analysis of proteins, including their relative abundance, distribution, post-translational modifications, functions, and interactions with other molecules (347). For example, it is possible to examine the expression of more than 1000 proteins by coupling mass spectrometry technology with several separation methods. Proteomic analysis of tissues involved in the pathogenesis of functional hyperandrogenism and PCOS, such as ovarian theca and granulosa cells, adrenal cortex, sc and omental adipose tissue, and muscle, are essential for our understanding of the complex interactions between the genome and the environment that underlie these disorders.

Ultimately, the identification of genes and proteins related to functional hyperandrogenism and PCOS, and their interactions with the environment, will be an essential step for the development of more precise diagnostic techniques, the identification of new therapeutic targets, and the identification of particular individuals that, because of their genetic background, may be predisposed to certain complications of the syndrome or respond differently to available treatments.

IX. Summary

Functional hyperandrogenism and PCOS appear to be complex multigenic disorders, arising from the interaction of predisposing and protective genetic variants that might have been selected during evolution because of a previous survival advantage, with environmental influences that play an important role in the expression of the hyperandrogenic phenotype.

Among others, genomic variants in genes pertaining to the regulation of androgen biosynthesis, insulin resistance, metabolic syndrome, and proinflammatory genotypes are involved in the genetic predisposition to functional hyperandrogenism and PCOS. Progress in this area requires adequately sized multicenter collaborative studies in which modifying environmental factors such as ethnicity, diet, and lifestyle are identified with precision.

In the future, classic molecular genetic techniques such as linkage analysis in the form of a whole-genome scan and large case-control studies, as well as modern genomic and proteomic approaches, will hopefully provide new insights into the pathogenesis of functional hyperandrogenism and PCOS, with the ultimate aim of improving the prevention, diagnosis, and treatment of these prevalent disorders.

Acknowledgments

Address all correspondence and requests for reprints to: Héctor F. Escobar-Morreale, M.D., Ph.D., Department of Endocrinology, Hospital Ramón y Cajal, Carretera de Colmenar km 9′1, Madrid E-28034, Spain. E-mail: hescobarm.hrc@salud.madrid.org

This work was supported by Grants FIS 02/0741 and RGDM G03/212 from the Fondo de Investigación Sanitaria, Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, and by Grant CAM 08.6/0010/2001 from the Consejería de Educación, Comunidad de Madrid, Spain.

References

1. **Carmina E, Lobo RA** 1999 Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 84:1897–1899
2. **Rosenfield RL** 1999 Ovarian and adrenal function in polycystic ovary syndrome. Endocrinol Metab Clin North Am 28:265–293
3. **Dunaif A** 1997 Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 18:774–800
4. **Achard MC, Thyers MJ** 1921 Le virilisme pilaire et son association à l'insuffisance glycolytique. (Diabète des femmes a barbe). Bull Acad Natl Med 86:51–64
5. **Stein IF, Leventhal LM** 1935 Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 29:181–191
6. **Amowitz LL, Sobel BE** 1999 Cardiovascular consequences of polycystic ovary syndrome. Endocrinol Metab Clin North Am 28:439–458
7. **Talbott EO, Zborowski JV, Sutton-Tyrrell K, McHugh-Pemu KP, Guzick DS** 2001 Cardiovascular risk in women with polycystic ovary syndrome. Obstet Gynecol Clin North Am 28:111–133
8. **Wild RA** 2002 Polycystic ovary syndrome: a risk for coronary artery disease? Am J Obstet Gynecol 186:35–43
9. **Zawadzki JK, Dunaif A** 1992 Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, eds. Polycystic ovary syndrome. Boston: Blackwell Scientific Publications; 377–384
10. **Homburg R** 2002 What is polycystic ovarian syndrome? A proposal for a consensus on the definition and diagnosis of polycystic ovarian syndrome. Hum Reprod 17:2495–2499
11. **The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group** 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 81:19–25
12. **The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus**

Workshop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41–47
13. **Azziz R, Carmina E, Sawaya ME** 2000 Idiopathic hirsutism. Endocr Rev 21:347–362
14. **Azziz R, Kashar-Miller MD** 2000 Family history as a risk factor for the polycystic ovary syndrome. J Pediatr Endocrinol Metab 13(Suppl 5):1303–1306
15. **Benitez R, Sir-Petermann T, Palomino A, Angel B, Maliqueo M, Perez F, Calvillan M** 2001 Prevalence of metabolic disorders among family members of patients with polycystic ovary syndrome. Rev Med Chil 129:707–712 (Spanish)
16. **Colilla S, Cox NJ, Ehrmann DA** 2001 Heritability of insulin secretion and insulin action in women with polycystic ovary syndrome and their first-degree relatives. J Clin Endocrinol Metab 86:2027–2031
17. **Legro RS** 1995 The genetics of polycystic ovary syndrome. Am J Med 98:9S–16S
18. **Franks S, Gharani N, Waterworth D, Batty S, White D, Williamson R, McCarthy M** 1997 The genetic basis of polycystic ovary syndrome. Hum Reprod 12:2641–2648
19. **Jahanfar S, Eden JA** 1996 Genetic and non-genetic theories on the etiology of polycystic ovary syndrome. Gynecol Endocrinol 10: 357–364
20. **Carey AH, Chan KL, Short F, White D, Williamson R, Franks S** 1993 Evidence for a single gene effect causing polycystic ovaries and male pattern baldness. Clin Endocrinol (Oxf) 38:653–658
21. **Govind A, Obhrai MS, Clayton RN** 1999 Polycystic ovaries are inherited as an autosomal dominant trait: analysis of 29 polycystic ovary syndrome and 10 control families. J Clin Endocrinol Metab 84:38–43
22. **Franks S, Gharani N, McCarthy M** 2001 Candidate genes in polycystic ovary syndrome. Hum Reprod Update 7:405–410
23. **Holte J** 1998 Polycystic ovary syndrome and insulin resistance: thrifty genes struggling with over-feeding and sedentary life style? J Endocrinol Invest 21:589–601
24. **Crosignani PG, Nicolosi AE** 2001 Polycystic ovarian disease: heritability and heterogeneity. Hum Reprod Update 7:3–7
25. **Cooper HE, Spellacy WN, Prem KA, Cohen WD** 1968 Hereditary factors in the Stein-Leventhal syndrome. Am J Obstet Gynecol 100:371–387
26. **Wilroy Jr RS, Givens JR, Wiser WL, Coleman SA, Andersen RN, Summitt RL** 1975 Hyperthecosis: an inheritable form of polycystic ovarian disease. Birth Defects Orig Artic Ser 11:81–85
27. **Givens JR** 1971 Familial polycystic ovarian hyperthecosis: a study of two families. Am J Obstet Gynecol 11:959–972
28. **Givens JR** 1988 Familial polycystic ovarian disease. Endocrinol Metab Clin North Am 17:771–783
29. **Ferriman D, Purdie AW** 1979 The inheritance of polycystic ovarian disease and a possible relationship to premature balding. Clin Endocrinol (Oxf) 11:291–300
30. **Hague WM, Adams J, Reeders ST, Peto TE, Jacobs HS** 1988 Familial polycystic ovaries: a genetic disease? Clin Endocrinol (Oxf) 29:593–605
31. **Lunde O, Magnus P, Sandvik L, Hoglo S** 1989 Familial clustering in the polycystic ovarian syndrome. Gynecol Obstet Invest 28:23–30
32. **Kahsar-Miller MD, Nixon C, Boots LR, Go RC, Azziz R** 2001 Prevalence of polycystic ovary syndrome (PCOS) in first-degree relatives of patients with PCOS. Fertil Steril 75:53–58
33. **Legro RS, Driscoll D, Strauss III JF, Fox J, Dunaif A** 1998 Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. Proc Natl Acad Sci USA 95:14956–14960
34. **Legro RS, Bentley-Lewis R, Driscoll D, Wang SC, Dunaif A** 2002 Insulin resistance in the sisters of women with polycystic ovary syndrome: association with hyperandrogenemia rather than menstrual irregularity. J Clin Endocrinol Metab 87:2128–2133
35. **Yildiz BO, Yarali H, Oguz H, Bayraktar M** 2003 Glucose intolerance, insulin resistance, and hyperandrogenemia in first degree relatives of women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:2031–2036
36. **Norman RJ, Masters S, Hague W** 1996 Hyperinsulinemia is common in family members of women with polycystic ovary syndrome. Fertil Steril 66:942–947
37. **Mao W, Li M, Zhao Y** 2000 Study on parents phenotypes in women with polycystic ovary syndrome. Zhonghua Fu Chan Ke Za Zhi 35:583–585 (Chinese)
38. **Legro RS** 2000 Is there a male phenotype in polycystic ovary syndrome families? J Pediatr Endocrinol Metab 13(Suppl 5):1307–1309
39. **Legro RS, Kunselman AR, Demers L, Wang SC, Bentley-Lewis R, Dunaif A** 2002 Elevated dehydroepiandrosterone sulfate levels as the reproductive phenotype in the brothers of women with polycystic ovary syndrome. J Clin Endocrinol Metab 87:2134–2138
40. **Jahanfar S, Eden JA, Warren P, Seppala M, Nguyen TV** 1995 A twin study of polycystic ovary syndrome. Fertil Steril 63:478–486
41. **Jahanfar S, Eden JA, Nguyen T, Wang XL, Wilcken DE** 1997 A twin study of polycystic ovary syndrome and lipids. Gynecol Endocrinol 11:111–117
42. **Ibáñez L, Valls C, Potau N, Marcos MV, de Zegher F** 2001 Polycystic ovary syndrome after precocious pubarche: ontogeny of the low-birthweight effect. Clin Endocrinol (Oxf) 55:667–672
43. **Cresswell JL, Barker DJ, Osmond C, Egger P, Phillips DI, Fraser RB** 1997 Fetal growth, length of gestation, and polycystic ovaries in adult life. Lancet 350:1131–1135
44. **Edozien L** 1998 Length of gestation and polycystic ovaries in adulthood. Lancet 351:295–296
45. **van Hooff MH, Lambalk CB** 1998 Length of gestation and polycystic ovaries in adulthood. Lancet 351:296
46. **Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R** 2002 Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord 26:883–896
47. **Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R** 1998 Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab 83:3078–3082
48. **Asuncion M, Calvo RM, San Millán JL, Sancho J, Avila S, Escobar-Morreale HF** 2000 A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab 85:2434–2438
49. **Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED, Bartzis MI** 1999 A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab 84:4006–4011
50. **Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA** 1992 Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? Am J Obstet Gynecol 167:1807–1812
51. **Dunaif A, Sorbara L, Delson R, Green G** 1993 Ethnicity and polycystic ovary syndrome are associated with independent and additive decreases in insulin action in Caribbean-Hispanic women. Diabetes 42:1462–1468
52. **Williamson K, Gunn AJ, Johnson N, Milsom SR** 2001 The impact of ethnicity on the presentation of polycystic ovarian syndrome. Aust NZ J Obstet Gynaecol 41:202–206
53. **Rodin DA, Bano G, Bland JM, Taylor K, Nussey SS** 1998 Polycystic ovaries and associated metabolic abnormalities in Indian subcontinent Asian women. Clin Endocrinol (Oxf) 49:91–99
54. **Wijeyaratne CN, Balen AH, Barth JH, Belchetz PE** 2002 Clinical manifestations and insulin resistance (IR) in polycystic ovary syndrome (PCOS) among South Asians and Caucasians: is there a difference? Clin Endocrinol (Oxf) 57:343–350
55. **Kasim-Karakas SE, Almario RU, Gregory L, Wong R, Todd H, Lasley BL** 2004 Metabolic and endocrine effects of a polyunsaturated fatty acid-rich diet in polycystic ovary syndrome. J Clin Endocrinol Metab 89:615–620
56. **Guttmacher AE, Collins FS** 2002 Genomic medicine–a primer. N Engl J Med 347:1512–1520
57. **Econs MJ, Speer MC** 1996 Genetic studies of complex diseases: let the reader beware. J Bone Miner Res 11:1835–1840
58. **Risch N, Merikangas K** 1996 The future of genetic studies of complex human diseases. Science 273:1516–1517
59. **Altshuler D, Daly M, Kruglyak L** 2000 Guilt by association. Nat Genet 26:135–137
60. **Lander E, Kruglyak L** 1995 Genetic dissection of complex traits:

guidelines for interpreting and reporting linkage results. Nat Genet 11:241–247
61. **Morton NE** 1998 Significance levels in complex inheritance. Am J Hum Genet 62:690–697
62. **Elston RC** 1998 Methods of linkage analysis–and the assumptions underlying them. Am J Hum Genet 63:931–934
63. **Kruglyak L** 1999 Prospects for whole-genome linkage disequilibrium mapping of common disease genes. Nat Genet 22:139–144
64. **Vink JM, Boomsma DI** 2002 Gene finding strategies. Biol Psychol 61:53–71
65. **Kelly TE** 1986 Clinical genetics, genetic counseling. 2nd ed. Chicago: Year Book Medical Publishers, Inc.; 175–246
66. **Kashar-Miller M, Azziz R** 1999 Heritability and the risk of developing androgen excess. J Steroid Biochem Mol Biol 69:261–268
67. **Legro RS, Strauss JF** 2002 Molecular progress in infertility: polycystic ovary syndrome. Fertil Steril 78:569–576
68. **Spielman RS, McGinnis RE, Ewens WJ** 1993 Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506–516
69. **Spielman RS, Ewens WJ** 1996 The TDT and other family-based tests for linkage disequilibrium and association. Am J Hum Genet 59:983–989
70. **Collins FS** 1995 Positional cloning moves from perditional to traditional. Nat Genet 9:347–350
71. **Seminara SB, Crowley WF** 2002 Genetic approaches to unraveling reproductive disorders: example of bedside to bench research in the genomic era. Endocr Rev 23:382–392
72. **Legro RS** 1999 Polycystic ovary syndrome. Phenotype to genotype. Endocrinol Metab Clin North Am 28:379–396
73. **Greenberg DA** 1993 Linkage analysis of "necessary" disease loci versus "susceptibility" loci. Am J Hum Genet 52:135–143
74. **Fall CH, Osmond C, Barker DJ, Clark PM, Hales CN, Stirling Y, Meade TW** 1995 Fetal and infant growth and cardiovascular risk factors in women. BMJ 310:428–432
75. **Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD** 1991 Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303:1019–1022
76. **Barker DJ, Osmond C** 1988 Low birth weight and hypertension. BMJ 297:134–135
77. **Barker DJ, Osmond C, Golding J, Kuh D, Wadsworth ME** 1989 Growth *in utero*, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 298:564–567
78. **Barker DJ, Bull AR, Osmond C, Simmonds SJ** 1990 Fetal and placental size and risk of hypertension in adult life. BMJ 301:259–262
79. **Barker DJ** 1995 Fetal origins of coronary heart disease. BMJ 311: 171–174
80. **Phipps K, Barker DJ, Hales CN, Fall CH, Osmond C, Clark PM** 1993 Fetal growth and impaired glucose tolerance in men and women. Diabetologia 36:225–228
81. **Eriksson JG, Forsen T, Tuomilehto J, Jaddoe VW, Osmond C, Barker DJ** 2002 Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. Diabetologia 45:342–348
82. **Hattersley AT, Tooke JE** 1999 The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789–1792
83. **Dunger DB, Ong KK, Huxtable SJ, Sherriff A, Woods KA, Ahmed ML, Golding J, Pembrey ME, Ring S, Bennett ST, Todd JA** 1998 Association of the INS VNTR with size at birth. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Nat Genet 19:98–100
84. **Bell GI, Selby MJ, Rutter WJ** 1982 The highly polymorphic region near the human insulin gene is composed of simple tandemly repeating sequences. Nature 295:31–35
85. **Kennedy GC, German MS, Rutter WJ** 1995 The minisatellite in the diabetes susceptibility locus IDDM2 regulates insulin transcription. Nat Genet 9:293–298
86. **Jowett NI, Williams LG, Hitman GA, Galton DJ** 1984 Diabetic hypertriglyceridaemia and related 5′ flanking polymorphism of the human insulin gene. Br Med J (Clin Res Ed) 288:96–99
87. **Weaver JU, Kopelman PG, Hitman GA** 1992 Central obesity and hyperinsulinaemia in women are associated with polymorphism in the 5′ flanking region of the human insulin gene. Eur J Clin Invest 22:265–270
88. **Bennett ST, Todd JA** 1996 Human type 1 diabetes and the insulin gene: principles of mapping polygenes. Annu Rev Genet 30:343–370
89. **Ong KK, Phillips DI, Fall C, Poulton J, Bennett ST, Golding J, Todd JA, Dunger DB** 1999 The insulin gene VNTR, type 2 diabetes and birth weight. Nat Genet 21:262–263
90. **Ong KK, Dunger DB** 2000 Thrifty genotypes and phenotypes in the pathogenesis of type 2 diabetes mellitus. J Pediatr Endocrinol Metab 13(Suppl 6):1419–1424
91. **Ibáñez L, Potau N, Francois I, de Zegher F** 1998 Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls: relation to reduced fetal growth. J Clin Endocrinol Metab 83:3558–3562
92. **Ibáñez L, de Zegher F, Potau N** 1998 Premature pubarche, ovarian hyperandrogenism, hyperinsulinism and the polycystic ovary syndrome: from a complex constellation to a simple sequence of prenatal onset. J Endocrinol Invest 21:558–566
93. **Ibáñez L, Potau N, De Zegher F** 1999 Endocrinology and metabolism after premature pubarche in girls. Acta Paediatr Suppl 88: 73–77
94. **Ibáñez L, Potau N, Dunger D, de Zegher F** 2000 Precocious pubarche in girls and the development of androgen excess. J Pediatr Endocrinol Metab 13(Suppl 5):1261–1263
95. **Ibáñez L, Dimartino-Nardi J, Potau N, Saenger P** 2000 Premature adrenarche–normal variant or forerunner of adult disease? Endocr Rev 21:671–696
96. **Ibáñez L, de Zegher F, Potau N** 1999 Anovulation after precocious pubarche: early markers and time course in adolescence. J Clin Endocrinol Metab 84:2691–2695
97. **Ibáñez L, Potau N, Marcos MV, De Zegher F** 2000 Adrenal hyperandrogenism in adolescent girls with a history of low birthweight and precocious pubarche. Clin Endocrinol (Oxf) 53:523–527
98. **Banerjee S, Raghavan S, Wasserman EJ, Linder BL, Saenger P, DiMartino-Nardi J** 1998 Hormonal findings in African-American and Caribbean Hispanic girls with premature adrenarche: implications for polycystic ovarian syndrome. Pediatrics 102:E36
99. **Jaquet D, Leger J, Chevenne D, Czernichow P, Levy-Marchal C** 1999 Intrauterine growth retardation predisposes to insulin resistance but not to hyperandrogenism in young women. J Clin Endocrinol Metab 84:3945–3949
100. **Laitinen J, Taponen S, Martikainen H, Pouta A, Millwood I, Hartikainen AL, Ruokonen A, Sovio U, McCarthy MI, Franks S, Jarvelin MR** 2003 Body size from birth to adulthood as a predictor of self-reported polycystic ovary syndrome symptoms. Int J Obes Relat Metab Disord 27:710–715
101. **Ibáñez L, Ong K, Potau N, Marcos MV, de Zegher F, Dunger D** 2001 Insulin gene variable number of tandem repeat genotype and the low birth weight, precocious pubarche, and hyperinsulinism sequence. J Clin Endocrinol Metab 86:5788–5793
102. **El Mkadem SA, Lautier C, Macari F, Molinari N, Lefebvre P, Renard E, Gris JC, Cros G, Daures JP, Bringer J, White MF, Grigorescu F** 2001 Role of allelic variants Gly^{972}Arg of IRS-1 and Gly^{1057}Asp of IRS-2 in moderate-to-severe insulin resistance of women with polycystic ovary syndrome. Diabetes 50:2164–2168
103. **Ehrmann DA, Tang X, Yoshiuchi I, Cox NJ, Bell GI** 2002 Relationship of insulin receptor substrate-1 and -2 genotypes to phenotypic features of polycystic ovary syndrome. J Clin Endocrinol Metab 87:4297–4300
104. **Ibáñez L, Marcos MV, Potau N, White C, Aston CE, Witchel SF** 2002 Increased frequency of the G972R variant of the insulin receptor substrate-1 (IRS-1) gene among girls with a history of precocious pubarche. Fertil Steril 78:1288–1293
105. **Lin WH, Chiu KC, Chang HM, Lee KC, Tai TY, Chuang LM** 2001 Molecular scanning of the human sorbin and SH3-domain-containing-1 (SORBS1) gene: positive association of the T228A polymorphism with obesity and type 2 diabetes. Hum Mol Genet 10:1753–1760
106. **Witchel SF, Trivedi RN, Kammerer C** 2003 Frequency of the T228A polymorphism in the SORBS1 gene in children with premature pubarche and in adolescent girls with hyperandrogenism. Fertil Steril 80:128–132

107. **Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC** 1995 Association of a polymorphism in the β_3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 333:348–351
108. **Witchel SF, Fagerli J, Siegel J, Smith R, Mitwally MF, Lewy V, Arslanian S, Lee PA** 2000 No association between body mass index and β_3-adrenergic receptor variant (W64R) in children with premature pubarche and adolescent girls with hyperandrogenism. Fertil Steril 73:509–515
109. **Ek J, Andersen G, Urhammer SA, Hansen L, Carstensen B, Borch-Johnsen K, Drivsholm T, Berglund L, Hansen T, Lithell H, Pedersen O** 2001 Studies of the Pro^{12}Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) gene in relation to insulin sensitivity among glucose tolerant Caucasians. Diabetologia 44:1170–1176
110. **Witchel SF, White C, Siegel ME, Aston CE** 2001 Inconsistent effects of the proline12 –> alanine variant of the peroxisome proliferator-activated receptor-γ2 gene on body mass index in children and adolescent girls. Fertil Steril 76:741–747
111. **Nayak S, Lee PA, Witchel SF** 1998 Variants of the type II 3β-hydroxysteroid dehydrogenase gene in children with premature pubic hair and hyperandrogenic adolescents. Mol Genet Metab 64:184–192
112. **Witchel SF, Lee PA, Suda-Hartman M, Hoffman EP** 1997 Hyperandrogenism and manifesting heterozygotes for 21-hydroxylase deficiency. Biochem Mol Med 62:151–158
113. **Witchel SF, Aston CE** 2000 The role of heterozygosity for CYP21 in the polycystic ovary syndrome. J Pediatr Endocrinol Metab 13(Suppl 5):1315–1317
114. **Witchel SF, Lee PA, Suda-Hartman M, Smith R, Hoffman EP** 1998 17α-Hydroxylase/17,20-lyase dysregulation is not caused by mutations in the coding regions of CYP17. J Pediatr Adolesc Gynecol 11:133–137
115. **Witchel SF, Smith R, Tomboc M, Aston CE** 2001 Candidate gene analysis in premature pubarche and adolescent hyperandrogenism. Fertil Steril 75:724–730
116. **Ibáñez L, Ong KK, Mongan N, Jaaskelainen J, Marcos MV, Hughes IA, De Zegher F, Dunger DB** 2003 Androgen receptor gene CAG repeat polymorphism in the development of ovarian hyperandrogenism. J Clin Endocrinol Metab 88:3333–3338
117. **Tomboc M, Witchel SF** 2003 Frequencies of the D85 and Y85 variants of UGT2B15 in children and adolescent girls with hyperandrogenism. J Pediatr Endocrinol Metab 16:719–726
118. **Zhang LH, Rodriguez H, Ohno S, Miller WL** 1995 Serine phosphorylation of human P450c17 increases 17,20-lyase activity: implications for adrenarche and the polycystic ovary syndrome. Proc Natl Acad Sci USA 92:10619–10623
119. **Rosenfield RL, Barnes RB, Cara JF, Lucky AW** 1990 Dysregulation of cytochrome P450c 17α as the cause of polycystic ovarian syndrome. Fertil Steril 53:785–791
120. **Rosenfield RL, Barnes RB, Ehrmann DA** 1994 Studies of the nature of 17-hydroxyprogesterone hyperresponsiveness to gonadotropin-releasing hormone agonist challenge in functional ovarian hyperandrogenism. J Clin Endocrinol Metab 79:1686–1692
121. **Escobar-Morreale H, Pazos F, Potau N, Garcia-Robles R, Sancho JM, Varela C** 1994 Ovarian suppression with triptorelin and adrenal stimulation with adrenocorticotropin in functional hyperandrogenism: role of adrenal and ovarian cytochrome P450c17α. Fertil Steril 62:521–530
122. **Escobar-Morreale HF, Serrano-Gotarredona J, Garcia-Robles R, Sancho JM, Varela C** 1997 Lack of an ovarian function influence on the increased adrenal androgen secretion present in women with functional ovarian hyperandrogenism. Fertil Steril 67:654–662
123. **Escobar-Morreale HF, Serrano-Gotarredona J, Garcia-Robles R, Sancho J, Varela C** 1997 Mild adrenal and ovarian steroidogenic abnormalities in hirsute women without hyperandrogenemia: does idiopathic hirsutism exist? Metabolism 46:902–907
124. **Wickenheisser JK, Quinn PG, Nelson VL, Legro RS, Strauss III JF, McAllister JM** 2000 Differential activity of the cytochrome P450 17α-hydroxylase and steroidogenic acute regulatory protein gene promoters in normal and polycystic ovary syndrome theca cells. J Clin Endocrinol Metab 85:2304–2311
125. **Carey AH, Waterworth D, Patel K, White D, Little J, Novelli P, Franks S, Williamson R** 1994 Polycystic ovaries and premature male pattern baldness are associated with one allele of the steroid metabolism gene CYP17. Hum Mol Genet 3:1873–1876
126. **Franks S** 1997 The 17α-hydroxylase/17,20 lyase gene (CYP17) and polycystic ovary syndrome. Clin Endocrinol (Oxf) 46:135–136
127. **Franks S, White D, Gilling-Smith C, Carey A, Waterworth D, Williamson R** 1996 Hypersecretion of androgens by polycystic ovaries: the role of genetic factors in the regulation of cytochrome P450c17α. Baillieres Clin Endocrinol Metab 10:193–203
128. **Diamanti-Kandarakis E, Bartzis MI, Zapanti ED, Spina GG, Filandra FA, Tsianateli TC, Bergiele AT, Kouli CR** 1999 Polymorphism T–>C (−34 bp) of gene CYP17 promoter in Greek patients with polycystic ovary syndrome. Fertil Steril 71:431–435
129. **Gharani N, Waterworth DM, Williamson R, Franks S** 1996 5′ Polymorphism of the CYP17 gene is not associated with serum testosterone levels in women with polycystic ovaries. J Clin Endocrinol Metab 81:4174
130. **Marszalek B, Lacinski M, Babych N, Capla E, Biernacka-Lukanty J, Warenik-Szymankiewicz A, Trzeciak WH** 2001 Investigations on the genetic polymorphism in the region of CYP17 gene encoding 5′-UTR in patients with polycystic ovarian syndrome. Gynecol Endocrinol 15:123–128
131. **Techatraisak K, Conway GS, Rumsby G** 1997 Frequency of a polymorphism in the regulatory region of the 17α-hydroxylase-17,20-lyase (CYP17) gene in hyperandrogenic states. Clin Endocrinol (Oxf) 46:131–134
132. **Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF, Spielman RS, Dunaif A** 1999 Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc Natl Acad Sci USA 96:8573–8578
133. **Liovic M, Prezelj J, Kocijancic A, Majdic G, Komel R** 1997 CYP17 gene analysis in hyperandrogenised women with and without exaggerated 17-hydroxyprogesterone response to ovarian stimulation. J Endocrinol Invest 20:189–193
134. **Qin KN, Rosenfield RL** 1998 Role of cytochrome P450c17 in polycystic ovary syndrome. Mol Cell Endocrinol 145:111–121
135. **Martens JW, Geller DH, Arlt W, Auchus RJ, Ossovskaya VS, Rodriguez H, Dunaif A, Miller WL** 2000 Enzymatic activities of P450c17 stably expressed in fibroblasts from patients with the polycystic ovary syndrome. J Clin Endocrinol Metab 85:4338–4346
136. **Nelson VL, Legro RS, Strauss III JF, McAllister JM** 1999 Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol 13:946–957
137. **Nelson VL, Qin Kn KN, Rosenfield RL, Wood JR, Penning TM, Legro RS, Strauss III JF, McAllister JM** 2001 The biochemical basis for increased testosterone production in theca cells propagated from patients with polycystic ovary syndrome. J Clin Endocrinol Metab 86:5925–5933
138. **Gilling-Smith C, Willis DS, Beard RW, Franks S** 1994 Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. J Clin Endocrinol Metab 79:1158–1165
139. **Franks S, Gilling-Smith C, Gharani N, McCarthy M** 2000 Pathogenesis of polycystic ovary syndrome: evidence for a genetically determined disorder of ovarian androgen production. Hum Fertil (Camb) 3:77–79
140. **Gharani N, Waterworth DM, Batty S, White D, Gilling-Smith C, Conway GS, McCarthy M, Franks S, Williamson R** 1997 Association of the steroid synthesis gene CYP11a with polycystic ovary syndrome and hyperandrogenism. Hum Mol Genet 6:397–402
141. **Diamanti-Kandarakis E, Bartzis MI, Bergiele AT, Tsianateli TC, Kouli CR** 2000 Microsatellite polymorphism (tttta) at −528 base pairs of gene CYP11a influences hyperandrogenemia in patients with polycystic ovary syndrome. Fertil Steril 73:735–741
142. **Daneshmand S, Weitsman SR, Navab A, Jakimiuk AJ, Magoffin DA** 2002 Overexpression of theca-cell messenger RNA in polycystic ovary syndrome does not correlate with polymorphisms in the cholesterol side-chain cleavage and 17α-hydroxylase/C(17–20) lyase promoters. Fertil Steril 77:274–280
143. **San Millán JL, Sancho J, Calvo RM, Escobar-Morreale HF** 2001 Role of the pentanucleotide (tttta)(n) polymorphism in the promoter of the CYP11a gene in the pathogenesis of hirsutism. Fertil Steril 75:797–802

144. **Calvo RM, Asuncion M, Telleria D, Sancho J, San Millán JL, Escobar-Morreale HF** 2001 Screening for mutations in the steroidogenic acute regulatory protein and steroidogenic factor-1 genes, and in CYP11A and dosage-sensitive sex reversal-adrenal hypoplasia gene on the X chromosome, gene-1 (DAX-1), in hyperandrogenic hirsute women. J Clin Endocrinol Metab 86:1746–1749
145. **Gaasenbeek M, Powell BL, Sovio U, Haddad L, Gharani N, Bennett A, Groves CJ, Rush K, Goh MJ, Conway GS, Ruokonen A, Martikainen H, Pouta A, Taponen S, Hartikainen AL, Halford S, Jarvelin MR, Franks S, McCarthy MI** 2004 Large-scale analysis of the relationship between CYP11A promoter variation, polycystic ovarian syndrome, and serum testosterone. J Clin Endocrinol Metab 89:2408–2413
146. **Azziz R, Bradley EL, Potter HD, Boots LR** 1995 Adrenal androgen excess in women: lack of a role for 17-hydroxylase and 17,20-lyase dysregulation. J Clin Endocrinol Metab 80:400–405
147. **Escobar-Morreale HF, San Millán JL, Smith RR, Sancho J, Witchel SF** 1999 The presence of the 21-hydroxylase deficiency carrier status in hirsute women: phenotype-genotype correlations. Fertil Steril 72:629–638
148. **Hague WM, Adams J, Algar V, Drummond V, Schwarz G, Bottazzo GF, Jacobs HS** 1990 HLA associations in patients with polycystic ovaries and in patients with congenital adrenal hyperplasia caused by 21-hydroxylase deficiency. Clin Endocrinol (Oxf) 32: 407–415
149. **Hague WM, Adams J, Rodda C, Brook CG, de Bruyn R, Grant DB, Jacobs HS** 1990 The prevalence of polycystic ovaries in patients with congenital adrenal hyperplasia and their close relatives. Clin Endocrinol (Oxf) 33:501–510
150. **Simard J, Moisan AM, Morel Y** 2002 Congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase deficiency. Semin Reprod Med 20:255–276
151. **Pang SY, Lerner AJ, Stoner E, Levine LS, Oberfield SE, Engel I, New MI** 1985 Late-onset adrenal steroid 3β-hydroxysteroid dehydrogenase deficiency. I. A cause of hirsutism in pubertal and postpubertal women. J Clin Endocrinol Metab 60:428–439
152. **Siegel SF, Finegold DN, Lanes R, Lee PA** 1990 ACTH stimulation tests and plasma dehydroepiandrosterone sulfate levels in women with hirsutism. N Engl J Med 323:849–854
153. **Eldar Geva T, Hurwitz A, Vecsei P, Palti Z, Milwidsky A, Rosler A** 1990 Secondary biosynthetic defects in women with late-onset congenital adrenal hyperplasia. N Engl J Med 323:855–863
154. **Azziz R, Bradley EJ, Potter HD, Boots LR** 1993 3β-Hydroxysteroid dehydrogenase deficiency in hyperandrogenism. Am J Obstet Gynecol 168:889–895
155. **Chang YT, Zhang L, Alkaddour HS, Mason JI, Lin K, Yang X, Garibaldi LR, Bourdony CJ, Dolan LM, Donaldson DL** 1995 Absence of molecular defect in the type II 3β-hydroxysteroid dehydrogenase (3β-HSD) gene in premature pubarche children and hirsute female patients with moderately decreased adrenal 3β-HSD activity. Pediatr Res 37:820–824
156. **Carbunaru G, Prasad P, Scoccia B, Shea P, Hopwood N, Ziai F, Chang YT, Myers SE, Mason JI, Pang S** 2004 The hormonal phenotype of nonclassic 3β-hydroxysteroid dehydrogenase (HSD3B) deficiency in hyperandrogenic females is associated with insulin-resistant polycystic ovary syndrome and is not a variant of inherited HSD3B2 deficiency. J Clin Endocrinol Metab 89:783–794
157. **Lindqvist A, Hughes IA, Andersson S** 2001 Substitution mutation C268Y causes 17β-hydroxysteroid dehydrogenase 3 deficiency. J Clin Endocrinol Metab 86:921–923
158. **Pang SY, Softness B, Sweeney III WJ, New MI** 1987 Hirsutism, polycystic ovarian disease, and ovarian 17-ketosteroid reductase deficiency. N Engl J Med 316:1295–1301
159. **Moghrabi N, Hughes IA, Dunaif A, Andersson S** 1998 Deleterious missense mutations and silent polymorphism in the human 17β-hydroxysteroid dehydrogenase 3 gene (HSD17B3). J Clin Endocrinol Metab 83:2855–2860
160. **Takayama K, Fukaya T, Sasano H, Funayama Y, Suzuki T, Takaya R, Wada Y, Yajima A** 1996 Immunohistochemical study of steroidogenesis and cell proliferation in polycystic ovarian syndrome. Hum Reprod 11:1387–1392
161. **Jakimiuk AJ, Weitsman SR, Brzechffa PR, Magoffin DA** 1998 Aromatase mRNA expression in individual follicles from polycystic ovaries. Mol Hum Reprod 4:1–8
162. **Mullis PE, Yoshimura N, Kuhlmann B, Lippuner K, Jaeger P, Harada H** 1997 Aromatase deficiency in a female who is compound heterozygote for two new point mutations in the P450arom gene: impact of estrogens on hypergonadotropic hypogonadism, multicystic ovaries, and bone densitometry in childhood. J Clin Endocrinol Metab 82:1739–1745
163. **Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, Lavery GG, Bedendo O, Ray DW, Laing I, Malunowicz E, White PC, Hewison M, Mason PJ, Connell JM, Shackleton CH, Stewart PM** 2003 Mutations in the genes encoding 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. Nat Genet 34:434–439
164. **Ehrmann DA, Barnes RB, Rosenfield RL** 1995 Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. Endocr Rev 16:322–353
165. **Furui K, Suganuma N, Tsukahara S, Asada Y, Kikkawa F, Tanaka M, Ozawa T, Tomoda Y** 1994 Identification of two point mutations in the gene coding luteinizing hormone (LH) β-subunit, associated with immunologically anomalous LH variants. J Clin Endocrinol Metab 78:107–113
166. **Okuda K, Yamada T, Imoto H, Komatsubara H, Sugimoto O** 1994 Antigenic alteration of an anomalous human luteinizing hormone caused by two chorionic gonadotropin-type amino-acid substitutions. Biochem Biophys Res Commun 200:584–590
167. **Nilsson C, Pettersson K, Millar RP, Coerver KA, Matzuk MM, Huhtaniemi IT** 1997 Worldwide frequency of a common genetic variant of luteinizing hormone: an international collaborative research. International Collaborative Research Group. Fertil Steril 67:998–1004
168. **Haavisto AM, Pettersson K, Bergendahl M, Virkamaki A, Huhtaniemi I** 1995 Occurrence and biological properties of a common genetic variant of luteinizing hormone. J Clin Endocrinol Metab 80:1257–1263
169. **Rajkhowa M, Talbot JA, Jones PW, Pettersson K, Haavisto AM, Huhtaniemi I, Clayton RN** 1995 Prevalence of an immunological LH β-subunit variant in a UK population of healthy women and women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 43:297–303
170. **Tapanainen JS, Koivunen R, Fauser BC, Taylor AE, Clayton RN, Rajkowa M, White D, Franks S, Anttila L, Pettersson KS, Huhtaniemi IT** 1999 A new contributing factor to polycystic ovary syndrome: the genetic variant of luteinizing hormone. J Clin Endocrinol Metab 84:1711–1715
171. **Bergendah M, Veldhuis JD** 2001 Is there a physiological role for gonadotrophin oligosaccharide heterogeneity in humans? III. Luteinizing hormone heterogeneity: a medical physiologist's perspective. Hum Reprod 16:1058–1064
172. **Ramanujam LN, Liao WX, Roy AC, Loganath A, Goh HH, Ng SC** 1999 Association of molecular variants of luteinizing hormone with menstrual disorders. Clin Endocrinol (Oxf) 51:243–246
173. **Elter K, Erel CT, Cine N, Ozbek U, Hacihanefioglu B, Ertungealp E** 1999 Role of the mutations Trp8 $\Rightarrow$ Arg and Ile15 $\Rightarrow$ Thr of the human luteinizing hormone β-subunit in women with polycystic ovary syndrome. Fertil Steril 71:425–430
174. **Liao WX, Roy AC, Chan C, Arulkumaran S, Ratnam SS** 1998 A new molecular variant of luteinizing hormone associated with female infertility. Fertil Steril 69:102–106
175. **Takahashi K, Karino K, Kanasaki H, Kurioka H, Ozaki T, Yonehara T, Miyazaki K** 2003 Influence of missense mutation and silent mutation of LHβ-subunit gene in Japanese patients with ovulatory disorders. Eur J Hum Genet 11:402–408
176. **Tong Y, Liao WX, Roy AC, Ng SC** 2000 Association of AccI polymorphism in the follicle-stimulating hormone β gene with polycystic ovary syndrome. Fertil Steril 74:1233–1236
177. **Tong Y, Liao WX, Roy AC, Ng SC** 2001 Absence of mutations in the coding regions of follicle-stimulating hormone receptor gene in Singapore Chinese women with premature ovarian failure and polycystic ovary syndrome. Horm Metab Res 33:221–226
178. **Takakura K, Takebayashi K, Wang HQ, Kimura F, Kasahara K, Noda Y** 2001 Follicle-stimulating hormone receptor gene mutations

are rare in Japanese women with premature ovarian failure and polycystic ovary syndrome. Fertil Steril 75:207–209
179. **Cohen DP, Stein EM, Li Z, Matulis CK, Ehrmann DA, Layman LC** 1999 Molecular analysis of the gonadotropin-releasing hormone receptor in patients with polycystic ovary syndrome. Fertil Steril 72:360–363
180. **Legro RS, Dietz GW, Comings DE, Lobo RA, Kovacs BW** 1994 Association of dopamine D2 receptor gene haplotypes with anovulation and fecundity in female Hispanics. Hum Reprod 9:1271–1275
181. **Legro RS, Muhleman DR, Comings DE, Lobo RA, Kovacs BW** 1995 A dopamine D3 receptor genotype is associated with hyperandrogenic chronic anovulation and resistant to ovulation induction with clomiphene citrate in female Hispanics. Fertil Steril 63:779–784
182. **Kahsar-Miller M, Boots LR, Azziz R** 1999 Dopamine D3 receptor polymorphism is not associated with the polycystic ovary syndrome. Fertil Steril 71:436–438
183. **Pugeat M, Crave JC, Tourniaire J, Forest MG** 1996 Clinical utility of sex hormone-binding globulin measurement. Horm Res 45:148–155
184. **Hogeveen KN, Cousin P, Pugeat M, Dewailly D, Soudan B, Hammond GL** 2002 Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. J Clin Invest 109:973–981
185. **Xita N, Tsatsoulis A, Chatzikyriakidou A, Georgiou I** 2003 Association of the (TAAAA)n repeat polymorphism in the sex hormone-binding globulin (SHBG) gene with polycystic ovary syndrome and relation to SHBG serum levels. J Clin Endocrinol Metab 88:5976–5980
186. **Cousin P, Calemard-Michel L, Lejeune H, Raverot G, Yessaad N, Emptoz-Bonneton A, Morel Y, Pugeat M** 2004 Influence of SHBG gene pentanucleotide TAAAA repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in hirsute women. J Clin Endocrinol Metab 89:917–924
187. **Calvo RM, Asunción M, Sancho JM, San Millán JL, Escobar-Morreale HF,** Analysis of the gene encoding the glucocorticoid receptor (GRL) in hirsute women presenting with functional adrenal hyperandrogenism. Program of the 82nd Annual Meeting of The Endocrine Society, Toronto, ON, Canada, 2000, p 402 (Abstract 1662)
188. **Witchel SF, Smith RR** 1999 Glucocorticoid resistance in premature pubarche and adolescent hyperandrogenism. Mol Genet Metab 66:137–141
189. **Kahsar-Miller M, Azziz R, Feingold E, Witchel SF** 2000 A variant of the glucocorticoid receptor gene is not associated with adrenal androgen excess in women with polycystic ovary syndrome. Fertil Steril 74:1237–1240
190. **Thigpen AE, Davis DL, Milatovich A, Mendonca BB, Imperato-McGinley J, Griffin JE, Francke U, Wilson JD, Russell DW** 1992 Molecular genetics of steroid 5α-reductase 2 deficiency. J Clin Invest 90:799–809
191. **Andersson S, Berman DM, Jenkins EP, Russell DW** 1991 Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism. Nature 354:159–161
192. **Jakimiuk AJ, Weitsman SR, Magoffin DA** 1999 5α-Reductase activity in women with polycystic ovary syndrome. J Clin Endocrinol Metab 84:2414–2418
193. **Lubahn DB, Joseph DR, Sullivan PM, Willard HF, French FS, Wilson EM** 1988 Cloning of human androgen receptor complementary DNA and localization to the X chromosome. Science 240: 327–330
194. **Brown CJ, Goss SJ, Lubahn DB, Joseph DR, Wilson EM, French FS, Willard HF** 1989 Androgen receptor locus on the human X chromosome: regional localization to Xq11–12 and description of a DNA polymorphism. Am J Hum Genet 44:264–269
195. **Carson-Jurica MA, Schrader WT, O'Malley BW** 1990 Steroid receptor family: structure and functions. Endocr Rev 11:201–220
196. **Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R** 1992 Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12:241–253
197. **Chamberlain NL, Driver ED, Miesfeld RL** 1994 The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 22:3181–3186
198. **Legro RS, Shahbahrami B, Lobo RA, Kovacs BW** 1994 Size polymorphisms of the androgen receptor among female Hispanics and correlation with androgenic characteristics. Obstet Gynecol 83:701–706
199. **Dowsing AT, Yong EL, Clark M, McLachlan RI, de Kretser DM, Trounson AO** 1999 Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. Lancet 354: 640–643
200. **Sawaya ME, Shalita AR** 1998 Androgen receptor polymorphisms (CAG repeat lengths) in androgenetic alopecia, hirsutism, and acne. J Cutan Med Surg 3:9–15
201. **Mifsud A, Ramirez S, Yong EL** 2000 Androgen receptor gene CAG trinucleotide repeats in anovulatory infertility and polycystic ovaries. J Clin Endocrinol Metab 85:3484–3488
202. **Vottero A, Stratakis CA, Ghizzoni L, Longui CA, Karl M, Chrousos GP** 1999 Androgen receptor-mediated hypersensitivity to androgens in women with nonhyperandrogenic hirsutism: skewing of X-chromosome inactivation. J Clin Endocrinol Metab 84:1091–1095
203. **Hickey T, Chandy A, Norman RJ** 2002 The androgen receptor CAG repeat polymorphism and X-chromosome inactivation in Australian Caucasian women with infertility related to polycystic ovary syndrome. J Clin Endocrinol Metab 87:161–165
204. **Calvo RM, Asuncion M, Sancho J, San Millán JL, Escobar-Morreale HF** 2000 The role of the CAG repeat polymorphism in the androgen receptor gene and of skewed X-chromosome inactivation, in the pathogenesis of hirsutism. J Clin Endocrinol Metab 85:1735–1740
205. **Jaatinen TA, Matinlauri I, Anttila L, Koskinen P, Erkkola R, Irjala K** 1995 Serum total renin is elevated in women with polycystic ovarian syndrome. Fertil Steril 63:1000–1004
206. **Morris RS, Wong IL, Hatch IE, Gentschein E, Paulson RJ, Lobo RA** 1995 Prorenin is elevated in polycystic ovary syndrome and may reflect hyperandrogenism. Fertil Steril 64:1099–1103
207. **Zhao SP, Tang XM, Shao DH, Dai HY, Dai SZ** 2003 Association study between a polymorphism of aldosterone synthetase gene and the pathogenesis of polycystic ovary syndrome. Zhonghua Fu Chan Ke Za Zhi 38:94–97 (Chinese)
208. **Ibáñez L, Potau N, Zampolli M, Prat N, Virdis R, Vicens-Calvet E, Carrascosa A** 1996 Hyperinsulinemia in postpubertal girls with a history of premature pubarche and functional ovarian hyperandrogenism. J Clin Endocrinol Metab 81:1237–1243
209. **Chang PL, Lindheim SR, Lowre C, Ferin M, González F, Berglund L, Carmina E, Sauer MV, Lobo RA** 2000 Normal ovulatory women with polycystic ovaries have hyperandrogenic pituitary-ovarian responses to gonadotropin-releasing hormone-agonist testing. J Clin Endocrinol Metab 85:995–1000
210. **Altuntas Y, Bilir M, Ozturk B, Gundogdu S** 2003 Comparison of various simple insulin sensitivity and β-cell function indices in lean hyperandrogenemic and normoandrogenemic young hirsute women. Fertil Steril 80:133–142
211. **Tucci S, Futterweit W, Concepcion ES, Greenberg DA, Villanueva R, Davies TF, Tomer Y** 2001 Evidence for association of polycystic ovary syndrome in Caucasian women with a marker at the insulin receptor gene locus. J Clin Endocrinol Metab 86:446–449
212. **Villuendas G, Escobar-Morreale HF, Tosi F, Sancho J, Moghetti P, San Millán JL** 2003 Association between the D19S884 marker at the insulin receptor gene locus and polycystic ovary syndrome. Fertil Steril 79:219–220
213. **Sorbara LR, Tang Z, Cama A, Xia J, Schenker E, Kohanski RA, Poretsky L, Koller E, Taylor SI, Dunaif A** 1994 Absence of insulin receptor gene mutations in three insulin-resistant women with the polycystic ovary syndrome. Metabolism 43:1568–1574
214. **Talbot JA, Bicknell EJ, Rajkhowa M, Krook A, O'Rahilly S, Clayton RN** 1996 Molecular scanning of the insulin receptor gene in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 81:1979–1983
215. **Siegel S, Futterweit W, Davies TF, Concepcion ES, Greensberg DA, Villanueva R, Tomer Y** 2002 A C/T single nucleotide polymorphism at the tyrosine kinase domain of the insulin receptor

gene is associated with polycystic ovary syndrome. Fertil Steril 78:1240–1243
216. **Dunaif A, Xia J, Book CB, Schenker E, Tang Z** 1995 Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. J Clin Invest 96:801–810
217. **Dunaif A, Wu X, Lee A, Diamanti-Kandarakis E** 2001 Defects in insulin receptor signaling in vivo in the polycystic ovary syndrome (PCOS). Am J Physiol Endocrinol Metab 281:E392—E399
218. **Conway GS, Avey C, Rumsby G** 1994 The tyrosine kinase domain of the insulin receptor gene is normal in women with hyperinsulinaemia and polycystic ovary syndrome. Hum Reprod 9:1681–1683
219. **Jellema A, Zeegers MP, Feskens EJ, Dagnelie PC, Mensink RP** 2003 $Gly^{972}Arg$ variant in the insulin receptor substrate-1 gene and association with type 2 diabetes: a meta-analysis of 27 studies. Diabetologia 46:990–995
220. **Mammarella S, Romano F, Di Valerio A, Creati B, Esposito DL, Palmirotta R, Capani F, Vitullo P, Volpe G, Battista P, Della Loggia F, Mariani-Costantini R, Cama A** 2000 Interaction between the G1057D variant of IRS-2 and overweight in the pathogenesis of type 2 diabetes. Hum Mol Genet 9:2517–2521
221. **Ehrmann DA, Sturis J, Byrne MM, Karrison T, Rosenfield RL, Polonsky KS** 1995 Insulin secretory defects in polycystic ovary syndrome. Relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. J Clin Invest 96:520–527
222. **Waterworth DM, Bennett ST, Gharani N, McCarthy MI, Hague S, Batty S, Conway GS, White D, Todd JA, Franks S, Williamson R** 1997 Linkage and association of insulin gene VNTR regulatory polymorphism with polycystic ovary syndrome. Lancet 349:986–990
223. **Michelmore K, Ong K, Mason S, Bennett S, Perry L, Vessey M, Balen A, Dunger D** 2001 Clinical features in women with polycystic ovaries: relationships to insulin sensitivity, insulin gene VNTR and birth weight. Clin Endocrinol (Oxf) 55:439–446
224. **Eaves IA, Bennett ST, Forster P, Ferber KM, Ehrmann D, Wilson AJ, Bhattacharyya S, Ziegler AG, Brinkmann B, Todd JA** 1999 Transmission ratio distortion at the INS-IGF2 VNTR. Nat Genet 22:324–325
225. **Calvo RM, Telleria D, Sancho J, San Millán JL, Escobar-Morreale HF** 2002 Insulin gene variable number of tandem repeats regulatory polymorphism is not associated with hyperandrogenism in Spanish women. Fertil Steril 77:666–668
226. **Vankova M, Vrbikova J, Hill M, Cinek O, Bendlova B** 2002 Association of insulin gene VNTR polymorphism with polycystic ovary syndrome. Ann NY Acad Sci 967:558–565
227. **Voutilainen R, Franks S, Mason HD, Martikainen H** 1996 Expression of insulin-like growth factor (IGF), IGF-binding protein, and IGF receptor messenger ribonucleic acids in normal and polycystic ovaries. J Clin Endocrinol Metab 81:1003–1008
228. **Giudice LC** 1999 Growth factor action on ovarian function in polycystic ovary syndrome. Endocrinol Metab Clin North Am 28:325–339
229. **San Millán JL, Corton M, Villuendas G, Sancho J, Peral B, Escobar-Morreale HF** 2004 Association of the polycystic ovary syndrome with genomic variants related to insulin resistance, type 2 diabetes mellitus, and obesity. J Clin Endocrinol Metab 89:2640–2646
230. **Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A, Lamberts SW, Oostra BA, Pols HA, van Duijn CM** 2001 A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes 50:637–642
231. **Meloni R, Fougerousse F, Roudaut C, Beckmann JS** 1992 Trinucleotide repeat polymorphism at the human insulin-like growth factor I receptor gene (IGF1R). Nucleic Acids Res 20:1427
232. **Smrzka OW, Fae I, Stoger R, Kurzbauer R, Fischer GF, Henn T, Weith A, Barlow DP** 1995 Conservation of a maternal-specific methylation signal at the human IGF2R locus. Hum Mol Genet 4:1945–1952
233. **Vafiadis P, Bennett ST, Todd JA, Grabs R, Polychronakos C** 1998 Divergence between genetic determinants of IGF2 transcription levels in leukocytes and of IDDM2-encoded susceptibility to type 1 diabetes. J Clin Endocrinol Metab 83:2933–2939
234. **O'Dell SD, Miller GJ, Cooper JA, Hindmarsh PC, Pringle PJ, Ford H, Humphries SE, Day IN** 1997 Apal polymorphism in insulin-like growth factor II (IGF2) gene and weight in middle-aged males. Int J Obes Relat Metab Disord 21:822–825
235. **Mesiano S, Katz SL, Lee JY, Jaffe RB** 1997 Insulin-like growth factors augment steroid production and expression of steroidogenic enzymes in human fetal adrenal cortical cells: implications for adrenal androgen regulation. J Clin Endocrinol Metab 82:1390–1396
236. **l'Allemand D, Penhoat A, Lebrethon MC, Ardevol R, Baehr V, Oelkers W, Saez JM** 1996 Insulin-like growth factors enhance steroidogenic enzyme and corticotropin receptor messenger ribonucleic acid levels and corticotropin steroidogenic responsiveness in cultured human adrenocortical cells. J Clin Endocrinol Metab 81:3892–3897
237. **Cara JF** 1994 Insulin-like growth factors, insulin-like growth factor binding proteins and ovarian androgen production. Horm Res 42:49–54
238. **Ghazeeri G, Kutteh WH, Bryer-Ash M, Haas D, Ke RW** 2003 Effect of rosiglitazone on spontaneous and clomiphene citrate-induced ovulation in women with polycystic ovary syndrome. Fertil Steril 79:562–566
239. **Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R** 1996 The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 81:3299–3306
240. **Nestler JE** 1997 Role of hyperinsulinemia in the pathogenesis of the polycystic ovary syndrome, and its clinical implications. Semin Reprod Endocrinol 15:111–122
241. **Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN** 2001 Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 86:1626–1632
242. **Romualdi D, Guido M, Ciampelli M, Giuliani M, Leoni F, Perri C, Lanzone A** 2003 Selective effects of pioglitazone on insulin and androgen abnormalities in normo- and hyperinsulinaemic obese patients with polycystic ovary syndrome. Hum Reprod 18:1210–1218
243. **Glueck CJ, Moreira A, Goldenberg N, Sieve L, Wang P** 2003 Pioglitazone and metformin in obese women with polycystic ovary syndrome not optimally responsive to metformin. Hum Reprod 18:1618–1625
244. **Ek J, Urhammer SA, Sorensen TI, Andersen T, Auwerx J, Pedersen O** 1999 Homozygosity of the Pro12Ala variant of the peroxisome proliferation-activated receptor-γ2 (PPAR-γ2): divergent modulating effects on body mass index in obese and lean Caucasian men. Diabetologia 42:892–895
245. **Hara M, Alcoser SY, Qaadir A, Beiswenger KK, Cox NJ, Ehrmann DA** 2002 Insulin resistance is attenuated in women with polycystic ovary syndrome with the $Pro^{12}Ala$ polymorphism in the PPARγ gene. J Clin Endocrinol Metab 87:772–775
246. **Korhonen S, Heinonen S, Hiltunen M, Helisalmi S, Hippelainen M, Koivunen R, Tapanainen JS, Laakso M** 2003 Polymorphism in the peroxisome proliferator-activated receptor-γ gene in women with polycystic ovary syndrome. Hum Reprod 18:540–543
247. **Orio F, Matarese G, Di Biase S, Palomba S, Labella D, Sanna V, Savastano S, Zullo F, Colao A, Lombardi G** 2003 Exon 6 and 2 peroxisome proliferator-activated receptor-γ polymorphisms in polycystic ovary syndrome. J Clin Endocrinol Metab 88:5887–5892
248. **bin Ali A, Zhang Q, Lim YK, Fang D, Retnam L, Lim SK** 2003 Expression of major HDL-associated antioxidant PON-1 is gender dependent and regulated during inflammation. Free Radic Biol Med 34:824–829
249. **Villuendas G, San Millán JL, Sancho J, Escobar-Morreale HF** 2002 The −597 G→A and −174 G→C polymorphisms in the promoter of the IL-6 gene are associated with hyperandrogenism. J Clin Endocrinol Metab 87:1134–1141
250. **Peral B, San Millán JL, Castello R, Moghetti P, Escobar-Morreale HF** 2002 The methionine 196 arginine polymorphism in exon 6 of the TNF receptor 2 gene (TNFRSF1B) is associated with the poly-

cystic ovary syndrome and hyperandrogenism. J Clin Endocrinol Metab 87:3977–3983
251. **Escobar-Morreale HF, Calvo RM, Villuendas G, Sancho J, San Millán JL** 2003 Association of polymorphisms in the interleukin 6 receptor complex with obesity and hyperandrogenism. Obes Res 11:987–996
252. **Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE** 2001 Effects of 5′ regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. Am J Hum Genet 68:1428–1436
253. **Fenkci V, Fenkci S, Yilmazer M, Serteser M** 2003 Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fertil Steril 80:123–127
254. **Rudich A, Kozlovsky N, Potashnik R, Bashan N** 1997 Oxidant stress reduces insulin responsiveness in 3T3–L1 adipocytes. Am J Physiol 272:E935–E940
255. **Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, Miller JE, Boulton AJ, Durrington PN** 1998 Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. Atherosclerosis 139:341–349
256. **Sakai T, Matsuura B, Onji M** 1998 Serum paraoxonase activity and genotype distribution in Japanese patients with diabetes mellitus. Intern Med 37:581–584
257. **James RW, Leviev I, Ruiz J, Passa P, Froguel P, Garin MC** 2000 Promoter polymorphism T(−107)C of the paraoxonase PON1 gene is a risk factor for coronary heart disease in type 2 diabetic patients. Diabetes 49:1390–1393
258. **Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD, Furlong CE** 2000 Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1(192) or PON1(55) genotype. Arterioscler Thromb Vasc Biol 20:2441–2447
259. **Solomon CG** 1999 The epidemiology of polycystic ovary syndrome. Prevalence and associated disease risks. Endocrinol Metab Clin North Am 28:247–263
260. **Nieters A, Becker N, Linseisen J** 2002 Polymorphisms in candidate obesity genes and their interaction with dietary intake of n-6 polyunsaturated fatty acids affect obesity risk in a sub-sample of the EPIC-Heidelberg cohort. Eur J Nutr 41:210–221
261. **Sreenan SK, Zhou YP, Otani K, Hansen PA, Currie KP, Pan CY, Lee JP, Ortega DM, Pugh W, Horikawa Y, Cox NJ, Hanis CL, Burant CF, Fox AP, Bell GI, Polonsky KS** 2001 Calpains play a role in insulin secretion and action. Diabetes 50:2013–2020
262. **Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI** 2000 Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 26:163–175
263. **Ehrmann DA, Schwarz PE, Hara M, Tang X, Horikawa Y, Imperial J, Bell GI, Cox NJ** 2002 Relationship of calpain-10 genotype to phenotypic features of polycystic ovary syndrome. J Clin Endocrinol Metab 87:1669–1673
264. **Haddad L, Evans JC, Gharani N, Robertson C, Rush K, Wiltshire S, Frayling TM, Wilkin TJ, Demaine A, Millward A, Hattersley AT, Conway G, Cox NJ, Bell GI, Franks S, McCarthy MI** 2002 Variation within the type 2 diabetes susceptibility gene calpain-10 and polycystic ovary syndrome. J Clin Endocrinol Metab 87:2606–2610
265. **Escobar-Morreale HF, Peral B, Villuendas G, Calvo RM, Sancho J, San Millán JL** 2002 Common single nucleotide polymorphisms in intron 3 of the calpain-10 gene influence hirsutism. Fertil Steril 77:581–587
266. **González A, Abril E, Roca A, Aragon MJ, Figueroa MJ, Velarde P, Royo JL, Real LM, Ruiz A** 2002 CAPN10 alleles are associated with polycystic ovary syndrome. J Clin Endocrinol Metab 87:3971–3976
267. **González A, Abril E, Roca A, Aragon MJ, Figueroa MJ, Velarde P, Ruiz R, Fayez O, Galan JJ, Herreros JA, Real LM, Ruiz A** 2003 Specific CAPN10 gene haplotypes influence the clinical profile of polycystic ovary patients. J Clin Endocrinol Metab 88:5529–5536
268. **Rajkhowa M, Talbot JA, Jones PW, Clayton RN** 1996 Polymorphism of glycogen synthetase gene in polycystic ovary syndrome. Clin Endocrinol (Oxf) 44:85–90
269. **Urbanek M, Du Y, Silander K, Collins FS, Steppan CM, Strauss III JF, Dunaif A, Spielman RS, Legro RS** 2003 Variation in resistin gene promoter not associated with polycystic ovary syndrome. Diabetes 52:214–217
270. **Oksanen L, Tiitinen A, Kaprio J, Koistinen HA, Karonen S, Kontula K** 2000 No evidence for mutations of the leptin or leptin receptor genes in women with polycystic ovary syndrome. Mol Hum Reprod 6:873–876
271. **Heinonen S, Korhonen S, Hippelainen M, Hiltunen M, Mannermaa A, Saarikoski S** 2001 Apolipoprotein E alleles in women with polycystic ovary syndrome. Fertil Steril 75:878–880
272. **Frishman WH** 1998 Biologic markers as predictors of cardiovascular disease. Am J Med 104:18S–27S
273. **Fernandez-Real JM, Ricart W** 2003 Insulin resistance and chronic cardiovascular inflammatory syndrome. Endocr Rev 24:278–301
274. **Zinman B, Hanley AJ, Harris SB, Kwan J, Fantus IG** 1999 Circulating tumor necrosis factor-α concentrations in a native Canadian population with high rates of type 2 diabetes mellitus. J Clin Endocrinol Metab 84:272–278
275. **Fernandez-Real JM, Broch M, Ricart W, Casamitjana R, Gutierrez C, Vendrell J, Richart C** 1998 Plasma levels of the soluble fraction of tumor necrosis factor receptor 2 and insulin resistance. Diabetes 47:1757–1762
276. **Hak AE, Pols HA, Stehouwer CD, Meijer J, Kiliaan AJ, Hofman A, Breteler MM, Witteman JC** 2001 Markers of inflammation and cellular adhesion molecules in relation to insulin resistance in nondiabetic elderly: the Rotterdam study. J Clin Endocrinol Metab 86:4398–4405
277. **Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM** 1995 Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 95:2409–2415
278. **Hotamisligil GS, Arner P, Atkinson RL, Spiegelman BM** 1997 Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance. Diabetes 46:451–455
279. **Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H, Hainque B** 2000 Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 85:3338–3342
280. **Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM** 1996 IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α- and obesity-induced insulin resistance. Science 271:665–668
281. **Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V** 2000 Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148:209–214
282. **Deshpande RR, Chang MY, Chapman JC, Michael SD** 2000 Alteration of cytokine production in follicular cystic ovaries induced in mice by neonatal estradiol injection. Am J Reprod Immunol 44:80–88
283. **Gallinelli A, Ciaccio I, Giannella L, Salvatori M, Marsella T, Volpe A** 2003 Correlations between concentrations of interleukin-12 and interleukin-13 and lymphocyte subsets in the follicular fluid of women with and without polycystic ovary syndrome. Fertil Steril 79:1365–1372
284. **Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N** 2001 Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 86:2453–2455
285. **Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O, Blumenfeld Z** 2004 Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. J Clin Endocrinol Metab 89:2160–2165
286. **Amato G, Conte M, Mazziotti G, Lalli E, Vitolo G, Tucker AT, Bellastella A, Carella C, Izzo A** 2003 Serum and follicular fluid cytokines in polycystic ovary syndrome during stimulated cycles. Obstet Gynecol 101:1177–1182
287. **González F, Thusu K, Abdel-Rahman E, Prabhala A, Tomani M, Dandona P** 1999 Elevated serum levels of tumor necrosis factor α in normal-weight women with polycystic ovary syndrome. Metabolism 48:437–441
288. **Escobar-Morreale HF, Calvo RM, Sancho J, San Millán JL** 2001

TNF-α and hyperandrogenism: a clinical, biochemical, and molecular genetic study. J Clin Endocrinol Metab 86:3761–3767
289. **Sayin NC, Gucer F, Balkanli-Kaplan P, Yuce MA, Ciftci S, Kucuk M, Yardim T** 2003 Elevated serum TNF-α levels in normal-weight women with polycystic ovaries or the polycystic ovary syndrome. J Reprod Med 48:165–170
290. **Escobar-Morreale HF, Villuendas G, Botella-Carretero JI, Sancho J, San Millán JL** 2003 Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk markers in pre-menopausal women. Diabetologia 46:625–633
291. **Mohlig M, Spranger J, Osterhoff M, Ristow M, Pfeiffer AF, Schill T, Schlosser HW, Brabant G, Schofl C** 2004 The polycystic ovary syndrome per se is not associated with increased chronic inflammation. Eur J Endocrinol 150:525–532
292. **Escobar-Morreale HF, Botella-Carretero JI, Villuendas G, Sancho J, San Millán JL** 2004 Serum interleukin-18 concentrations are increased in the polycystic ovary syndrome: relationship to insulin resistance and to obesity. J Clin Endocrinol Metab 89:806–811
293. **Spaczynski RZ, Arici A, Duleba AJ** 1999 Tumor necrosis factor-α stimulates proliferation of rat ovarian theca-interstitial cells. Biol Reprod 61:993–998
294. **Roby KF, Terranova PF** 1990 Effects of tumor necrosis factor-α *in vitro* on steroidogenesis of healthy and atretic follicles of the rat: theca as a target. Endocrinology 126:2711–2718
295. **Kaipia A, Chun SY, Eisenhauer K, Hsueh AJ** 1996 Tumor necrosis factor-α and its second messenger, ceramide, stimulate apoptosis in cultured ovarian follicles. Endocrinology 137:4864–4870
296. **Milner CR, Craig JE, Hussey ND, Norman RJ** 1999 No association between the −308 polymorphism in the tumor necrosis factor α (TNFα) promoter region and polycystic ovaries. Mol Hum Reprod 5:5–9
297. **Mao W, Yu L, Chen Y** 2000 Study on the relationship between a polymorphism of tumor necrosis factor-α gene and the pathogenesis of polycystic ovary syndrome. Zhonghua Fu Chan Ke Za Zhi 35:536–539 (Chinese)
298. **Korhonen S, Romppanen EL, Hiltunen M, Mannermaa A, Punnonen K, Hippelainen M, Heinonen S** 2002 Lack of association between C-850T polymorphism of the gene encoding tumor necrosis factor-α and polycystic ovary syndrome. Gynecol Endocrinol 16:271–274
299. **Bazzoni F, Beutler B** 1996 The tumor necrosis factor ligand and receptor families. N Engl J Med 334:1717–1725
300. **Fernandez-Real JM, Vendrell J, Ricart W, Broch M, Gutierrez C, Casamitjana R, Oriola J, Richart C** 2000 Polymorphism of the tumor necrosis factor-α receptor 2 gene is associated with obesity, leptin levels, and insulin resistance in young subjects and diet-treated type 2 diabetic patients. Diabetes Care 23:831–837
301. **Geurts JM, Janssen RG, van Greevenbroek MM, van der Kallen CJ, Cantor RM, Bu X, Aouizerat BE, Allayee H, Rotter JI, de Bruin TW** 2000 Identification of TNFRSF1B as a novel modifier gene in familial combined hyperlipidemia. Hum Mol Genet 9:2067–2074
302. **Glenn CL, Wang WY, Benjafield AV, Morris BJ** 2000 Linkage and association of tumor necrosis factor receptor 2 locus with hypertension, hypercholesterolemia and plasma shed receptor. Hum Mol Genet 9:1943–1949
303. **Benjafield AV, Wang XL, Morris BJ** 2001 Tumor necrosis factor receptor 2 gene (TNFRSF1B) in genetic basis of coronary artery disease. J Mol Med 79:109–115
304. **Benjafield AV, Glenn CL, Wang XL, Colagiuri S, Morris BJ** 2001 TNFRSF1B in genetic predisposition to clinical neuropathy and effect on HDL cholesterol and glycosylated hemoglobin in type 2 diabetes. Diabetes Care 24:753–757
305. **Papanicolaou DA, Vgontzas AN** 2000 Interleukin-6: the endocrine cytokine. J Clin Endocrinol Metab 85:1331–1333
306. **Fernandez-Real JM, Broch M, Vendrell J, Richart C, Ricart W** 2000 Interleukin-6 gene polymorphism and lipid abnormalities in healthy subjects. J Clin Endocrinol Metab 85:1334–1339
307. **Fernandez-Real JM, Broch M, Vendrell J, Gutierrez C, Casamitjana R, Pugeat M, Richart C, Ricart W** 2000 Interleukin-6 gene polymorphism and insulin sensitivity. Diabetes 49:517–520
308. **Fernandez-Real JM, Vayreda M, Richart C, Gutierrez C, Broch M, Vendrell J, Ricart W** 2001 Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. J Clin Endocrinol Metab 86:1154–1159
309. **Omu AE, Al-Azemi MK, Makhseed M, Al-Oattan F, Ismail AA, Al-Tahir S, Al-Busiri N** 2003 Differential expression of T-helper cytokines in the peritoneal fluid of women with normal ovarian cycle compared with women with chronic anovulation. Acta Obstet Gynecol Scand 82:603–609
310. **Haan C, Heinrich PC, Behrmann I** 2002 Structural requirements of the interleukin-6 signal transducer gp130 for its interaction with Janus kinase 1: the receptor is crucial for kinase activation. Biochem J 361:105–111
311. **Shimonaka M, Inouye S, Shimasaki S, Ling N** 1991 Follistatin binds to both activin and inhibin through the common subunit. Endocrinology 128:3313–3315
312. **Mather JP, Moore A, Li RH** 1997 Activins, inhibins, and follistatins: further thoughts on a growing family of regulators. Proc Soc Exp Biol Med 215:209–222
313. **Shibata H, Kanzaki M, Takeuchi T, Miyazaki J, Kojima I** 1996 Two distinct signaling pathways activated by activin A in glucose-responsive pancreatic β-cell lines. J Mol Endocrinol 16:249–258
314. **Findlay JK** 1993 An update on the roles of inhibin, activin, and follistatin as local regulators of folliculogenesis. Biol Reprod 48: 15–23
315. **Guo Q, Kumar TR, Woodruff T, Hadsell LA, DeMayo FJ, Matzuk MM** 1998 Overexpression of mouse follistatin causes reproductive defects in transgenic mice. Mol Endocrinol 12:96–106
316. **Roberts VJ, Barth S, el-Roeiy A, Yen SS** 1994 Expression of inhibin/activin system messenger ribonucleic acids and proteins in ovarian follicles from women with polycystic ovarian syndrome. J Clin Endocrinol Metab 79:1434–1439
317. **Jaatinen TA, Penttila TL, Kaipia A, Ekfors T, Parvinen M, Toppari J** 1994 Expression of inhibin α, βA and βB messenger ribonucleic acids in the normal human ovary and in polycystic ovarian syndrome. J Endocrinol 143:127–137
318. **Urbanek M, Wu X, Vickery KR, Kao LC, Christenson LK, Schneyer A, Legro RS, Driscoll DA, Strauss III JF, Dunaif A, Spielman RS** 2000 Allelic variants of the follistatin gene in polycystic ovary syndrome. J Clin Endocrinol Metab 85:4455–4461
319. **Liao WX, Roy AC, Ng SC** 2000 Preliminary investigation of follistatin gene mutations in women with polycystic ovary syndrome. Mol Hum Reprod 6:587–590
320. **Calvo RM, Villuendas G, Sancho J, San Millán JL, Escobar-Morreale HF** 2001 Role of the follistatin gene in women with polycystic ovary syndrome. Fertil Steril 75:1020–1023
321. **Cigolini M, Tonoli M, Borgato L, Frigotto L, Manzato F, Zeminian S, Cardinale C, Camin M, Chiaramonte E, De Sandre G, Lunardi C** 1999 Expression of plasminogen activator inhibitor-1 in human adipose tissue: a role for TNF-α? Atherosclerosis 143:81–90
322. **Samad F, Uysal KT, Wiesbrock SM, Pandey M, Hotamisligil GS, Loskutoff DJ** 1999 Tumor necrosis factor α is a key component in the obesity-linked elevation of plasminogen activator inhibitor 1. Proc Natl Acad Sci USA 96:6902–6907
323. **Sakamoto T, Woodcock-Mitchell J, Marutsuka K, Mitchell JJ, Sobel BE, Fujii S** 1999 TNF-α and insulin, alone and synergistically, induce plasminogen activator inhibitor-1 expression in adipocytes. Am J Physiol 276:C1391–C1397
324. **Ehrmann DA, Schneider DJ, Sobel BE, Cavaghan MK, Imperial J, Rosenfield RL, Polonsky KS** 1997 Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome. J Clin Endocrinol Metab 82:2108–2116
325. **Hoffstedt J, Andersson IL, Persson L, Isaksson B, Arner P** 2002 The common −675 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene is strongly associated with obesity. Diabetologia 45:584–587
326. **Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L** 1999 Metformin-induced resumption of normal menses in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. Metabolism 48:511–519
327. **Tsanadis G, Vartholomatos G, Korkontzelos I, Avgoustatos F, Kakosimos G, Sotiriadis A, Tatsioni A, Eleftheriou A, Lolis D** 2002 Polycystic ovarian syndrome and thrombophilia. Hum Reprod 17:314–319

328. **Orio F, Jr., Palomba S, Di Biase S, Colao A, Tauchmanova L, Savastano S, Labella D, Russo T, Zullo F, Lombardi G** 2003 Homocysteine levels and C677T polymorphism of methylenetetrahydrofolate reductase in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:673–679
329. **Korhonen S, Romppanen EL, Hiltunen M, Helisalmi S, Punnonen K, Hippelainen M, Heinonen S** 2003 Two exonic single nucleotide polymorphisms in the microsomal epoxide hydrolase gene are associated with polycystic ovary syndrome. Fertil Steril 79:1353–1357
330. **Shimasaki S, Moore RK, Erickson GF, Otsuka F** 2003 The role of bone morphogenetic proteins in ovarian function. Reprod Suppl 61:323–337
331. **Takebayashi K, Takakura K, Wang H, Kimura F, Kasahara K, Noda Y** 2000 Mutation analysis of the growth differentiation factor-9 and -9B genes in patients with premature ovarian failure and polycystic ovary syndrome. Fertil Steril 74:976–979
332. **Fernandez-Real JM, Ricart W** 1999 Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness. Diabetologia 42:1367–1374
333. **Witchel SF, Lee PA, Suda-Hartman M, Trucco M, Hoffman EP** 1997 Evidence for a heterozygote advantage in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. J Clin Endocrinol Metab 82:2097–2101
334. **Parsons P** 1997 Success in mating: a coordinated approach to fitness through genotypes incorporating genes for stress resistance and heterozygous advantage under stress. Behav Genet 27:75–81
335. **Stearns SC, Ackermann M, Doebeli M, Kaiser M** 2000 Experimental evolution of aging, growth, and reproduction in fruitflies. Proc Natl Acad Sci USA 97:3309–3313
336. **Eaton SB, Konner M** 1985 Paleolithic nutrition. A consideration of its nature and current implications. N Engl J Med 312:283–289
337. **Eaton SB, Konner M, Shostak M** 1988 Stone agers in the fast lane: chronic degenerative diseases in evolutionary perspective. Am J Med 84:739–749
338. **Wendorf M, Goldfine ID** 1991 Archaeology of NIDDM. Excavation of the "thrifty" genotype. Diabetes 40:161–165
339. **Ordovas JM** 2003 Cardiovascular disease genetics: a long and winding road. Curr Opin Lipidol 14:47–54
340. **Guo SW** 1998 Inflation of sibling recurrence-risk ratio, due to ascertainment bias and/or overreporting. Am J Hum Genet 63: 252–258
341. **Epstein MP, Lin X, Boehnke M** 2002 Ascertainment-adjusted parameter estimates revisited. Am J Hum Genet 70:886–895
342. **Cooper RS** 2003 Gene-environment interactions and the etiology of common complex disease. Ann Intern Med 139:437–440
343. **Wang J** 2004 Sibship reconstruction from genetic data with typing errors. Genetics 166:1963–1979
344. **Hosking L, Lumsden S, Lewis K, Yeo A, McCarthy L, Bansal A, Riley J, Purvis I, Xu CF** 2004 Detection of genotyping errors by Hardy-Weinberg equilibrium testing. Eur J Hum Genet 12:395–399
345. **Kang H, Qin ZS, Niu T, Liu JS** 2004 Incorporating genotyping uncertainty in haplotype inference for single-nucleotide polymorphisms. Am J Hum Genet 74:495–510
346. **McKusick V, Ruddle F** 1985 A new discipline, a new name, a new journal. Genomics 1:1–2
347. **Mooser V, Ordovas JM** 2003 'Omic' approaches and lipid metabolism: are these new technologies holding their promises? Curr Opin Lipidol 14:115–119
348. **Michael NL** 1999 Host genetic influences on HIV-1 pathogenesis. Curr Opin Immunol 11:466–474
349. **Mouradian MM** 2002 Recent advances in the genetics and pathogenesis of Parkinson disease. Neurology 58:179–185
350. **St George-Hyslop PH** 1999 Molecular genetics of Alzheimer disease. Semin Neurol 19:371–383
351. **Glazier AM, Nadeau JH, Aitman TJ** 2002 Finding genes that underlie complex traits. Science 298:2345–2349
352. **Wood JR, Nelson VL, Ho C, Jansen E, Wang CY, Urbanek M, McAllister JM, Mosselman S, Strauss III JF** 2003 The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem 278:26380–26390
353. **Anderson L, Seilhamer J** 1997 A comparison of selected mRNA and protein abundances in human liver. Electrophoresis 18:533–537

***Endocrine Reviews* is published bimonthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**