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Case Report

Raspberry Leaf and Hypoglycemia in Gestational Diabetes Mellitus

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BACKGROUND: Raspberry leaf is commonly consumed by pregnant women. Hypoglycemic effects have been documented with other species within the plant family. Whether raspberry leaf affects glycemic control in gestational diabetes mellitus (GDM) is unknown.

CASE: A 38-year-old nulliparous woman with GDM developed hypoglycemia requiring lowered insulin dose after consuming raspberry leaf tea at 32 weeks of gestation. The temporal relationship was confirmed by the patient's self-withdrawal and reintroduction of the herb. Fetal surveillance and growth were reassuring. A cesarean delivery was performed at 39 weeks of gestation. The neonate did not experience hypoglycemia or other complications. Placental biopsy revealed normal findings.

CONCLUSION: Consumption of raspberry leaf may lead to reduced insulin requirements in GDM. Women with GDM should be cautioned about its use and their glucose levels more closely monitored.

(Obstet Gynecol 2016;128:1421–4)
DOI: 10.1097/AOG.0000000000001757

Teaching Points

1. Clinicians should interview pregnant patients about use of alternative medicine.
2. Use of raspberry leaf may lead to hypoglycemia and lowering of insulin requirements in gestational diabetes mellitus treated with insulin.
3. Women with gestational diabetes mellitus should be cautioned about use of raspberry leaf, and their glucose levels should be more closely monitored.

The use of alternative medicine in pregnancy has gained interest from both patients and health care providers. Among U.S. pregnant women, 29% reported herbal medicine use,[1] and 93.9% of midwives reported recommending alternative medicine to their pregnant patients.[2] Raspberry leaf (*Rubus idaeus,* or *Rubus strigosus,* plant family *Rosaceae*) is a commonly used herbal product during pregnancy[3] on the belief that it could shorten labor.[4] Based on our MEDLINE search (1964–2016) with terms "glucose" (MeSH term), "diabetes, gestational" (MeSH term), and raspberry (all fields), "rubus" (MeSH term), "rubus idaeus" (all fields), "rubus strigosus" (all fields), the effect of raspberry leaf on glycemic control in women with gestational diabetes (GDM) has never been documented. We present a case of hypoglycemia and reduced insulin requirements in a woman with GDM using raspberry leaf tea in the third trimester.

CASE

A 38-year-old Asian woman, gravida 2 para 0010, with GDM presented at her routine prenatal visit at 32 weeks of gestation and reported hypoglycemic episodes. Gestational diabetes mellitus was suspected at 10 weeks of gestation when her glucose was positive with urine dipstick. Her 50-g glucose challenge yielded 199 mg/dL (1 hour). Hemoglobin A1c drawn on the same day was 5.4%, suggesting she did not have pre-existing diabetes. Her 3-hour glucose challenge results were 84 mg/dL (fasting), 184 mg/dL (1 hour), 156 mg/dL (2 hours), and 94 mg/dL (3 hours). Her glucose had been under excellent control with medical nutrition therapy, neutral protamine Hagedorn insulin (NPH), and preprandial insulin lispro (dosed by carbohydrate counting). Self-monitoring of glucose was performed at fasting, before and after meals, and at bedtime. Her insulin requirements had been increasing throughout the pregnancy. At 13 weeks of gestation, her lispro requirement was 1 unit/15 g of carbohydrate without any NPH. Her last dose increase was at 30 weeks of gestation (Table 1), 2 weeks before her report of hypoglycemic episodes (to NPH 22 units twice daily and lispro 1 unit/3 g of carbohydrates). She reported no change in dietary or physical activity habits other than the addition of two servings of raspberry leaf tea (dried red raspberry leaf as the only ingredient) 3 days prior (at 32 2/7 weeks of gestation). She initiated the raspberry leaf tea in hopes that it might shorten her labor. Examination of her glucose logs revealed good

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Financial Disclosure
The authors did not report any potential conflicts of interest.

ISSN: 0029-7844/16

Table 1. Temporal Relationship Between Raspberry Leaf Consumption and Glycemic Control (From the Patient's Glucose and Food Log)

Gestational Week	Insulin Dose*	Raspberry Leaf Tea Consumption†	Glucose Reading‡ (mg/dL)
30 0/7 to 32 1/7	NPH 22 units; lispro 1 unit/3 g carb	No	At goal
32 2/7		Yes	At goal
32 3/7		Yes	2 h PP **52–66;** bedtime **48**
32 4/7		Yes	2 h PP **54–63;** bedtime **55**
32 5/7§	Dose change: NPH 22 units; lispro 1 unit/4 g carb	Yes	At goal
32 6/7 to 33 2/7§		Yes	At goal
33 3/7		No	1 h PP 118–145
33 4/7		No	2 h PP **155–163**
33 5/7		Yes	2 h PP 114–144
33 6/7		Yes	At goal
34		No	1 h PP 128–147
34 1/7		No	2 h PP **157–176**
34 2/7	Dose change: NPH 22 units; lispro 1 unit/3 g carb	No	At goal
34 4/7§ to 35 3/7		No	At goal
35 4/7	Dose change: NPH 22 units; lispro 1 unit/4 g carb	Yes	At goal
Remainder of pregnancy	Dose gradually changed to NPH 18 units; lispro 1 unit/5 g carb for morning meals, 1 unit/4 g carb for afternoon and evening	Yes	At goal

NPH, neutral protamine Hagedorn; carb, carbohydrate; PP, postprandial, 1- or 2-hour postprandial as indicated (patient alternated between checking 1-hour and 2-hour postmeal values).
Bold indicates hypoglycemia or hyperclycemia.
* NPH was administered twice daily at the listed dose.
† Raspberry leaf consumed was two servings per day.
‡ Glycemic goal was less than 90 mg/dL (premeal), less than 130 mg/dL (1 hour), less than 120 mg (2 hours).
§ Day of prenatal visit or fetal surveillance.

glycemic control since the last insulin adjustment 2 weeks prior, and hypoglycemia was experienced only since the addition of raspberry leaf tea with 2-hour postprandial glucose of 52–66 mg/dL and bedtime glucose values as low as 48 mg/dL. She reported tachycardia and mild diaphoresis with her hypoglycemia, which were self-treated with orange juice.

Fetal surveillance was reassuring with moderate variability, present accelerations, and absent decelerations. There was no evidence of uteroplacental insufficiency; however, weekly fetal surveillance was recommended as a result of concern about the hypoglycemic episodes. Prandial insulin was decreased to lispro 1 unit/4 g of carbohydrates.

At 33 2/7 weeks of gestation, a biophysical profile was performed without a nonstress test, again with reassuring findings. The biophysical profile score was 8 of 8. The ultrasonogram revealed a viable, singleton intrauterine fetal gestation in cephalic position with normal amniotic fluid, four quadrant amniotic fluid index of 144 mm, placental grade I, and appropriate fetal growth (66th percentile). The patient reported that the hypoglycemic episodes had resolved.

On the next prenatal visit at 34 4/7 weeks of gestation, the patient reported she had discontinued the raspberry leaf tea twice (Table 1), because she suspected that its use may have led to the hypoglycemia and reduced insulin needs. Each time on the day after abstaining from raspberry leaf tea, she experienced postprandial hyperglycemia (Table 1) using the same dose of lispro. She self-adjusted her prandial insulin coverage to lispro 1 unit/3 g of carbohydrate to prevent further hyperglycemia.

Over the remaining course of her pregnancy, the patient resumed drinking two servings of raspberry leaf tea per day. Her lispro was adjusted to an insulin-to-carbohydrate ratio of one to five in the morning and one to four in the afternoon with NPH 18 units twice daily. Repeat ultrasonography at 37 and 39 weeks of gestation revealed normal findings. One week before delivery, her NPH was decreased to 18 units at bedtime only. Her glucose was well controlled throughout the remainder of the pregnancy. Her weekly fetal surveillance tests remained reassuring.

At 39 weeks of gestation, the patient underwent labor augmentation with oxytocin as a result of prolonged rupture of membranes and meconium-stained amniotic fluid. A cesarean delivery was performed because of active-phase arrest in the setting of suspected chorioamnionitis, which was treated with intrapartum antibiotics. There was no intrapartum hyper- or hypoglycemia in the

mother. The patient delivered a live female neonate weighing 3,490 g (75th percentile for gestational age)[5] with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. The neonate did not experience hypoglycemia or other complications but did receive prophylactic antibiotics. Biopsy of the placenta revealed a term placenta and fetal membranes with meconium-laden macrophages but no additional abnormalities. Both the mother and neonate were discharged home after routine postpartum care. The mother's 2-hour glucose challenge at her 8-week postpartum visit yielded glucose of 79 mg/dL (fasting), 192 mg/dL (1 hour), and 148 mg/dL (2 hours).

DISCUSSION

During pregnancy, placental production of various anti-insulin hormones, cytokines, and growth factors[6] exacerbate insulin resistance.[7] Women with GDM using insulin typically have progressive increments in insulin need as pregnancy advances.[8] Falling insulin requirements have been associated with placental dysfunction, preeclampsia, small-for-gestational-age neonates, and a higher incidence of neonatal intensive care admissions.[9]

Our case describes the occurrence of reduced insulin requirements as gestation advances not resulting from placental insufficiency. We initially did not suspect the patient's self-medication with raspberry leaf could have led to hypoglycemia because such effects have not been documented in the literature. However, using the Naranjo algorithm[10] to assess causality, use of raspberry leaf tea probably had led to the hypoglycemic episodes in this case. The hypoglycemic effects followed a reasonable temporal sequence after its use and were confirmed by the patient's self-withdrawal and reintroduction of the herb. In addition, alternative causes of hypoglycemia have been ruled out. To wit, the patient reported no changes in physical activity or dietary habits. Her insulin dose had been stable for 2 weeks before the hypoglycemia. The placenta was grossly normal on inspection as well as microscopically.

There is a growing use of complementary and alternative medicine during pregnancy. In the United States, 29% of pregnant women reported self-medication with herbal medicine.[1] Most patients assume that herbal therapy has the advantage of being "natural." However, there are often insufficient efficacy and safety data for their use.[13]

Raspberry leaf (*R ideus*) has been used during pregnancy primarily on the belief that it can shorten labor,[4] but available studies have shown no difference in the need for labor augmentation, instrument-assisted births, or duration of labor.[4,12]

The risks associated with raspberry leaf have not been well studied. Blackberry leaves (*Rubus fructicosis*) and raspberry leaves *(R ideus)* belong to the same plant family, Rosaceae, and may have similar chemical properties.[13] Hypoglycemic effects of blackberry leaves have been documented in animal models and may be relevant to raspberry leaves. In diabetic rats, *R fructicosis* extract significantly reduced glucose 2 hours after a single oral dose ($P<.001$) and persisted up to 6 hours postdose.[14] On continuous administration for 9 days, *R fructicosis* reduced blood glucose to normal levels in diabetic rats ($P<.001$). In another study, supplementation of rubus extract in rats on a standard diet increased adipose tissue lipolytic activity and decreased plasma glucose.[15] Decreased insulin concentrations were also seen in female rats, suggesting increased insulin sensitivity.

The extent to which hypoglycemic effects of blackberry leaves can be extrapolated to raspberry leaf tea in the amount typically consumed by pregnant women is unknown. We did not initially suspect the patient's hypoglycemia was the result of use of raspberry leaf tea because literature on its glycemic effect is nonexistent. Additional fetal surveillance was performed as a result of the unexpected reduction in insulin requirements. We suggest that in women with GDM using insulin, use of raspberry leaf may require closer glucose monitoring to prevent inadvertent hypoglycemic episodes. It will be prudent to interview patients about use of alternative medicine when hypoglycemic episodes occur. Given the paucity of data supporting the use of raspberry leaf in shortening labor, women with GDM should be cautioned about its use. For patients who prefer nondrug options and whose GDM remain uncontrolled with medical nutrition therapy, prospective clinical trials may be considered to evaluate the role of raspberry leaf in GDM.

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0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(12):4546–4556

doi: 10.1210/jc.2007-1549

POSITION STATEMENT: Glucose Intolerance in Polycystic Ovary Syndrome—A Position Statement of the Androgen Excess Society

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Objectives: Women with polycystic ovarian syndrome (PCOS) are at increased risk for developing glucose intolerance and type 2 diabetes mellitus (DM). Recommendations for the timing and method of screening have varied. The purpose of this statement is to determine the optimal screening method, timing of screening, and treatment modalities for impaired glucose tolerance (IGT) among women with PCOS.

Participants: The expert panel was appointed by the Androgen Excess Society (AES) to review the literature and make recommendations based on the available evidence. Meetings were open, and there was no funding for the panel.

Evidence: A systematic review was conducted of the published, peer-reviewed medical literature using MEDLINE to identify studies that addressed the prevalence, risk factors, testing, and treatment for IGT in both adults and adolescents with PCOS. Unpublished data were not considered.

Consensus Process: The panel held meetings to review the literature and draft the statement as a committee. The AES board members reviewed and critiqued the manuscript, and changes were made based on their comments.

Conclusions: The panel recommends that all patients with PCOS be screened for IGT with a 2-h oral glucose tolerance test. A few members of the AES board recommend alternatively screening women with PCOS for IGT and type 2 DM using an oral glucose tolerance test only in patients with a body mass index of 30 kg/m^2 or greater or in lean patients with additional risk factors. Patients with normal glucose tolerance should be rescreened at least once every 2 yr, or more frequently if additional risk factors are identified. Those with IGT should be screened annually for development of type 2 DM. PCOS patients with IGT should be treated with intensive lifestyle modification and weight loss and considered for treatment with insulin-sensitizing agents. **(*J Clin Endocrinol Metab* 92: 4546–4556, 2007)**

THE POLYCYSTIC ovarian syndrome (PCOS) is a common endocrinopathy, affecting approximately 5–10% of women of reproductive age (1–4). In its classical form, the syndrome is characterized by oligo- or anovulation, biochemical or clinical hyperandrogenism, and polycystic ovarian morphology on ultrasonography (5). Although much remains unknown regarding the underlying pathophysiology of PCOS, a form of insulin resistance intrinsic to the syndrome appears to play a central role in its development. Among many women with PCOS, the observed insulin resistance is partially explained by excess adiposity; however, it is increasingly recognized that even lean women with PCOS have increased insulin resistance compared with normal controls (6).

Given the significant metabolic burden of insulin resistance seen in women with PCOS, affected women may have an increased risk of impaired glucose tolerance (IGT) and type 2 diabetes mellitus (DM). IGT is a known risk factor for type 2 DM and the development of cardiovascular disease (7). Because IGT is often asymptomatic, the screening of women with PCOS for IGT has been recommended; however, recommendations have varied regarding the timing and method of screening for IGT (8, 9). Because patients with PCOS are at high risk for developing IGT, the early identification of affected patients and institution of lifestyle changes or pharmacological treatments may help delay the progression to type 2 DM. The following consensus recommendations attempt to determine the optimal screening method, timing of screening, and treatment modalities for IGT among women with PCOS based on the currently available medical literature.

Abbreviations: ADA, American Diabetes Association; AES, Androgen Excess Society; BMI, body mass index; CI, confidence interval; DM, diabetes mellitus; DPP, Diabetes Prevention Program; GDM, gestational diabetes mellitus; IDPP, Indian Diabetes Prevention Program; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; MeSH, Medical Subject Headings; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PCOS, polycystic ovarian syndrome; WHO, World Health Organization.

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

Process

A systematic review was conducted of the published, peer-reviewed medical literature to identify studies assessing the prevalence and risk factors for IGT in patients with PCOS, as well as the testing and treatment of IGT in both adults and adolescents using MEDLINE databases.

To review the natural history of PCOS and IGT, MEDLINE was searched from 1966 through 2007. Medical Subject Headings (MeSH) used included polycystic ovary syndrome

(which includes polycystic ovarian disease) or ovarian hyperandrogenism and diabetes, IGT, β-cell dysfunction, gestational diabetes, or metabolic syndrome. Additional references identified from these initial articles were also considered.

To examine risk factors for IGT in PCOS, the MEDLINE MeSH headings used were PCOS or ovarian hyperandrogenism, and glucose intolerance and risk factors, with the following limitations: major topic (PCOS) and English and humans. Cross-referenced studies were also reviewed.

To review the measurements of IGT, MEDLINE was searched using the terms IGT and measure. PCOS or ovarian hyperandrogenism were added in a subsequent search. Furthermore, the MeSH heading glucose intolerance with the subheading diagnosis was searched by itself and combined with the MeSH heading polycystic ovary syndrome. Supplementary references were obtained from initial citations.

To review treatments for IGT, MEDLINE was searched using the terms: type 2 diabetes prevention; PCOS and diabetes prevention; ovarian hyperandrogenism and diabetes prevention; and IGT prevention, and IGT treatment with the limits (clinical trial, metaanalysis, or randomized-controlled trial). During evaluation, particular emphasis was placed on identifying prospective randomized, controlled studies that enrolled at least 100 subjects, included women as part of their study population, involved an intervention and follow-up period of at least 1 yr, and clearly defined the prevalence of glucose intolerance at baseline and the end of the study period.

To examine the development, measurement, and treatment of IGT in adolescents, MEDLINE was searched using the terms PCOS, glucose intolerance, and adolescents, and ovarian hyperandrogenism and glucose intolerance. Insulin resistance was also used as a search term, but only studies that assessed IGT were reviewed.

Unpublished data or data published only in abstract form were not included in the review.

Development of IGT and Type 2 DM

Insulin resistance is present in both lean and obese women with PCOS compared with their body mass index (BMI) and age-matched counterparts. A seminal study conducted by Dunaif *et al.* (6) evaluated insulin sensitivity using the hyperinsulinemic-euglycemic clamp technique in lean and obese women with and without PCOS. In this study women with PCOS were more insulin resistant than women without the disorder, at equivalent degrees of obesity. Insulin resistance has been identified as a major risk factor for the development of type 2 DM, and likely contributes to the high prevalence of glucose intolerance in women with PCOS.

Prevalence of glucose intolerance in women with PCOS

In two of the largest studies (>100 women) to date that documented the prevalence of IGT and type 2 DM in women with PCOS, it is estimated that IGT is present in 31–35% of women with PCOS (10, 11). In addition, type 2 DM, classified according to the World Health Organization (WHO) criteria, is present in 7.5–10% of women with PCOS. Compared with the prevalence of IGT (1.6%) and DM (2.2%) found in U.S. women of similar age in the Third National Health and Nutrition Survey (12), the rates in women with PCOS are considerably higher. In addition, IGT and type 2 DM are also highly prevalent among adolescents with PCOS. In one study, IGT was present in eight of 27 (29.6%), and type 2 DM was present in two of 27 (7.4%) adolescent girls with PCOS (13).

The majority of U.S. studies evaluating the prevalence of glucose intolerance in PCOS primarily included obese women, which aggravates their risk for glucose intolerance. Studies on the prevalence of glucose intolerance are limited in Europe where women with PCOS are substantially leaner. However, it has been shown that glycemic abnormalities are not restricted to Caucasian women with PCOS. A high prevalence of abnormal glucose tolerance has been documented in Chinese (20.5%) and Thai (20.3%) women with PCOS (14, 15). Current studies also support abnormal glucose homeostasis in Japanese women with PCOS (16, 17), although one study (17) suggests that obesity may have a stronger effect than the existence of PCOS. In Indian populations, women with PCOS appear to have worse glucose tolerance than Caucasian populations (18).

Conversion rates to IGT and type 2 DM

The conversion from IGT to frank diabetes is also substantially enhanced in women with PCOS. In an uncontrolled study, Norman *et al.* (19) assessed 77 Australian women with PCOS. During an average 6.2-yr follow-up, five of 54 (9.3%) women with normal glucose tolerance (NGT) at baseline developed IGT, and another four women (7.4%) progressed from normoglycemia to type 2 DM. Among the 13 women with IGT at baseline, seven of them (5.4%) developed DM at follow-up. Furthermore, BMI at baseline appeared to be an independent predictor of worsening glycemic control.

The enhanced rate of deterioration in glucose tolerance was corroborated by Legro *et al.* (20), who assessed the changes in glucose tolerance over time in 71 U.S. women with PCOS and 23 control women who had baseline NGT. The mean follow-up period was approximately 3 yr. In this study, of 35 women with PCOS with NGT at baseline, 17 converted to IGT, equivalent to a NGT to IGT conversion rate of 16% per year. In addition, in this study the conversion rate from IGT to DM among PCOS women was 2% per year. Conversely, seven women with PCOS who had abnormal glucose tolerance at baseline reverted in their WHO glucose tolerance category. The conversion rate from NGT to IGT in the control women is less prominent. Of 23 control women who had NGT at baseline, only five converted to IGT, which is less than half the rate of women with PCOS. There are little data on conversion rates from European countries.

Development of gestational DM (GDM)

Besides converting to IGT or type 2 DM, women with PCOS are also at high risk for developing GDM. Polycystic ovarian morphology is a common finding among women with a history of GDM (21, 22). In a metaanalysis of 720 women with PCOS and 4505 controls, PCOS women have a 2.94 times [confidence interval (CI) for odds ratio 1.70–5.08] higher risk of developing GDM than control women (23).

This risk estimate was recently confirmed by a large database study performed using a multiethnic population in the Northern California Kaiser Permanente program (24).

Mechanisms of glucose intolerance in PCOS

Several mechanisms have been postulated to account for the predisposition to the development of type 2 DM among women with PCOS. Dunaif *et al.* (6, 25) demonstrated that women with PCOS are insulin resistant, independent of obesity. Although the nature of insulin resistance in PCOS is currently unclear, defects in insulin receptor or post-receptor signal transduction (25), altered adipocyte lipolysis (26, 27), decreased glucose transporter 4 in adipocytes (28), and impaired release of a D-chiro-inositol mediator (29–31) have all been implicated. Furthermore, many women with PCOS exhibit β-cell dysfunction (32–35), rendering insulin response to a glucose load insufficient for the degree of insulin resistance in PCOS.

Current Controversies in Screening for Glucose Intolerance

Given the presence of significant insulin resistance in the syndrome, several organizations have made recommendations regarding screening for glucose intolerance in patients with PCOS (Table 1).

A number of risk factors, including family history, advanced age, increased BMI, and a history of GDM, has increased the risk of glucose intolerance in patients with PCOS. Legro *et al.* (11) prospectively studied 254 women with PCOS using the oral glucose tolerance test (OGTT) and showed that PCOS women with a first-degree relative with DM were at an increased risk for developing glucose intolerance. In a smaller study of 122 women with PCOS, Ehrmann *et al.* (10) found that those with type 2 DM were 2.6 times more likely to have a first-degree relative with type 2 DM than patients with NGT. In a separate study evaluating a population of 408 premenopausal women with PCOS, Ehrmann *et al.* (36) also found a family history of type 2 DM in a first-degree relative to be associated with a significantly higher risk for IGT and type 2 DM in women with PCOS.

In addition to evaluating family history as a risk factor, Legro *et al.* (11) also showed an increased risk for IGT in women with advanced age, increased BMI, and increased waist to hip ratios, which are identical risk factors for the general population in developing IGT. This was corroborated in a cross-sectional study of 91 women with PCOS by Trolle and Lauszus (37), who found women who were older and had a higher BMI were more likely to have elevated fasting glucose levels. In women with a history of GDM, Koivunen *et al.* (38) found an increased prevalence of an abnormal OGTT as well as a higher prevalence of PCOS (39.4 *vs.* 16.7%; $P = 0.03$) when compared with controls.

There remains some controversy in the practicality of screening all patients with PCOS for IGT. Due to the time-consuming nature of the OGTT, Mohlig *et al.* (39) investigated the use of decision tree modeling in 118 women with PCOS to determine whether the number of patients with PCOS who should undergo the OGTT could be decreased. The best decision tree used the homeostasis assessment model for estimating insulin resistance, the proinsulin to insulin ratio, proinsulin, 17-OH progesterone, and the ratio of LH to FSH. The sensitivity of this tree was 100% and the specificity was 74%, and it cut down on the number of OGTTs by about 60%. The most suitable decision tree using medical history and clinical parameters only used BMI (>25.7 kg/m^2), waist circumference (>76 cm), and waist to hip ratio (>0.77). Applying the clinical data tree alone to a stratified screening algorithm reduced the number of OGTTs in patients with PCOS by about 25%. This decision tree yielded a sensitivity for the detection of IGT of 100%, with a specificity of 32.3%. Thus, the use of this decision tree correctly identified all women with IGT. However, the widespread application of this tree needs to be confirmed by larger studies.

Measurement of Glucose Intolerance

Presently, the only clinical method of identifying individuals with IGT is by an OGTT, typically performed as a 2-h OGTT (40). The WHO describes this test as a measure of venous plasma glucose 2 h after a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water (41). The most current recommendations for diagnosing IGT from the WHO and American Diabetes Association (ADA) slightly

TABLE 1. Screening for glucose intolerance in patients with PCOS—organization recommendations

Organization	Recommendations
American Association of Clinical Endocrinologists	Women with PCOS should have glucose levels measured. An oral glucose challenge may be considered, particularly in obese women with PCOS and those with a family history of type 2 DM (75).
American College of Obstetrics and Gynecology	Screening for glucose intolerance should be performed in all patients with PCOS with a fasting glucose level followed by a 2-h glucose level obtained after a 75-g glucose load (76).
ADA	Screening for DM should be performed in asymptomatic individuals under the age of 45 yr if they are overweight (BMI 25 kg/m^2) and have additional risk factors, which include PCOS. The recommended screening test is the fasting plasma glucose; an OGTT may be considered in patients with IFG to define better the risk of diabetes (77).
American Society of Reproductive Medicine and the European Society of Human Reproduction and Endocrinology PCOS Consensus Workshop Group	Obese women with PCOS should be screened for the metabolic syndrome, including screening for glucose intolerance with an OGTT. Screening should be considered for nonobese PCOS women with PCOS if there are additional risk factors for insulin resistance, such as a family history of insulin resistance (78).

differ (Table 2). The WHO criteria recommend first measuring a fasting plasma glucose level, followed by a 2-h OGTT only in individuals with impaired fasting glucose (IFG), *i.e.* fasting plasma glucose level between 110 and 125 mg/dl (6.1–6.9 mmol/liter). IGT is then defined as a 2-h fasting glucose equal to or more than 140 mg/dl and less than 199 mg/dl (7.8–11.0 mmol/liter) (40). The ADA also defines IGT as a 2-h plasma glucose of 140–199 mg/dl (7.8–11.0 mmol/liter) but does not require a fasting glucose before the performance of the OGTT (9).

Both the ADA and the WHO recommend using fasting plasma glucose as the initial screening test for DM because it is more convenient to patients, less costly, more reproducible, and easier to administer than the 2-h OGTT (9, 40). Despite these disadvantages, the 2-h OGTT is more sensitive and moderately more specific in diagnosing DM compared with fasting plasma glucose (9). In addition to providing information on both β-cell secretion and peripheral insulin action, the OGTT provides a better assessment of IGT than homeostatic techniques such as fasting glucose to insulin ratio, fasting insulin, and homeostatic model assessment (42). Reproducibility of the 2-h OGTT can be enhanced by paying attention to the carbohydrate intake of the last meal before a 2-h OGTT because low-carbohydrate intake may falsely result in a diagnosis of IGT (43, 44), and by ensuring that the 2-h sample is collected within 120 ± 5 min (40).

Several studies have shown that the fasting plasma glucose and the OGTT do not identify the same group of patients (45–48). The Diabetes Epidemiology: Collaborative Analysis Of Diagnostic Criteria in Europe (DECODE) study (45) demonstrated in a group of 1517 individuals with newly diagnosed DM that 40% met the criteria by fasting plasma glucose only, 31% met the criteria by a 2-h OGTT only, and 28% met both criteria. Therefore, nearly one third of individuals with DM would have been missed using fasting plasma glucose only. In a study of 5023 Pima Indians, Gabir *et al.* (48) reported that IGT was more common than IFG (15 *vs.* 5%). In fact, IGT, measured by an OGTT, is typically more common in women, whereas IFG, measured by fasting plasma glucose, is more common in men (40). In women with PCOS, Legro *et al.* (11) revealed that the majority of PCOS women with IGT have normal fasting glucose levels. In the PCOS population studied, fasting plasma glucose measurements by ADA criteria failed to diagnose 58% of women with DM diagnosed by a 2-h OGTT. Therefore, in PCOS, measurement of fasting blood glucose misses even more persons with IGT and DM than in the general population.

IGT is a strong predictor for DM, as well as risk of cardiovascular disease and premature mortality (7, 48–51). A 7-yr cohort study reported that IGT, but not IFG, is a risk factor for cardiovascular disease (5). In the Diabetes Epidemiology: Collaborative Analysis Of Diagnostic Criteria in Europe study (45), hazard ratios (95% CI) for DM diagnosed by a fasting plasma glucose were 1.6 (1.4–1.8) for all-cause mortality, 1.6 (1.3–1.9) for cardiovascular mortality, and 1.6 (1.4–1.9) for noncardiovascular mortality, respectively. The corresponding hazard ratios for DM by a 2-h OGTT were 2.0 (1.7–2.3), 1.9 (1.5–2.4), and 2.1 (1.7–2.5). In terms of prevention, the ADA acknowledges that although the efficacy of interventions for primary prevention of type 2 DM has been well recognized in individuals with IGT, there are presently no data regarding individuals with IFG who do not also have IGT (9). Data are also nonexistent regarding primary prevention of premature mortality and cardiovascular disease in individuals with IFG only (40).

The OGTT is a simple test and can be performed in an office laboratory setting. Based on current evidence and because the majority of women with PCOS have normal fasting plasma glucose, the 2-h OGTT is the best screening measure for glucose intolerance and diagnosis of type 2 DM in women with PCOS.

Prevention and Treatment of Glucose Intolerance in PCOS

A systematic review of the published peer-reviewed medical literature did not reveal high-quality, prospective, randomized-controlled trials addressing the prevention and treatment of IGT specifically in women with PCOS. Consequently, recommendations regarding the roles of lifestyle modification and pharmacological therapy in the prevention of type 2 DM in PCOS are primarily derived from studies involving broader subject populations.

Lifestyle modification

The characteristics and results of five studies evaluating the role of lifestyle modification, including dietary modification and regular moderate activity, in preventing the development of type 2 DM among high-risk individuals are outlined in Table 3. Of note, there were significant variations

TABLE 2. Current WHO and ADA criteria for defining hyperglycemia

	WHO (2006)	ADA (2007)
2-h glucose/OGTT		
NGT	<140 mg/dl (7.8 mmol/liter)	<140 mg/dl (7.8 mmol/liter)
IGT	FG < 126 mg/dl (7.0 mmol/liter) if measured and 2-h glucose ≥ 140 mg/dl (7.8 mmol/liter) and < 200 mg/dl (11.1 mmol/liter)	140 mg/dl (7.8 mmol/liter) to 199 mg/dl (11.0 mmol/liter)
Diabetes	=200 mg/dl (11.1 mmol/liter)	≥200 mg (11.1 mmol/liter)
FG		
Normal FG	<110 mg/dl (6.1 mmol/liter)	<100 mg/dl (5.6 mmol/liter)
IFG	110 mg/dl (6.1 mmol/liter) to 125 mg/dl (6.9 mmol/liter)	100 mg/dl (5.6 mmol/liter) to 125 mg/dl (6.9 mmol/liter)
Diabetes	=126 mg/dl (7.0 mmol/liter)	≥126 mg/dl (7.0 mmol/liter)

FG, Fasting glucose.

TABLE 3. Summary of randomized, controlled trials evaluating the role of lifestyle modification in preventing progression to DM among high-risk populations

Study	Knowler *et al.* (52)	Pan *et al.* (54)	Ramachandran *et al.* (55)	Tuomilehto *et al.* (53)	Wein *et al.* (56)	Wing *et al.* (57)
No. of randomized subjects	3234[a]	577[b]	531[a]	522	200	154[b]
Inclusion criteria	Males and females, BMI $\geq$ 24 kg/m^2 (22 kg/m^2 in Asians), IFG and IGT	Males and females, IGT	Males and females, IGT	Males and females, BMI $>$ 25 kg/m^2, ages 40–65 yr, IGT	Women with history of GDM, IGT	Males and females, 30–100% over ideal body weight, 40–55 yr, parent with DM
IGT/DM definition	ADA 1997	WHO 1985	WHO 1999	WHO 1985	WHO 1985	WHO
Follow-up duration (yr)	2.8	6	3	3.2	4.25	2
Age (yr)	50	45	45–46	55	25	46
BMI (kg/m^2)	34	26	25–26	31	38–40	36
Intervention	Diet and exercise, >7% weight loss, 16-lesson curriculum	Diet and exercise, small group support sessions	Diet and exercise	Diet and exercise, >5% weight loss	Diet alone	Diet and exercise
Dietary education	Hypocaloric, low-fat diet	Hypocaloric diet to induce 0.5–1.0 kg/month weight loss in BMI $\geq$ 25 kg/m^2	Hypocaloric, low-fat, high-fiber diet	Low-fat, high-fiber diet, sessions with nutritionist	Standard dietary advice, nutrition telephone follow-up every 3 months	Hypocaloric, low-fat diet, multidisciplinary nutrition sessions
Exercise education	Moderate activity (150 min/wk)	Increase physical activity 1–2 U/d	Moderate (30 min/d) exercise	Moderate (30 min/d) exercise, supervised circuit resistance training		1500 kcal/wk moderate activity
Control intervention	Standard lifestyle modification (single education session)	General information regarding DM and IGT		General verbal and written diet and exercise information	Standard dietary advice	Provided self-help manual
Diabetes incidence intervention	4.8/100 person/yr	9.6/100 person/yr, 46.0%	39.3%	27/265, 3.2/100 person/yr, 10.2%	26.8%	5/32, 15.6%
Diabetes incidence controls	11/100 person/yr	15.7/100 person/yr, 67.7%	55.0%	59/257, 7.8/100 person/yr, 23.0%	28.1%	2/31, 6.5%
RRR (95% CI)	58% (48–66%)	38%	28.5% (20.5–37.3%)	58%	NS	NS
Hazard ratio (95% CI)				0.4 (0.3–0.7)		

NS, Nonsignificant; RRR, relative risk reduction.
[a] Included a group randomized to treatment with medication.
[b] Included groups randomized to diet alone and exercise alone.

in the reported effects of lifestyle modification on the conversion of IGT to DM among high-risk populations.

Two large intervention studies, the Diabetes Prevention Program (DPP) (52) and the Finnish Diabetes Prevention Study (53), demonstrated strikingly similar reductions (58% relative risk reduction) in the conversion rate to DM among overweight men and women with IGT randomized to treatment with intensive lifestyle modification compared with controls. Importantly, the study populations had mean BMI measurements more than 30 kg/m^2, and the goal of both lifestyle treatment programs was a 5–7% reduction in body weight. The specific interventions involved in the DPP included a hypocaloric low-fat diet and a minimum of 150-min moderate intensity physical activity a week.

Studies by Pan *et al.* (54) and Ramachandran *et al.* (55) also demonstrated a significant but less dramatic relative risk reduction (28–38%) in the conversion rate from IGT to diabetes with intensive lifestyle modification. The discrepancies among the reported differences in risk reduction with lifestyle modification may be partially explained by differences in the study populations. The mean BMI measurements in the studies by Pan *et al.* (54) and Ramachandran *et al.* (55) were 25–26 kg/m^2, lower than the mean BMI measurements in the DPP and Finnish Diabetes Prevention Study.

A study by Wein *et al.* (56) involving 200 women with a history of GDM and current IGT demonstrated a small but nonsignificant reduction in the diabetes conversion rate; however, the study intervention included dietary modification alone. Finally, one smaller study by Wing *et al.* (57) failed to show a significant difference in the development of diabetes with diet alone, exercise alone, or a combined intervention in overweight subjects with a family history of diabetes.

Little is known regarding the role of exercise in preventing the development of IGT and DM in nonobese women with PCOS.

Pharmacological intervention

Pharmacological therapies, including insulin-sensitizing agents, have also been shown to decrease the conversion rate to overt DM among subjects with IGT, and randomized-controlled trials evaluating pharmacotherapy that met the specified criteria are outlined in Table 4. Treatment with medications from a variety of different drug classes, including the biguanide, metformin (52, 55), thiazolidinediones (58,

59), the α-glucosidase inhibitor, acarbose (60), and the lipoprotein lipase inhibitor, orlistat (61, 62), has prevented the development of DM among high-risk populations with IGT.

In addition to intensive lifestyle modification, the DPP evaluated the role of the insulin-sensitizing agent metformin in the prevention of DM (52). In the DPP, the treatment arm randomized to receive metformin 850 mg twice daily demonstrated a 31% reduction in the relative risk of developing DM compared with placebo treatment. Notably, the risk reduction in the metformin treatment arm was less robust than the response reported in the group receiving intensive lifestyle modification (31 *vs.* 58%, respectively). Although the DPP represents a large, well-designed control trial evaluating the efficacy of lifestyle modification and pharmacotherapy in preventing DM, there were several limitations to the study. First, there was no treatment arm in the DPP that evaluated the combined effect of intensive lifestyle modification and metformin therapy in high-risk individuals. Second, some experts have suggested that treatment with metformin may merely mask the development of DM as opposed to preventing the disease. In response to this debate, the DPP Research Group published follow-up results reporting repeat OGTTs in a subset of subjects who received metformin therapy after a washout period of 1–2 wk (63). Although the reported incidence of DM increased in the metformin treatment group after the washout period, the incidence of DM in the metformin arm was still reduced by 25% compared with the placebo group.

Similar to the DPP, the Indian Diabetes Prevention Program (IDPP) evaluated the role of lifestyle modification and metformin in the prevention of DM among overweight Asian Indian men and women with IGT (55). The IDPP results revealed significant, 28.5 and 26.4%, relative risk reductions for the development of DM with intensive lifestyle modification and metformin treatment, respectively. Although both lifestyle modification and metformin therapy reduced the incidence of DM in the IDPP, there was no added benefit from the combination lifestyle modification and metformin compared with either treatment alone. As outlined previously, the less robust reduction in DM risk reported with lifestyle modification in the IDPP compared with the DPP may be explained by differences in subject BMIs at baseline between the two studies (25.7 ± 3.3 kg/m^2 in the IDPP compared with 33.9 ± 6.8 kg/m^2 in the DPP).

Similar to metformin, the thiazolidinediones improve insulin sensitivity and may prevent or delay the development of DM in high-risk individuals. Randomized, placebo-controlled trials involving the prevention of DM using thiazolidinediones demonstrated a 62–89% relative risk reduction with this class of medications (58, 59). Unfortunately, these studies did not compare treatment with thiazolidinediones with intensive lifestyle modification or other medications. The DPP initially contained a treatment arm that was randomized to receive the thiazolidinedione, troglitazone; however, given concerns of hepatotoxicity, the troglitazone arm was discontinued in 1998 (64). Before its discontinuation, the incidence of DM among the 585 subjects receiving troglitazone for a mean duration of 0.9 yr was statistically lower than the incidence in both the placebo and metformin groups. Furthermore, there was no statistical difference in the progression rate to DM between the troglitazone and intensive lifestyle modification treatment arms. Despite these findings, longer term studies are needed to compare the efficacy of thiazolidinediones and other insulin-sensitizing agents and lifestyle modification in preventing DM.

Prevention of glucose intolerance in PCOS

Although there are no published prospective, randomized-controlled trials that evaluate the prevention or treatment of IGT specifically in women with PCOS, several small studies do address the potential role of insulin-sensitizing agents in this high-risk population. Unluhizarci *et al.* (65) reported the impact of treatment with metformin 500 mg twice daily for 3 months on IGT in 17 adult women with PCOS (mean age 24.4 ± 1.4 yr; mean BMI 29.7 ± 1.4 kg/m^2). At baseline, five (31.6%) of the women demonstrated IGT. Of these, two patients (40%) showed NGT after treatment with metformin. In a study by Arslanian *et al.* (66), 15 obese adolescents with PCOS and IGT (mean age 14.0 ± 0.8 yr; mean BMI 38.1 ± 1.6 kg/m^2) were treated with metformin 850 mg twice daily for 3 months. At the end of the relatively brief treatment period, approximately one half (n = 8) of the adolescents had reverted back to NGT on repeat testing. In a study by Dereli *et al.* (67), 40 women with PCOS, a BMI less than 27 kg/m^2, and IGT were randomized to treatment with rosiglitazone with either 2 (n = 20) or 4 mg daily (n = 20) for 8 months. In addition to decreases in free testosterone levels and improvements in ovulatory dysfunction in both treatment groups, 19 (95%) and 15 (75%) of the women receiving rosiglitazone 4 and 2 mg daily, respectively, had reverted to NGT after 8-month treatment.

Finally, using a retrospective study design, Sharma and Nestler (68) evaluated the role of metformin in preventing the progression of glucose intolerance in PCOS. At baseline, 11 (22%) of the 50 nondiabetic women with PCOS had evidence of IGT according to an OGTT. After a mean treatment period of 2.4 yr, approximately half (n = 6) the women with IGT at baseline had reverted to NGT with metformin therapy. Furthermore, while receiving metformin (average treatment period of 3.6 yr), only two (5.1%) of the 39 women with NGT at baseline had converted to IGT, representing an annual conversion rate of 1.6%. None of the women with IGT at baseline converted to overt DM during treatment with metformin. When compared with the 16% annual conversion rate from NGT to IGT among drug-naive PCOS women reported by Legro *et al.* (20), treatment with metformin appeared to lead to an 8-fold decrease in the annual conversion rate. Despite the limitations of these four studies, including small sample size, lack of control groups, and the question of whether glucose intolerance was prevented or merely being masked, their results support the potential role of insulin-sensitizing agents in the prevention of IGT and DM in PCOS. However, well-designed, prospective, randomized-controlled trials are needed to evaluate more fully the specific roles of lifestyle modification and insulin-sensitizing agents in the prevention of IGT and its progression to type 2 DM among women with PCOS.

TABLE 4. Summary of randomized, controlled trials evaluating the role of pharmacotherapy in preventing progression to DM among high-risk populations

Study	Bosch *et al.* (69)	Chaisson *et al.* (60)	Durbin (58)	Gerstein *et al.* (59)	Heymsfield *et al.* (61)	Knowler *et al.* (52)	Ramachandran *et al.* (55)	Torgerson et al. (62)
No. of randomized subjects	5269	1429	172	5269	675, 120 with IGT	3234[a]	531[a]	3305, 694 with IGT
Inclusion criteria	Males and females, ≥ 30 yr, IFG or IGT ± IFG	Males and females, 40–70 yr, BMI 25–40 kg/m^2, IGT	Males and females, IFG and IGT	Males and females, ≥ 30 yr, IFG or IGT ± IFG	Males and females, BMI 30–43 kg/m^2, NGT, IGT, DM	Males and females, ≥ 25 yr, BMI 24 kg/m^2 (22 kg/m^2 in Asians), IFG and IGT	Males and females, IGT	Males and females, BMI ≥ 30 kg/m^2, 30–60 yr, IFG and IGT
IGT/DM definition	WHO	WHO 1985	ADA	WHO	WHO 1985	ADA 1997	WHO 1999	WHO 1994
Follow-up duration (yr)	3.0	3.3	3.0	3.0	2.0	2.8	3.0	4.0
Age (yr)	55	54	54–60	55	44	50	45–46	43
BMI (kg/m^2)	31	31	N/A	31	36	34	25–26	37
Intervention	Ramipril titrated to 15 mg daily	Acarbose 100 mg three times daily	Troglitazone 400 mg daily × 10 months, pioglitazone/ rosiglitazone	Rosiglitazone 8 mg daily	Orlistat 120 mg three times daily, hypocaloric diet × 1 yr	Metformin 850 mg twice daily, standard lifestyle modifications	Metformin 500–250 mg twice daily	Orlistat 120 mg three times daily; hypocaloric, low-fat diet; increase exercise by 1 km/d
Control	Placebo	Placebo	Placebo	Placebo	Placebo, hypocaloric diet × 1 yr	Placebo, standard lifestyle modifications	Standard health care advice	Placebo; hypocaloric, low-fat diet; increase exercise by 1 km/d
Diabetes incidence intervention	449/2623, 17.5%	221/682, 32%	1.4/100 person/ yr, 3.0%	280/2635, 10.6%	2/67, 3.0%	7.8/100 person/yr	40.5%	6.2%
Diabetes incidence controls	489/2646, 18.5%	285/686, 42%	9.4/100 person/ yr, 26.8%	658/2634, 25.0%	4/53, 7.6%	11/100 person/yr	55%	9.2%
RRR (95% CI)	NS	25%	88.9%	62%	47%	31% (17–43%)	26.4% (19.1–35.1%)	37.3%
Hazard ratio (95% CI)	0.91 (0.8–1.03)	0.75		0.38 (0.33–0.44)	$P < 0.04$			0.63 (0.46–0.86)
Adverse events	Cough	Gastrointestinal symptoms	Not formally reported	Heart failure	Not formally reported	Gastrointestinal symptoms	Symptoms of hypoglycemia, gastrointestinal symptoms	Gastrointestinal symptoms

N/A, Not available; NS, nonsignificant, RRR, relative risk reduction.

[a] Included an additional group randomized to intensive lifestyle modification.

Glucose Intolerance in Adolescents with PCOS

Development of IGT in adolescents

Data on glucose intolerance in adolescents with PCOS are limited, and studies are difficult to interpret with confidence, given the small numbers of participants in each study. As in adult women, adolescents with PCOS are at increased risk for developing glucose intolerance and DM compared with their non-PCOS counterparts (11); however, the exact prevalence of IGT in young women with PCOS is less clear. For example, a Canadian study of 22 obese adolescents with PCOS revealed baseline IGT in only one participant (4.5%) (70). In contrast, small studies involving obese adolescents with PCOS in the United States report rates of IGT as high as 33 (13) to 52% (35). These differences may be accounted for by many factors, including, but not limited to, family history, diet, BMI, and exercise habits. Even less is known regarding the risk of IGT in nonobese adolescents with evidence of PCOS or ovarian hyperandrogenism. In two small studies involving a total of 39 nonobese adolescent girls with PCOS by Ibanez *et al*. (71) and Silfen *et al*. (72), none of the nonobese adolescents demonstrated IGT on the OGTT.

Measurements of glucose intolerance in adolescents

As in adults, screening for IGT with a fasting glucose level is not reliable in adolescents, and tests of insulin resistance such as the fasting glucose to insulin ratio and the homeostasis assessment model for estimating insulin resistance are poor predictors of IGT and DM in adolescents with PCOS (13). Therefore, the most reliable screening test for IGT in PCOS adolescents is the 2-h OGTT after a 75-g glucose load, interpreted using ADA guidelines. Although the most appropriate screening interval is not clearly defined, the conversion from NGT to type 2 DM can occur in as little as 5 yr (73), most likely because of the strong correlation of PCOS and insulin resistance.

Treatment of IGT in adolescents

Although the literature regarding treatment of IGT specific to adolescents is sparse, it seems reasonable to use a similar approach to that used in adult women with PCOS. Diet and exercise appear to be the most important aspects of treating IGT and reducing progression to type 2 DM. As demonstrated in the DPP (52), lifestyle intervention comprised of a low-fat diet and 150-min exercise per week reduced the progression from IGT to type 2 DM by 58% compared with placebo and was more successful than metformin therapy, which reduced progression by 31%.

A small randomized-controlled trial comparing metformin to placebo for 12 wk in 22 adolescents with PCOS showed no significant difference in IGT (70). Of note, however, the only subject with baseline IGT was in the metformin group and showed persistence of her IGT at the study end. There were no subjects in the placebo arm with baseline IGT, but one developed IGT by the end of the 12-wk study. Clearly, this study was underpowered, and the duration was not sufficient to detect a true difference. Conversely, the only study evaluating PCOS adolescents with baseline IGT showed that treatment with metformin (850 mg twice daily) resulted in conversion back to NGT in eight of the 15 subjects after 3-month treatment (66). Limitations of this study include small sample size and lack of a control group; nonetheless, metformin may be a promising treatment for PCOS adolescents with IGT.

Conclusion and Recommendations

Although the strengths of the studies reviewed vary considerably, the expert panel concludes that there is sufficient evidence to recommend support for the following recommendations (Table 5). Because of the high prevalence of glucose intolerance among patients with PCOS, screening is a necessary part of the care of these patients who are at a markedly increased risk for the development of type 2 DM. Because an increased prevalence of both glucose intolerance and type 2 DM has been found in various ethnic populations, screening should be done regardless of ethnicity. Although numerous risk factors such as obesity and age increase the risk of glucose intolerance, women with PCOS of all ages and weights appear to be at greater risk for glucose intolerance than normal controls. Consequently, the panel recommends that all women with PCOS be screened, even in the absence of additional risk factors and regardless of BMI.

Multiple studies have shown that fasting glucose concentrations are not sufficiently sensitive to detect all patients with PCOS who have IGT. Therefore, an OGTT is recommended as the standard screening tool for IGT in these patients and should initially be performed at diagnosis. Although prior studies have suggested women with PCOS and NGT at baseline should be periodically rescreened for the development of IGT, the ideal interval for screening remains uncertain.

Acknowledging the presence of limited data, studies suggest a high (16–19%) annual conversion rate from NGT to IGT in PCOS, and the panel recommends screening PCOS women with NGT at baseline and at least once every 2 yr or earlier if additional risk factors are identified. However, given the high risk of progression to overt diabetes, women with PCOS who have IGT should be screened annually using an OGTT.

Intensive lifestyle modification should be considered the mainstay of treatment in all women with PCOS who have

TABLE 5. Androgen Excess Society screening and treatment recommendations for IGT in PCOS

- All patients with PCOS, regardless of BMI, should be screened for IGT using a 2-h OGTT.[a]
- Patients with NGT should be rescreened at least once every 2 yr or earlier if additional risk factors are identified.
- Patients with IGT should be screened annually for the development of DM.
- The mainstay of treatment for all patients with PCOS and IGT should be intensive lifestyle modification as well as weight loss in obese patients.
- Insulin-sensitizing agents, such as metformin and thiazolidinediones, should be considered in patients with PCOS and IGT.
- Adolescents with PCOS should be screened for IGT using a 2-h OGTT repeated once every 2 yr. If IGT develops, they should be treated with intensive lifestyle modification, and treatment with metformin should be considered.

[a] See *Minority Report*.

IGT to prevent progression to DM. Despite insufficient data in lean women, it is reasonable to recommend that, in all women with PCOS, a lifestyle modification program should consist of at least 30-min moderate activity 5 d/wk. Furthermore, in overweight and obese women with PCOS, a hypocaloric diet is recommended to achieve a minimum of 5–7% weight loss. However, many overweight and obese women with PCOS find significant weight loss difficult to achieve and maintain, and weight loss is not an option for lean women with PCOS. Consequently, the addition of insulin-sensitizing agents such as metformin and thiazolidinediones should be considered in women with PCOS and documented IGT if weight loss attempts fail or are not possible.

Adolescents with PCOS, like their adult counterparts, should be screened for IGT using an OGTT at least once every 2 yr after a normal screen and more frequently after an abnormal screen. Adolescents should also be treated with intensive lifestyle modification, including diet and moderate exercise as initial therapy. The use of metformin or other insulin-sensitizing agents to treat or prevent progression to IGT may be considered but should not be mandated until there have been well-designed, randomized-controlled trials demonstrating their efficacy.

Minority Report

Notwithstanding the aforementioned recommendation to screen all women with PCOS with a 2-h OGTT, it should be noted that a few members of the Androgen Excess Society Board did not agree with this recommendation. Indeed, evidence regarding risk of IGT in lean PCOS women is limited and still emerging (74). Therefore, these Board members recommend screening for IGT and type 2 DM using an OGTT only in obese PCOS patients with a BMI equal to or more than 30 kg/m^2, or alternatively, screening lean patients only if they have at least one additional risk factor for DM, including advanced age, family history of DM, or a personal history of GDM.

Future Directions

The panel also identified that additional research is needed in several key areas. Large studies are needed to determine the ideal frequency for rescreening women with both NGT and IGT at baseline. Investigation into the utility of stratifying women with PCOS to determine who should be screened for IGT should be examined, such as the role of decision tree modeling. It would be ideal to have a registry of patients seen at PCOS clinics that contained information on more patients than a single investigator's cohort that could be a valuable research resource to address some of these questions. In particular, further information is needed regarding the risk of IGT and progression to DM in nonobese women with PCOS. In addition, research is needed to determine the long-term role of insulin-sensitizing medications in preventing the progression to IGT and type 2 DM in both lean and obese women with PCOS. Because the literature in adolescents with PCOS is limited, the panel found several areas that need to be investigated further. Large, multicenter studies are needed to determine a more accurate incidence of adolescents with PCOS and IGT. Randomized-controlled trials are needed to investigate the efficacy of insulin-sensitizing agents *vs.* lifestyle modification *vs.* placebo in the prevention of IGT and type 2 DM in this population, and studies are needed to be done on the effect of oral contraceptives on conversion to IGT and diabetes in adolescents with PCOS.

Acknowledgments

We thank the Androgen Excess Society Board members for their review, comments, and critiques of this manuscript: Drs. Ricardo Azziz, Enrico Carmina, Evanthia Diamanti-Kandarakis, Walter Futterweit, Onno E. Janssen, Jan McAllister, Robert Norman, Ann E. Taylor, and Selma Witchel.

Received July 11, 2007. Accepted September 27, 2007.

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Disclosure Statement: The authors have nothing to disclose.

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

Endocrine (2012) 42:526–534
DOI 10.1007/s12020-012-9701-4

NEW HORIZONS IN

Emerging concepts about prenatal genesis, aberrant metabolism and treatment paradigms in polycystic ovary syndrome

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Received: 29 November 2011 / Accepted: 10 May 2012 / Published online: 4 June 2012

Abstract The interactive nature of the 8th Annual Meeting of the Androgen Excess and PCOS Society Annual Meeting in Munich, Germany (AEPCOS 2010) and subsequent exchanges between speakers led to emerging concepts in PCOS regarding its genesis, metabolic dysfunction, and clinical treatment of inflammation, metabolic dysfunction, anovulation and hirsutism. Transition of care in congenital adrenal hyperplasia from pediatric to adult providers emerged as a potential model for care transition involving PCOS adolescents.

Keywords Developmental programming · Inflammation · Advanced glycated end products · Congenital adrenal hyperplasia · Hirsutism · Statins · Clomiphene citrate · Aromatase inhibitors · Metformin · Lifestyle intervention

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Introduction

Depending on the diagnostic criteria, PCOS is found in ~6–10 % (NIH) or ~15 % (Rotterdam) of women in their reproductive years [1] and is characterized by androgen excess, ovulatory dysfunction, and polycystic ovaries [2]. While PCOS contributes to ~75 % of anovulation-related infertility [3], it also accounts for 12–28 % of overweight and obese (BMI ≥ 25) women, 15–36 % of women with type 2 diabetes mellitus (type 2 DM) [4–7], and doubling of the lifetime risk for cardiovascular disease [8]. Women with "classic" PCOS, diagnosed by NIH criteria alone, have greater risk of cardiovascular disease and type 2 DM than those diagnosed by non-NIH criteria [8, 9]. Genetic susceptibility loci for PCOS, identified by recent genome-wide association studies, particularly implicate genes involved in cytoplasmic function in multiple organ systems, including thyroid adenoma associated protein (THADA) [10–12] and DENN/MADD domain containing 1A (DENND1A) [10, 11]. Polymorphisms of genes involved in glucose homeostasis, including adipocyte fatty acid binding protein (FABP4) [13] and adiponectin [14], are also associated with PCOS, but PCOS-linked variants differ from those associated with type 2 DM [15].

Typical of a complex disease, however, progress in PCOS treatment has been limited due to incomplete knowledge of its pathogenesis, despite its high heritability [16, 17]. What is clear, nevertheless, is that pre- or peri-pubertal metabolic dysfunction is one of the first phenotypic traits observed in adolescent girls likely to develop PCOS [18, 19]. This is alarming as obesity now affects ~15 % of American children [20, 21]. Obesity commonly associates with peri-pubertal hyperandrogenemia in girls [22–26], a trait combination increasingly considered antecedent to PCOS [27, 28].

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AEPCOS 2010 provided an update regarding multiple aspects of PCOS, including its potential genesis, inflammation as an initiator of and/or contributor to PCOS, and emerging treatment paradigms related to inflammation, metabolic dysfunction, anovulation, and hirsutism. While the major focus of the meeting related to PCOS, three other relevant areas were also highlighted: (1) transition of care in congenital adrenal hyperplasia (CAH) from pediatric to reproductive/internal medicine endocrinology [29, 30] that may provide a model for clinical transition of PCOS adolescents, (2) androgen influences on the pilo-sebaceous unit and treatment options [31], and (3) the recently formed Australian National Polycystic Ovary Syndrome Alliance (http://www.adelaide.edu.au/robinson-institute/mediareleases/pcos/), an organization comprising PCOS patients and their close relatives, together with researchers and health care professionals, that is developing evidence-based guidelines and multidisciplinary care models that promise comprehensive care solutions targeted on prevention of PCOS complications [32].

Prenatal genesis of PCOS: is there a role for prenatal androgens?

Female mammalian fetuses exposed to fetal male levels of testosterone (T) express PCOS-like reproductive and metabolic traits in adulthood [2, 33–35]. Exposing fetal males to similar gestational treatment [36, 37] induces abnormalities in male reproductive and metabolic function. Reproductive consequences in T-exposed rams include reduced number and motility of spermatozoa [38], accompanied by higher numbers of Sertoli cells and fewer germ cells per seminiferous tubule [39]. Endocrine abnormalities accompanying male germ cell abnormalities include increases in (1) FSH responsiveness to GnRH analog treatment, (2) amplitude of spontaneously occurring LH pulses, and (3) testicular receptor expression for FSH and transforming growth factor-β1 (TGF-β1), together with a negative correlation between anti-mullerian hormone (AMH) and TGF-β3 expression [38, 39]. Taken together, these findings suggest that prenatal exposure to exogenous T in males may act as an endocrine disruptor, leading to an altered adult testicular environment that includes disruption of the blood–testis barrier and diminished spermatogenesis. In humans, close male relatives of women with PCOS have comparable metabolic dysfunction to their PCOS female kin, including dyslipidemia and insulin resistance that are accompanied by elevated DHEAS concentrations [40–44]. Results from T-exposed rams suggest that male relatives of women with PCOS warrant investigation of their fertility.

In T-exposed female monkeys, daily subcutaneous injections of T propionate into their dams contribute to transient hyperglycemia derived from mild-to-moderate

maternal glucose intolerance [45]. T-exposed females exhibit subtle increases in both fetal and neonatal body size and a degree of transient newborn hypoglycemia. T-exposed female infants have a relative hyperinsulinemic response to glucose [45]. Such insulin hypersecretion in insulin sensitive, T-exposed female infants may explain their modest weight gain, due to insulin's anabolic actions, that may lead to increased weight accumulation prior to puberty. As preadolescent and adolescent daughters of women with PCOS show subtle hyperinsulinemia from insulin resistance before manifesting an obvious PCOS phenotype [46, 47], prepubertal insulin defects may provide important developmental precursors in the expression of adult PCOS [2], as suggested by both PA monkey and sheep models [34, 45]. Evidence of fetal T-exposure preceding PCOS in women, however, is still inconclusive with studies of umbilical cord blood showing increased [48] or decreased [49] androgen levels among newborn daughters of women with PCOS. Interestingly, an epigenomics study of PA monkeys implicates altered TGF-β signaling in the most significantly differentially methylated pathways in both infants and adults [50], suggesting that PA monkeys may epigenetically mimic PCOS in women. In support of this notion, a dinucleotide repeat (D19S884) that maps to intron 55 within the fibrillin 3 (*FBN3*) gene has been the most consistent genetic region associated with familial PCOS [51, 52]. Since the degree and type of fibrillin expression contributes to differences in elasticity of cell-extracellular matrix interactions and storage of TGF-β, fibrillins may provide gestationally relevant [53], tissue-specific foundations for cell-mediated engagement of extracellular matrix-stored TGF-β in proliferation, differentiation, and apoptosis [54, 55] that may engender PCOS.

Pediatric to adult transition of endocrine clinical care in CAH: lessons for management of adolescent girls with PCOS

In order to enable patients with CAH to become productive, responsible citizens, transition from pediatric to adult health care needs to be a purposeful, planned transfer between providers [56–58]. An assessment of patient readiness should consider medical, psychosocial, educational, cognitive, emotional, and vocational needs. Expectations and differences in health care delivery systems between pediatric and adult care will be experienced, including a shift in treatment goals from a focus on linear growth and "on-time" puberty to concerns related to fertility, sexuality, bone health, and risks for cardiovascular disease.

Adolescents may feel uncomfortable in both pediatric and adult waiting rooms. Pediatricians, staff, and parents may have difficulty "letting go" and transferring their long-term relationship to adult healthcare providers. Over-involvement of parents can lead to adolescents feeling excluded and thwarted from participating in their own health care; ultimately this undermines the adolescent's emerging autonomy and self-responsibility [59]. Parents need to make the transition from being the "CEO" of their child's health care to becoming the consultant, and eventually, a bystander while the child ascends from a consumer to the "CEO" [60]. There are thus relationship reconfigurations from doctor–parent–patient to doctor–patient.

Patient behaviors such as texting and playing games on cell phones, increased risk-taking behavior, poor adherence to recommendations, and disinterest in participating in discussions frustrate health care providers and parents. Adult health care providers assume that their adolescent and young adult patients are active partners who are knowledgeable about their disorder, are autonomous, and are capable of negotiating the health care system. Some adolescents, however, especially those with chronic disorders, may be unprepared and unready to participate as an active partner in their own health care. Suboptimal transitions can lead to mediocre connections with adult healthcare providers that can culminate in "drop-out" from healthcare.

Knowledge about self-management does not always predict good adherence. For some CAH adolescents and young adults, non-adherence to recommendations may represent a conscious decision to minimize the intrusiveness of the disease. Problem solving or brainstorming discussions to find creative solutions regarding adherence to the recommended management regimens and transition to adult healthcare may be invaluable, and may enable care transition in hyperandrogenic adolescent girls at risk for PCOS [61], in addition to those who manifest CAH.

Inflammation in PCOS: is it the initiator or just a contributor to metabolic dysfunction?

While obesity provides a considerable proinflammatory contribution to PCOS [62], elevated levels of proinflammatory markers, including monocyte chemo-attractant protein-1, are discernible independent of obesity [63–65]. Other features include increased lipid peroxidation, increased markers of endothelial dysfunction, and decreased haptoglobin concentrations. Evidence is accumulating that inflammation may contribute to the genesis of PCOS [66]. Hyperandrogenism, whether extant in women with PCOS or induced by androgen therapy in normal women, enhances an inflammatory response in mononuclear (pre-macrophage) leukocytes when they are exposed to a hyperglycemic environment equivalent to

glucose intolerance or poorly controlled diabetes [67]. Such inflammatory-prone macrophages can infiltrate both ovaries and adipose depots, enhancing androgen biosynthesis and cytokine release, respectively [66].

Not surprisingly, therefore, inflammation and metabolic dysfunction are frequent co-morbidities among women with PCOS. Advanced glycated end products (AGEs) are a class of nutrients implicated in the pathogenesis of cardiometabolic disturbances, insulin resistance, and possibly, direct ovarian dysfunction. AGEs are products of non-enzymatic glycation and oxidation of proteins from endogenous or exogenous (dietary) sources that are known to accumulate in diverse clinical conditions or developmental stages, including type 2 DM, renal failure, and aging [68]. In women with PCOS, circulating AGEs concentrations are increased, independently of obesity, and are positively correlated with T and AMH concentrations [69, 70]. Immunohistochemical localization of (AGEs) and their receptor (RAGE) are increased in polycystic compared to normal ovaries [71]. In the ovarian microenvironment of PCOS, AGEs may contribute to insulin resistance, dysregulation of folliculogenesis, ovarian steroidogenesis and altered collagen synthesis [70, 71]. In the latter regard, as AGEs stimulate extracellular matrix production and abnormal collagen cross-linking in the ovary [72], they may contribute to altered TGF-β signaling in the PCOS ovary. Reduced dietary intake of AGEs improves metabolic and reproductive aspects of PCOS [69], and may thus ameliorate progression of PCOS symptomatology.

Emerging treatment paradigms in PCOS: approaches to ameliorate metabolic aberration, anovulatory infertility and hirsutism

Metabolic aberration

Statins, competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, interfere with cholesterol synthesis, ultimately improving lipid profiles and decreasing inflammation. Primarily due to statin-induced inhibition of isoprenylation, in vitro studies have demonstrated that statins inhibit proliferation of, and diminish androgen production in, ovarian theca-interstitial cells [73–75]. Statins may thus offer many advantages for women with PCOS. Specifically, statins reduce hyperandrogenism, improve lipid profiles, and decrease levels of several markers of inflammation and endothelial dysfunction including C-reactive protein, soluble vascular cell adhesion molecule-1 (sVCAM), IL-1β, IL-6, and TNF-α [76–79].

The Diabetes Prevention Program study demonstrates, that in the general population, both metformin and intensive lifestyle modification reduce incident type 2 DM, and when successful, lifestyle modification is more efficacious than metformin [80]. Lifestyle intervention, involving dietary action, exercise and psychological help, remains an important first-line therapy for management of the metabolic syndrome in women with PCOS [32, 81]. Measurement of AMH appears to be a predictor of response to lifestyle intervention [82]. Metformin treatment, alone or in combination with lifestyle modification, has been helpful to ameliorate metabolic syndrome risk factors among women with PCOS [83, 84]. Specifically, obese women with PCOS, and those with features of the metabolic syndrome at presentation, appear to particularly benefit from metformin treatment in terms of higher HDL cholesterol, lower diastolic blood pressure, and lower BMI [84]. In the future, genetic polymorphisms may categorize women as potential responders and non-responders to metformin treatment [84, 85]. The magnitude of weight loss, rather than the specific agent or modality for lifestyle intervention, appears to be more important for metabolic improvement [86, 87].

Anovulatory infertility

With regard to enabling ovulation induction, metformin in addition to clomiphene citrate (CC) and aromatase inhibitors (AIs), provides distinct clinical opportunities and challenges. The advantages of CC as the first-line option for ovulation induction [88] include extensive, long-term experience with its use, low cost, and efficacy. Yet, its mixed agonist/antagonist profile, together with an increased potential for multiple births and their sequelae, encourage consideration of other options [89]. Laparoscopic ovarian drilling (LOD) is considered a second-line treatment option for PCOS women who are CC resistant, and appears equally effective as gonadotropins, but with fewer multiple pregnancies [32]. LOD in PCOS women with less pronounced hyperandrogenism and insulin resistance improves subsequent ovulatory responses to CC in about one-third of cases [90].

One alternative to CC involves using the AIs, letrozole and anastrozole, which decrease estrogen concentrations without affecting estrogen receptor action [91]. The efficient estrogen-lowering properties of AIs temporarily release the hypothalamus from the negative feedback effect of estrogen inducing an increased discharge of FSH. Although the end result of an increased discharge of FSH is common to both AIs and CC, AIs have no direct effect on estrogen receptors conferring several potential advantages for ovulation induction including: (1) no deleterious effect on cervical mucus or endometrium; (2) the negative feedback mechanism remains intact enabling regulation of the FSH discharge when estrogen is produced, thus reducing the prevalence of multiple follicle development, and consequently, of multiple pregnancies when compared to CC;

and (3) shorter half-life. Available data indicate that letrozole is as effective or more effective than CC, with fewer multiple pregnancies [91, 92] in terms of ovulation rate and pregnancy [93]. Inexplicably, letrozole remains off-label for induction of ovulation in most countries, despite clear evidence that it produces fewer congenital abnormalities than CC [94].

Metformin has multiple direct and indirect actions that benefit women with PCOS. Its effects on ovulation are likely to depend on several factors, such as the degree of insulin resistance and prevailing hyperinsulinemia [95], obesity [96], duration of treatment, influence of cytokines on insulin secretion, and direct action at the level of the ovary [97]. Although metformin should not be considered as a pro-ovulatory drug, short-term use may restore ovulation in ~20 % of anovulatory women with PCOS. In PCOS patients with anovulatory infertility who have not been previously treated, the administration of metformin plus CC is no more effective than CC alone [98, 99]. Treatment with metformin, however, may be helpful for clomiphene-resistant, non-obese women [32, 100]. Nevertheless, questions regarding its mechanism(s) of action, treatment protocol, and efficacy in ovulation induction remain to be addressed [101]. Randomized controlled trials are necessary to establish optimal ovulation induction treatment.

Hirsutism

Androgens affect several functions of human skin, such as sebaceous gland growth and differentiation, hair growth, epidermal barrier homeostasis, and wound healing [102]. Not surprisingly, therefore, PCOS is one of the most common diagnoses associated with hirsutism (excessive hair growth in androgen-dependent areas). Hirsutism, quantified by a modified Ferriman–Gallway scoring system, is attributed to increases in circulating androgen concentrations, sensitivity of the pilo-sebaceous unit to androgens, or a combination of these. A thorough medical history and physical examination is necessary, however, to discriminate hirsutism from generalized excessive hair growth (hypertrichosis). The skin and the pilo-sebaceous unit express steroid hormone receptors and are capable of synthesizing several hormones, including androgens [31]. Over the past decade, steroid hormones, phospholipid hormones, and retinoids have all been shown to play pivotal roles in the development of pilo-sebaceous units, the lipogenesis of sebaceous glands, and hair cycling [103]. Common "cutaneous hyperandrogenism" skin disorders such as acne, androgenetic alopecia and seborrhea involve overexpression of androgen biosynthetic enzymes and hyper-responsiveness of androgen receptors [104], as the most patients exhibit normal circulating androgen levels [102, 105].

Hirsutism is not only a cosmetic problem, but can also diminish self-esteem while engaging anxiety and depression [106]. A multidisciplinary and individualized therapeutic approach is thus required [107]. Lifestyle changes and cosmetic measures are first-line therapeutic modalities. Pharmacological treatment includes topical eflornithine, oral contraceptive pills, anti-androgens, and insulin sensitizers [108]. Topical eflornithine may be used in women suffering from facial hirsutism as a single agent or as an adjuvant to medical treatment. Oral contraceptive pills are recommended for women with mild hirsutism or may be added to an antiandrogen drug as an adjuvant agent to reduce the hirsutism score and to prevent pregnancy. Oral contraceptives decrease ovarian androgen production, increase sex hormone binding globulin concentration, and decrease free T-concentrations [109]. The specific progestin used in an oral contraceptive influences the extent of the anti-androgenic action. Despite recent concerns about clustering of cardiovascular risk factors among women with PCOS, no unequivocal data showing adverse cardiometabolic outcomes with the use of oral contraceptives have been reported. Adjunctive anti-androgens include spironolactone, cyproterone acetate, finasteride and flutamide. Metformin is not particularly useful in treating hirsutism. Overall, systemic therapies reduce hair growth in <50 % of patients, thus hirsute women frequently require additional cosmetic measures [110].

Androgenic alopecia is another manifestation of androgen excess in which the pattern of hair loss differs between women and men. Women tend to show generalized thinning whereas men lose hair in distinct regions. Treatment includes reduction of DHT synthesis, androgen receptor blockade and attenuation of steroidogenesis [111]. Both hirsutism and androgenic alopecia would benefit from the development of specific inhibitors to 17,20 lyase, a key step in androgen biosynthesis beyond cortisol production, that would provide relief for androgen overproduction without diminishing endogenous glucocorticoid release [112].

Conclusions

Studies into the pathogenesis of PCOS identify androgenic, glycemic, and inflammatory contributions that implicate disordered TGF-β signaling and suggest mechanistic commonality in affected male kin. Novel approaches add and diversify the therapeutic options available to treat infertility in women with PCOS. PCOS signs and symptoms, including metabolic dysfunction and hirsutism, are ameliorated by lifestyle and/or therapeutic intervention. Enabling engaged and interactive transition from adolescent to adult healthcare in CAH patients provides insight

for care transition in PCOS. AEPCOS, within a brief period of 8 years, has encouraged an integrated understanding of androgen excess and PCOS through international conferences supporting educational discussion of major advances in the field, including those summarized herein.

Conflict of interest The authors have no conflicts of interest.

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16 Polycystic Ovary Syndrome

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Learning Objectives

1. Describe criteria used for the diagnosis of polycystic ovary syndrome (PCOS).
2. Evaluate clinical manifestations and long-term risks associated with PCOS.
3. Explain the pathophysiologic abnormalities in PCOS involving gonadotropin secretion, androgen production, and insulin resistance with hyperinsulinemia.
4. Compare and contrast medications used for the treatment of PCOS, including oral contraceptives, insulin sensitizers, antiandrogens, and ovulation-induction agents.
5. Formulate a comprehensive clinical plan including nonpharmacologic and pharmacologic therapy for a patient with PCOS.

Polycystic ovary syndrome (PCOS) affects approximately 6% to 8% (or 1 in 15) women of reproductive age, making it the most common endocrine abnormality and the leading cause of anovulatory **infertility** for this age group.[1] The clinical presentation will vary among individual women and can make management intriguing, complex, and challenging. Women with PCOS may have poor self-image because of hirsutism, acne (which can cause scarring), or obesity despite rigorous diet and exercise plans. The syndrome can also have major impact throughout life on the reproductive, metabolic, and cardiovascular health of affected women. Healthcare providers are well aligned to interact with this patient population by providing assessment and assisting in the decision-making process for appropriate treatment.

This syndrome was first described in 1935 by Stein and Leventhal when they reported infertility and amenorrhea in seven women with enlarged cystic ovaries.[2] Stein later added excessive male-patterned hair growth and obesity to the description.[3] Although it has been called Stein-Leventhal syndrome, polycystic ovary, polycystic ovarian disease, hyperandrogenic chronic anovulatory syndrome, and functional ovarian **hyperandrogenism**, the name *polycystic ovary syndrome* has been widely accepted to describe the heterogeneous nature of this disorder.

Clinical Presentation

Women with PCOS typically have a clinical presentation that involves hyperandrogenism, menstrual disturbances, and possible obesity. Common clinical signs of hyperandrogenism include hirsutism, acne, and alopecia. Hirsutism is the most common of these characteristics and occurs in 60% to 70% of women with PCOS.[4] It is defined as an excess of thickly pigmented body hair in a male distribution and commonly found on the upper lip, around the chin, on lower abdomen, around the nipples, on the inner aspects of the thighs, and on the lower back. Acne affects 15% to 25% of women with PCOS, but this incidence may not be different from the general population. Alopecia presents as scalp hair loss in the crown and vertex areas and occurs in approximately 5% of the PCOS population.[5]

Ovulatory dysfunction in PCOS is typically described as **oligoovulation** or **anovulation**, which means the patient has irregular menstrual cycles (eight or fewer per year). Overall, 60% to 85% of patients with PCOS and oligoovulation have menstrual dysfunction, usually oligomenorrhea or amenorrhea.[1] These menstrual disturbances usually begin in the peripubertal years. A laboratory finding that may provide evidence for irregular ovulation and menses is an increased luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio greater than 2 or 3; however, LH and FSH levels

CASE PRESENTATION

Patient Case: Part 1

J.C. is a 24-year-old obese woman who presents to clinic today for a co-consult for suspected PCOS with a physician and clinical pharmacist. She has mild hair growth above her upper lip, mild acne, and a history of irregular menstrual periods. Her irregular periods were not bothersome until the last 2 years when she and her husband have tried to get pregnant. She is interested in learning more about why they may be having difficulty and what treatments are available to help her become pregnant. Her husband has been checked, and he has a normal semen analysis.

HPI: Since age 12, J.C. has had six to seven periods per year that occur every 30 to 90 days. When she does have a period, she considers them to be normal, without pain or excessive bleeding. She states that the hair on her upper lip has always been bothersome, but she gets it waxed routinely. She struggles with her obesity because she has been unable to lose weight despite walking vigorously for 20 minutes 5 days/week over the past 6 months.

PMH: Occasional headaches when stressed.

Family history: Mother—diabetes, hypertension; father—diabetes, hypertension, dyslipidemia.

Social history: No tobacco, occasional alcohol, walks 20 min five days/week.

Medications: Benzoyl peroxide 5% topical twice daily; acetaminophen 1,000 mg as needed for headaches; multivitamin daily.

Allergies: No known drug allergies.

Vitals: 5′7″, 195 lb, body mass index (BMI) 30.5, blood pressure 114/80, heart rate 70, temperature 98.6°F, respiration rate 18.

Physical exam: Normal, with the exception of noted excessive facial hair, mild acne, and acanthosis nigricans.

Laboratory values: Pertinent findings include fasting glucose 102 mg/dL, total cholesterol 236, low-density lipoprotein 150 mg/dL, high-density lipoprotein 40 mg/dL, triglyceride 210 mg/dL, serum creatinine 0.9 mg/dL.

fluctuate throughout the menstrual cycle so this measurement may be inaccurate and is not considered diagnostic for PCOS.[4]

Obesity occurs in approximately 30% to 60% of women with PCOS.[1] A pattern of central or abdominal obesity is typically seen, which is a risk factor for many obesity-related health risks (e.g., diabetes, heart disease), worsens other clinical features of PCOS (e.g., anovulation, hyperandrogenism), and suggests that insulin resistance may be present.[6] Therefore, lifestyle modification with appropriate diet and exercise is a foundation of treatment for many patients with PCOS.

Pathophysiology

The pathophysiology of PCOS is complex and important to understand for appropriate treatment decisions. The primary defect in PCOS is unknown, but there appear to be three possible mechanisms acting alone or synergistically to create the presentation seen with PCOS. These mechanisms include inappropriate gonadotropin secretion, insulin resistance with hyperinsulinemia, and excessive androgen production. These mechanisms are closely integrated, as seen in Figure 16-1.

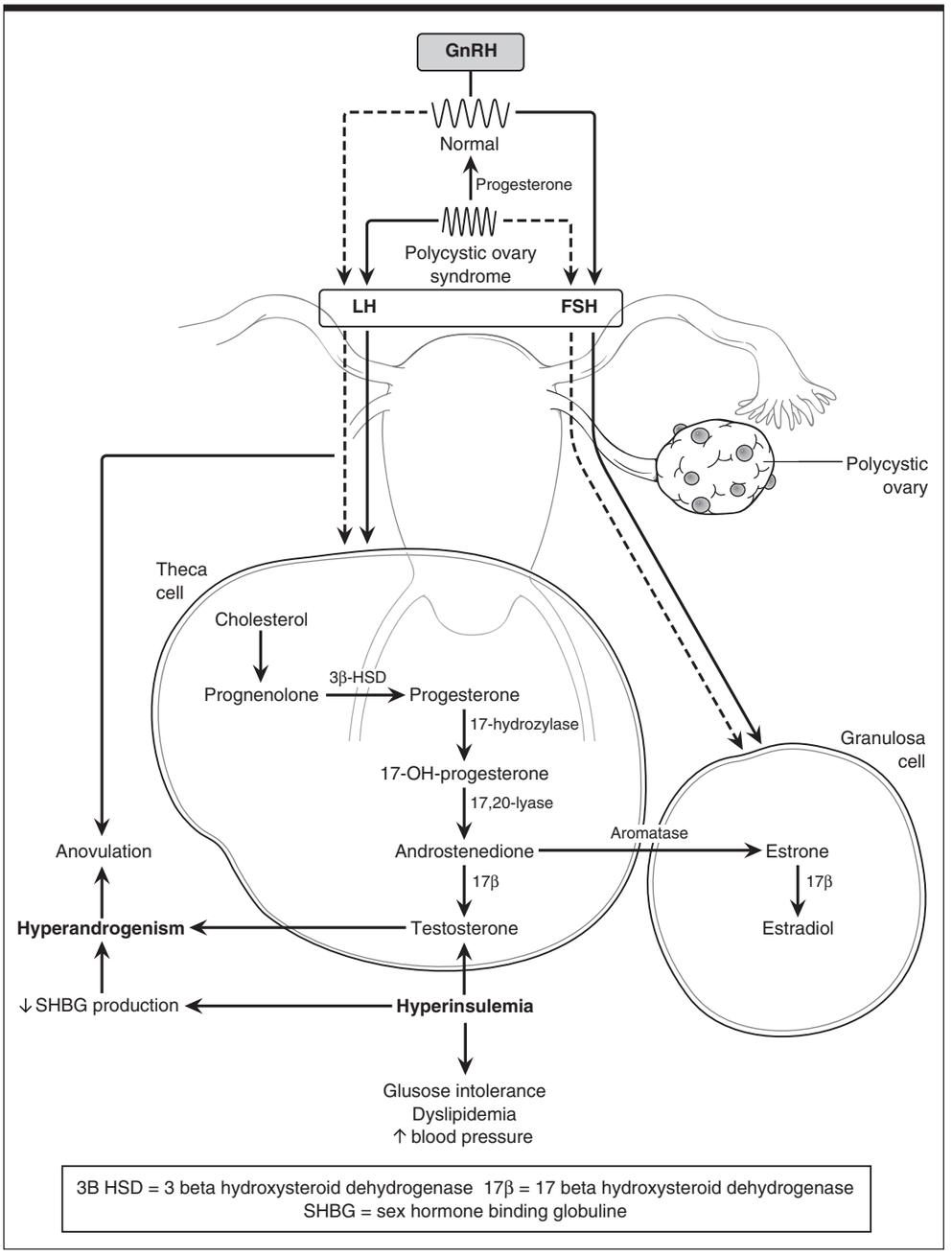
A genetic basis for PCOS has been postulated, but its mode of transmission is unclear.[7] Hypotheses have ranged from an autosomal dominant model to a polygenic model with genetic-environmental interactions. More than 50 candidate genes have been proposed as potential contributors to PCOS.

GONADOTROPIN SECRETION

In normal menstrual cycles, the hypothalamus produces a neurohormone called gonadotropin-releasing hormone (GnRH), which regulates the release of FSH and LH from the anterior pituitary in a pulsatile fashion every 60–90 minutes. FSH stimulates growth of ovarian follicles, and LH is critical for ovulation and sex steroid production. Typically, when FSH concentrations rise, a small group of follicles develop, then a dominant follicle emerges in the follicular phase. Estrogen concentrations also rise during this time and create an LH surge to cause ovulation. In the luteal phase that follows, progesterone is synthesized and secreted, which is necessary for successful implantation of an embryo. If pregnancy does not occur, progesterone concentrations decrease and menstruation occurs.

In PCOS, there is an increased frequency of GnRH stimulation, which results in an increase in LH pulse frequency and amplitude; FSH secretion remains normal. The development of a dominant follicle does not occur because LH secretion occurs before a dominant follicle can emerge. Therefore, a woman is left with several immature follicles and will not

Figure 16-1. Pathophysiology of PCOS.



typically ovulate. It is unclear whether the abnormal pulse frequency of GnRH is an intrinsic problem in the GnRH pulse generator or a result of relatively low progesterone concentrations from infrequent ovulation.[8] A woman with this abnormality does not enter the luteal phase of her menstrual cycle, leaving estrogen unopposed. This can create endometrial hyperplasia and increase risk for endometrial cancer. Increased LH stimulation also leads to increased steroidogenesis in the ovary, leading to excess androgen production.

INSULIN RESISTANCE

In addition to gonadotropin secretion, another mechanism for increased androgen production in the ovary is through insulin resistance. Insulin resistance with compensatory hyperinsulinemia occurs in 50% to 70% of women with PCOS.[1] Insulin resistance can occur in both obese and nonobese women and is associated with reproductive and metabolic abnormalities in PCOS. Although the nature of insulin resistance in PCOS is currently unclear, defects that have been implicated include insulin receptor or postreceptor signal transduction, altered adipocyte lipolysis, decreased GLUT-4 glucose transporters in adipocytes, and impaired release of a D-chiro-inositol mediator.[9-18] Regardless of the mechanism, it appears that insulin resistance in PCOS is a selective, tissue-specific process whereby insulin sensitivity is increased in the ovarian androgenic pathway (causing hyperandrogenism). However, in other tissues involved in carbohydrate metabolism, specifically in the fat and muscle, there is tissue resistance to insulin. Hyperinsulinemia results through the compensatory increase in insulin secretion to maintain euglycemia secondary to insulin resistance.

Insulin acts directly and indirectly in PCOS. In the ovary, insulin alone or with LH increases androgen production in theca cells. In the liver, insulin inhibits synthesis of sex-hormone binding globulin (SHBG), which binds to testosterone, causing an increase in the free fraction of androgens available for biologic activity. Therefore, hyperinsulinemia is a major contributor to hyperandrogenism and **hyperandrogenemia** in PCOS. Treatments targeted to improve insulin resistance in women with PCOS have shown improvements in ovulatory function, hirsutism, androgen levels, and metabolic profiles.[19,20] Indirectly, insulin may enhance the amplitude of LH pulses, advancing further the gonadotropin secretion defect in PCOS.[21]

EXCESS ANDROGEN PRODUCTION

Androgen production occurs in the theca cell of the ovary to facilitate follicular growth while estradiol synthesis occurs in the granulosa cell. In women with PCOS, hypersecretion of LH and insulin increase the production of androgens in the theca cell causing abnormal sex steroid synthesis, hyperandrogenism, and hyperandrogenemia. The dysregulation in steroid synthesis and metabolism is believed to primarily result from a dysfunction of the cytochrome P450c17 enzyme in the ovaries, an enzyme with 17-hydroxylase and 17,20-lyase activities that are required to form androstenedione.[8,22] Androstenedione is then converted to testosterone or is aromatized by the aromatase enzyme to form estrone. Theca cells in women with PCOS are more efficient at the conversion to testosterone than normal theca cells.[23] A similar steroid pathway simultaneously occurs in the adrenal cortex, and when hyperandrogenism or hyperinsulinemic states exist, androgen production is exacerbated.

Elevated androgen levels are seen in approximately 60% to 80% of women with PCOS, evidenced mostly as increased free testosterone concentrations.[4] However, assays for testosterone tend to be highly variable and inaccurate, so measurement of androgen concentrations, if used, should be used with other additional criteria for diagnosis. Clinical assessment will be the primary tool for assessment of excess androgen.

Long-term Complications

IMPAIRED GLUCOSE TOLERANCE AND DIABETES

Studies have shown that women with PCOS have a higher prevalence of impaired glucose tolerance (IGT), diabetes, and insulin resistance compared with women without the syndrome.[24] A family history further increases the risk of these conditions. In a study of 254 women with PCOS, 38.6% were found to have either impaired glucose tolerance or undiagnosed diabetes.[25] The prevalence of IGT and diabetes was significantly higher in both obese and nonobese (BMI <27 km/m^2) women with PCOS compared with those without PCOS. The most clinically important predictors of glucose intolerance were waist-to-hip ratio and BMI. Additionally, women with PCOS who have IGT appear to progress to type 2 diabetes at higher rates than the general population.[24] Therefore, screening and diagnosis of these conditions are important.

Glucose tolerance should be assessed in all women with PCOS using a fasting and 2-hour oral (75 g) glucose tolerance test (OGTT).[24] The American Association of Clinical Endocrinologists suggests routine screening for diabetes with an OGTT that should be performed for all women with PCOS by age 30 years.[26] The Androgen Excess and PCOS Society recommends routine screening for IGT with an OGTT in all women with PCOS, and screenings should be repeated at least every 2 years.[27] The American Diabetes Association or World Health Organization criteria should be followed to appropriately diagnose IGT or diabetes. Insulin concentrations are typically not obtained in clinical settings because insulin assays

Therapeutic Challenge:

Who should receive routine screening with OGTT, and how often should it be performed?

are not standardized and determination of insulin levels is not helpful in the management of PCOS.

Despite endorsement from professional societies for screening with an OGTT in women with PCOS, there is still considerate debate about whether all women with PCOS should be screened or whether the test should be restricted to PCOS women with other metabolic abnormalities, such as obesity. In addition, the optimal frequency of repeat screenings needs to be further evaluated.

METABOLIC SYNDROME AND CARDIOVASCULAR RISK

Approximately 30% to 50% of women with PCOS have the metabolic syndrome using the National Cholesterol Education Panel-Adult Treatment Panel III (NCEP-ATP III) criteria.[28-31] The rates in women with PCOS are significantly higher compared with the general U.S. population (45% vs. 6% ages 20–29 years, 53% vs. 14% ages 30–39 years) and are independent of body weight.[28] It is believed that insulin resistance is the primary contributing factor to metabolic syndrome in women with PCOS.[32] Insulin resistance in the metabolic syndrome has been associated with a twofold increased risk of cardiovascular disease and fivefold increased risk of type 2 diabetes.[33] Characteristics of the metabolic syndrome seen in women with PCOS include low high-density lipoprotein cholesterol (HDL-C) (68%), increased BMI and waist circumference (67%), high blood pressure (45%), hypertriglyceridemia (35%), and elevated fasting glucose (4%).[29] Elevated fasting insulin concentrations, obesity, and a family history of diabetes also appear to confer a higher risk of metabolic syndrome in women with PCOS.[31]

Women with PCOS have a higher prevalence of cardiovascular risk factors, including hypertension, dyslipidemia, and surrogate markers for early atherosclerosis (e.g., increased C-reactive protein concentrations) compared with women without PCOS.[32] Postmenopausal women with PCOS have a twofold increased risk of hypertension compared with age-matched controls.[34] Dyslipidemia in women with PCOS typically presents as decreased HDL-C, elevated triglycerides, elevated low-density lipoprotein cholesterol (LDL-C), and higher LDL-to-HDL ratios.[35] Women with PCOS may have more atherogenic, small, dense LDL-C compared with controls, and this substantially increases cardiovascular risk.[36] Surrogate markers for early atherosclerosis and cardiovascular disease, impaired endothelial dysfunction, and other markers of cardiovascular risk (e.g., coronary artery calcifications, carotid intima-media thickness) are also elevated in women with PCOS.[32] It appears that increased cardiovascular risk exists, with a recent study demonstrating an approximate twofold increase in cardiovascular events in postmenopausal women with a history of PCOS compared with those without PCOS.[37] Cardiovascular studies have not demonstrated an increase in overall mortality, but the methodology of these studies has been debated.

OBSTRUCTIVE SLEEP APNEA

Obstructive sleep apnea is cessation of breathing that occurs during sleep. Patients may not be aware they have symptoms of sleep apnea, such as snoring and a gasping or snorting when breathing resumes. Obstructive sleep apnea causes sleep disruption and daytime fatigue. The prevalence of obstructive sleep apnea in PCOS is higher than expected and cannot be explained by obesity alone.[38-40] The strongest predictor of sleep apnea appears to be insulin resistance, more so than age, BMI, or the circulating testosterone concentration.[38]

ENDOMETRIAL HYPERPLASIA AND CANCER

Anovulation in PCOS causes prolonged exposure of estrogen to the endometrium without the appropriate exposure to progesterone. Therefore, PCOS is recognized as a risk factor for endometrial hyperplasia. It is unknown if this translates into an increased risk for endometrial cancer because it is a rare occurrence in women of this age (4% of all cases of women <40 years).[41] It is considered prudent management to induce artificial withdrawal bleeds at least every 3 months in women with PCOS and amenorrhea or oligomenorrhea to prevent endometrial hyperplasia.[42] If this is not done, ultrasound scans can also be used to measure endometrial thickness and morphology every 6–12 months.

Diagnosis of PCOS

The diagnosis of PCOS can be difficult because presenting signs and symptoms vary among women. Additionally, precise and uniform criteria for diagnosis have not been established. Major diagnostic

Table 16-1. Criteria for Defining Polycystic Ovary Syndrome

	Proposed Definitions		
Clinical Criteria	**NIH 1990**[43]	**ESHRE/ASRM (Rotterdam 2003)**[44]	**Androgen Excess and PCOS Society**[4]
1. Hyperandrogenism or hyperandrogenemia	X	X	X
2. Oligo-ovulation or anovulation	X	X	X
3. Polycystic ovaries (by ultrasound)		X	
4. Exclusion of related disorders	X	X	X
Diagnostic criteria	**1, 2, and 4**	**2 of first 3 criteria and 4**	**1, 2, and 4**

Source: NIH, National Institutes of Health; ESHRE, European Society for Human Reproduction and Embryology; ASRM, American Society for Reproductive Medicine.

criteria for PCOS have been proposed by three different organizations (Table 16-1).

The initial criteria were developed in 1990 during an expert conference sponsored by the National Institutes of Health (NIH) and National Institute of Child Health and Human Development (NICHD). They concluded that the major criteria for PCOS should include (in order of importance) 1) hyperandrogenism (clinical signs of hyperandrogenism, such as hirsutism) and/or hyperandrogenemia (biochemical signs of hyperandrogenism, such as elevated testosterone levels); 2) oligo-ovulation (infrequent or irregular ovulation with fewer than nine menses per year); and 3) exclusion of other known disorders, such as hyperprolactinemia, thyroid abnormalities, and congenital adrenal hyperplasia.[43] Another set of diagnostic criteria for PCOS was proposed at an expert conference in Rotterdam sponsored by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) in 2003.[44] They concluded that the presence of two of the following three features after exclusion of related disorders confirmed the diagnosis of PCOS: 1) oligo-ovulation or anovulation, 2) clinical and/or biochemical signs of hyperandrogenism, or 3) polycystic ovaries (as evidenced by 12 or more small follicles in one ovary on ultrasound examination). Great controversy exists about whether women fitting this new Rotterdam definition are metabolically similar to those identified using the 1990 NICHD guidelines. In addition, most U.S. centers lack the radiologic expertise to satisfactorily assess the ovaries on ultrasound in the highly rigorous manner required by the guidelines. The third criteria were defined by a task force of the Androgen Excess and PCOS Society in 2006.[4] They concluded that hyperandrogenism and/or hyperandrogenemia (highest priority) and ovarian dysfunction as evidenced by oligo-ovulation and/or polycystic ovaries must be present after exclusion of other androgen excess or related disorders. There are advantages and disadvantages to each of the criteria proposed, but it is clear that the definition of and diagnostic criteria for PCOS will continue to evolve.

Before a diagnosis of PCOS can be made, a detailed history, physical, and laboratory testing have to be performed to exclude other related disorders. A history suggestive of PCOS includes chronic anovulation with mild to moderate clinical signs of hyperandrogenism (e.g., hirsutism, hair loss, acne), usually with onset at puberty or after weight gain. Hypothalamic anovulation can be ruled out by a detailed history of lifestyle and psychosocial problems, whereas premature ovarian failure can be ruled out by menstrual history and FSH concentrations. In addition, very high testosterone and dehydroepiandrosterone sulfate concentrations, when associated with virilizing clinical signs, may suggest an androgen-secreting neoplasm of ovarian or adrenal origins, respectively. Other laboratory assessments should

include prolactin, thyroid-stimulating hormone, and 17-hydroxyprogesterone concentrations to rule out hyperprolactinemia, hypothyroidism, and congenital adrenal hyperplasia, respectively. PCOS is primarily diagnosed by clinical assessment; however, a transvaginal ultrasound can be performed to determine if polycystic ovaries are present, defined as more than eight follicles per ovary that are <10 mm (usually 2–8 mm) in diameter or ovarian volume >10 mL.

Guidelines and Position Statements

Guidelines and position statements exist regarding diagnosis of PCOS (discussed above), but no guideline or position statement sufficiently addresses treatment or other aspects of PCOS.

Treatment Goals

The primary goals for PCOS are to maintain a normal endometrium, block the actions of androgens on target tissues, reduce insulin resistance and hyperinsulinemia, reduce weight, and prevent long-term complications. Other goals of treatment in patients with PCOS may include prevention of pregnancy, correcting anovulation or oligo-ovulation, and/or improving fertility.

Treatment goals should encompass both long- and short-term targets because response to nonpharmacologic and pharmacologic therapy is slow, often requiring 3–9 months. Setting long-term goals can minimize the risk for future complications, and specifying short-term goals can improve motivation and adherence to therapy.

Nonpharmacologic Treatment

WEIGHT LOSS

Weight-reduction programs designed for a modest weight loss (5% to 10%) with the incorporation of fitness are effective in improving ovulation and metabolic disease in women with PCOS. A minimum 5% weight loss has consistently demonstrated restoration of menstrual cyclicity and ovulation in overweight and obese women with PCOS.[8,45,46] Weight loss improves pregnancy rates and reduces miscarriage rates in women with PCOS.[47] When lifestyle modification is implemented, free testosterone concentrations decrease, but the effect on acne and hirsutism are not often reported.[6] Obesity in PCOS is associated with a higher risk of developing endometrial cancer, but there is very limited evidence to determine the impact of weight loss on the incidence of endometrial cancer.[8] Studies of weight loss in women without PCOS indicate a 25% to 50% reduced risk of endometrial cancer, so it is logical that addressing weight reduction in women with PCOS may lower the risk as well.[48,49] Studies specifically evaluating cardiovascular improvements with weight loss in women with PCOS are limited, but improvements in dyslipidemia and insulin sensitivity have been noted.

DIET

Although there is not one specific diet proven to be ideal for women with PCOS, a diet low in saturated fat and high in fiber from mostly low-glycemic-index-carbohydrate foods may be suitable and is recommended.[6,50] Low-glycemic-index foods include bran cereals, mixed grain breads, broccoli, peppers, lentils, and soy. High-glycemic-index foods (i.e., those that should be minimized) include white rice and bread, potatoes, chips, and foods containing simple sugars (e.g., juice). In women with PCOS, oral glucose intake caused larger fluctuations in plasma glucose and increased hyperinsulinemia; protein was found to be a preferred nutrient over glucose.[51] Diet should be individualized in women with PCOS to promote adherence and achieve specific goals.

EXERCISE

Exercise is a key component in the attainment and maintenance of weight loss. Exercise with muscle strengthening improves insulin sensitivity, a benefit for women with PCOS.[6] The American Heart Association recommends 1 hour daily of physical activity for weight reduction and 30 minutes daily for all adults. The American Diabetes Association recommends at least 150 minutes per week of moderate to vigorous activity spread over at least 3 days for individuals with IGT and 1 hour daily of exercise for long-term weight loss.

Diet and exercise are efficient, cost-effective, and safe ways to produce weight loss and improve the endocrine and metabolic parameters of PCOS. Weight reduction should be considered first-line therapy in all overweight or obese women with PCOS. It is important to note that even in controlled settings with motivated participants in clinical trials, attrition rates of lifestyle modification have been high, with 26% to

39% discontinuation in women with PCOS over 1–4 months compared with 8% to 9% discontinuation in patients without PCOS over 4 months.[46,52-54] It should be stressed that frequent, continued follow-up is necessary for sustained weight loss.

Pharmacologic Therapy

Successful treatment of PCOS requires an understanding of presenting symptoms and patient-specific goals. For example, sexually active women who are hirsute and do not desire pregnancy may benefit from hormonal contraception. Women with glucose impairment or diabetes may benefit from insulin sensitizer therapy. Women wanting to improve fertility may need ovulation induction agents. Fortunately, several different pharmacologic options are available for women with PCOS (Table 16-2).

ORAL CONTRACEPTIVES

A combined oral contraceptive (COC) containing an estrogen and progestin is the treatment of choice for women seeking menstrual cyclicity, relief of acne and hirsutism, and pregnancy prevention. The estrogen component of the COC suppresses LH, resulting in a reduction of androgen production as well as increased hepatic production of SHBG, which reduces free testosterone. The progestin components in COCs possess variable androgenic effects, so COC selection is important.

COC therapy in PCOS should be initiated with a formulation that contains a low or very low dose of estrogen (≤35 mcg ethinyl estradiol) and a low androgen or antiandrogen progestin. Most COCs have low or very low estrogen doses. Desogestrel and norgestimate are low androgen progestins, and drospirenone is an antiandrogen. These COCs are

Table 16-2. Selected Treatment Options for Polycystic Ovary Syndrome

Drug Class (Example)	Purpose of Therapy	Mechanism of Action	Effective Dosage	Select Side Effects	Pregnancy Category
Combined oral contraceptive (estrogen and progestin)	Menstrual cyclicity, hirsutism, acne	Suppresses LH (and FSH) and thus ovarian androgen	1 tablet orally daily for 21 (or 24) days, then 7-day (or 4-day) pill-free interval	Breast tenderness, breakthrough bleeding, mood swings, libido changes	X
Progestins (medroxyprogesterone)	Menstrual cyclicity	Creates withdrawal bleeding by transforming proliferative endometrium into secretory endometrium	5–10 mg orally daily for 10–14 days at least every 3 mo	Breakthrough bleeding, spotting, mood swings	X
Biguanide (metformin)	Menstrual cyclicity, ovulation induction, hirsutism, acne, insulin lowering	Decreases hepatic glucose production, secondarily reducing insulin levels; may have direct effects on steroidogenesis	500 mg orally 3 times daily (up to 2,550 mg/day)	Gastrointestinal problems, diarrhea, abdominal pain, weight loss	B
Thiazolidinediones (pioglitazone or rosiglitazone)	Menstrual cyclicity, ovulation induction, hirsutism, acne, insulin lowering	Improve insulin sensitivity at target-tissue level (muscle, adipocyte); may have direct effects on steroidogenesis	Pioglitazone: 45 mg orally daily Rosiglitazone: 4 mg orally twice daily	Edema, headache, fatigue, weight gain	C
Antiandrogen (spironolactone)	Hirsutism, acne	Inhibit androgens from binding to androgen receptor	50–100 mg orally twice daily	Hyperkalemia, polymenorrhea, headache, fatigue	C

(*Continued on next page*)

Drug Class (Example)	Purpose of Therapy	Mechanism of Action	Effective Dosage	Select Side Effects	Pregnancy Category
Antiestrogen (clomiphene citrate)	Ovulation induction	Increases GnRH secretion, which induces rise in FSH and LH	50 mg orally daily for 5 days; may increase up to 150 mg in subsequent cycles; not recommended to exceed six total cycles	Vasomotor symptoms, gastrointestinal problems	X
Antiprotozoal (eflornithine)	Hirsutism	Irreversible inhibitor of ornithine decarboxylase, which catalyzes the conversion of ornithine to polyamines necessary for cell growth and differentiation of the hair follicle	13.9% cream, topical application to facial area twice daily	Mild irritation and folliculitis	C

LH = luteinizing hormone; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone.

especially beneficial in women with hirsutism and acne.

A COC can be taken in various ways for menstrual cyclicity. For monthly cycles, a patient can take a 21/7 regimen (21 days active pill, 7 days inactive pill) or a 24/4 regimen (24 days active pill, 4 days inactive pill). Although not specifically evaluated in patients with PCOS, monophasic COCs (those containing the same amounts of estrogen and progestin throughout the cycle) can be used in an extended manner. This means that the active pills are taken daily on a continuous basis with no inactive pills, and women will not have a menstrual cycle. Regardless of the COC selected, a long-term benefit is a 50% reduction in endometrial cancer risk, even up to two decades after discontinuation.[55-57]

The effects of COCs on insulin resistance, glucose tolerance, and lipids should be considered when choosing a progestin.[58,59] COCs should be used with caution in those who have insulin resistance, a high propensity to develop type 2 diabetes, or abnormal lipid profiles. Additionally, hyperkalemia has been noted in patients using COCs containing drospirenone and should be used cautiously in patients susceptible to increased potassium concentrations.

Therapeutic Challenge:

How might COCs interact with the metabolic manifestations in PCOS, and does their benefit outweigh their risk?

INSULIN SENSITIZERS

Insulin sensitizers reduce insulin levels and can ameliorate the consequences of hyperinsulinemia and hyperandrogenemia. Currently available insulin sensitizers include metformin, rosiglitazone, and pioglitazone. More efficacy data are available for metformin than the thiazolidinediones in patients with PCOS. In addition, most pregnancy outcome data in humans are reported for patients taking metformin and suggest that it is not teratogenic. For these reasons, metformin tends to be the preferred insulin sensitizer for women with PCOS.

Metformin

Metformin inhibits hepatic glucose output and reduces insulin concentrations and androgen production in the ovary. Metformin also appears to influence ovarian steroidogenesis directly.[60] Most studies demonstrate that metformin improves menstrual cyclicity, ovulation, and fertility in both obese and lean patients with PCOS.[61] In one study of nonobese women with PCOS, metformin was superior for ovulation induction compared with rosiglitazone.[62] Metformin has been considered in ovulation induction protocols and

may be very effective when used alone or with clomiphene citrate for ovulation.[63,64]

Metformin has been shown to improve pregnancy outcomes. In a retrospective study, women with PCOS who achieved pregnancy while receiving metformin and continued taking metformin throughout their pregnancy had lower rates of early pregnancy loss (EPL), as compared with women with PCOS who conceived but were never exposed to metformin.[65] Other evidence suggests that metformin may not need to be administered throughout pregnancy, but only during conception, to confer protection from EPL.[66] However, metformin use for prevention of EPL is controversial, as metformin did not result in lower rates of EPL in the largest prospective study to date.[67]

Current data indicate that metformin use is not teratogenic. Pregnancy and fetal outcomes of women with PCOS who took metformin throughout pregnancy have been described in several historical cohorts.[68-70] Metformin use during pregnancy was not associated with maternal lactic acidosis nor neonatal or maternal hypoglycemia.[69,70] In infants studied up to 18 months of life, metformin did not appear to affect birth weight, length, motor-social development, or growth.[70] In addition, metformin use throughout pregnancy may reduce the incidence of gestational diabetes in the mother.[70,71]

Data also indicate that insulin and free testosterone concentrations may be decreased 20% to 50% with metformin in women with PCOS.[72,73] Metformin may also reduce BMI, especially in obese patients.[61] However, its efficacy in established hirsutism may be limited, and hirsutism should not be the sole indication for using metformin.[61] In women with PCOS whose hirsutism has been improved by antiandrogen treatment, it may be feasible to use metformin for maintenance of clinical improvement.

The minimal effective dosage of metformin in PCOS is 500 mg 3 times daily. It should be titrated slowly to this dosage and then evaluated for efficacy. Dosages up to 2,000 mg daily or 2,550 mg daily may be necessary for individual circumstances. Typical transient, dose-related side effects include diarrhea, nausea, vomiting, and abdominal bloating, but do not require discontinuation of the drug. Serum creatinine should be evaluated at least annually and when clinically prudent (e.g., some cause for change in renal function) in women using metformin; it is contraindicated in women who have a serum creatinine >1.4 mg/dL.[74]

Therapeutic Challenge:

If metformin is used for ovulation induction and pregnancy occurs, should metformin be stopped, continued for the first trimester, or continued for the entire pregnancy?

Thiazolidinediones

Rosiglitazone and pioglitazone have been evaluated in women with PCOS, but relatively few studies have been published. Thiazolidinediones improve insulin action in the liver, skeletal muscle, and adipose tissue and appear to directly affect ovarian steroid synthesis.[75] Rosiglitazone and pioglitazone reduce insulin and androgen concentrations and have modest effects on hirsutism.[8] Rosiglitazone has demonstrated significantly better ovulation rates in women with PCOS compared with placebo, but not better than metformin.[62] Other abstracts have indicated that rosiglitazone may be beneficial for menstrual regularity, hyperandrogenism, and insulin sensitivity. Pioglitazone was as effective as metformin in small studies of women with PCOS and may be especially beneficial when used in combination with metformin for clinical and biochemical improvements.[76,77]

Recommended dosages for rosiglitazone and pioglitazone are up to 4 mg twice daily and up to 45 mg once daily, respectively. Adverse effects include edema, headache, fatigue, potential liver enzyme elevations, and weight gain. Liver enzymes should be measured before initiation of treatment and periodically thereafter. Rosiglitazone also appears to have a negative effect on the lipid profile, an unwanted parameter considering the potential long-term consequences of PCOS. Clinical experiences with the thiazolidinediones during conception and pregnancy have not yet been reported; they are pregnancy category C. Thus, this class of agents should be used with caution in women with PCOS not taking contraception.

AGENTS FOR HIRSUTISM

Antiandrogenic agents are potential therapeutic options for hirsutism and acne in women with PCOS. However, antiandrogens are not approved for female hirsutism or acne in the U.S. They are often used off label alone or in combination with COCs for moderate to severe hirsutism or acne. Spironolactone is an antiandrogen often used for hirsutism, but

drospirenone is also an antiandrogen found in COCs, is structurally related to spironolactone, and has been evaluated in the long-term treatment of hirsutism.[78] Spironolactone reduces hair growth in women with PCOS by 40% to 88% and may take 6–9 months for improvement.[79] The usual effective spironolactone dosage is 50–100 mg twice daily for 6–12 months. It is recommended that spironolactone be used with COCs to avoid teratogenicity and polymenorrhea (more frequent menses) and provide additional benefit for the signs and symptoms of PCOS.

Eflornithine hydrochloride cream 13.9% is an agent that has also been used for hirsutism in women with PCOS. It is an irreversible inhibitor of ornithine decarboxylase that helps to slow hair growth. Therefore, it reduces the amount of unwanted facial hair by slowing terminal hair growth, but does not remove the hair. It is applied twice daily to the affected areas of the face.

OVULATION INDUCTION AGENTS

Clomiphene Citrate

Clomiphene citrate is the ovulation induction agent of choice in women with PCOS. Ovulation induction occurs in 50% to 80% and conception occurs in 35% to 40% of women with PCOS using clomiphene citrate. Essentially, clomiphene citrate helps to correct the gonadotropin secretion abnormality in PCOS by providing an antiestrogenic effect on the hypothalamus. GnRH secretion is increased, which leads to increased LH and FSH production. The increased FSH concentrations cause appropriate follicle development and estrogen secretion, which produces a positive feedback on the hypothalamic-pituitary system to create an LH surge for ovulation.

The usual initial dosage of clomiphene citrate is 50 mg orally daily for 5 days, initiated on day 5 after the start of a spontaneous or progestin-induced menses. Ovulation is then confirmed through laboratory testing or ultrasound monitoring. If ovulation does not occur, experts recommend a dosage increase to 100 mg daily, and subsequently to 150 mg daily (for 5 days) in the next cycles. However, dosages >100 mg daily for 5 days are not recommended by the manufacturers.[80] A repeat cycle can occur as early as 30 days after the previous cycle as long as pregnancy has not occurred. If conception does not occur, most patients can attempt three to four cycles before considering another regimen. Long-term cyclic therapy is not recommended beyond a total of six cycles because of potential ovarian cancer risk. Most women respond to clomiphene citrate within three to four ovulatory cycles, but 5% to 10% demonstrate clomiphene resistance and need to consider other options.[47,80]

It had previously been suggested that the combination of clomiphene citrate plus metformin produced higher ovulation rates than either agent alone.[63] However, live-birth rates had not been evaluated until recently. In a randomized controlled study to determine live-birth rates in 626 women with PCOS taking metformin, clomiphene citrate, or both, as initial therapy for ovulation induction and pregnancy, the live-birth rate was 22.5% in the clomiphene citrate group, 7.2% in the metformin group, and 26.8% in the combination group ($p < 0.001$ for metformin vs. clomiphene citrate and combination therapy).[67] The authors concluded that clomiphene citrate was superior to metformin in achieving live birth rates, but it should be noted that multiple births occurred in 6% (3/50) of live births. This study suggests that the combination of metformin and clomiphene citrate is not superior to clomiphene alone as the initial therapy in women with PCOS seeking pregnancy.

Other Agents and Procedures

Other regimens for ovulation induction include metformin (alone or in combination with clomiphene citrate), dexamethasone (in combination with clomiphene citrate), aromatase inhibitors (e.g., letrozole, anastrozole), ovarian drilling, or controlled ovarian stimulation with gonadotropins.[81-84] In clomiphene-resistant patients, combination clomiphene citrate and metformin would be an appropriate next step.[80] If that did not prove successful, then the following alternatives could be tried: dexamethasone 0.25 mg at bedtime can be used in combination with clomiphene citrate, aromatase inhibitors, ovarian drilling, administration of gonadotropins, or in vitro fertilization.[80]

Monitoring and Follow-up

Women with PCOS should be monitored for clinical signs or symptoms at least annually and more frequently when changes in appearance, menstrual cycles, or medication occur. Several laboratory tests are needed initially to rule out other possible disorders, but all of these tests do not require routine monitoring once the diagnosis of PCOS has been made. Routine laboratory tests include an OGTT (at least every 2 years) and fasting lipid panel to detect any

endocrine or metabolic abnormalities. Appropriate follow-up for women with PCOS may include quality-of-life measures, laboratory monitoring when necessary (e.g., testosterone), and medication adherence monitoring.

Patient Education

Adherence to therapeutic regimens for PCOS can often be difficult, especially because the drug(s) can take weeks to months for improvements to occur. The emotional and physical factors of PCOS can also affect the ability of women to follow appropriate regimens. Treatment regimens can fail when women are not educated about the rationale for therapy (e.g., using an insulin sensitizer for PCOS) or the importance of adhering to therapy for an extended time. Women need to be counseled on side effects of medications and potential alternatives if they occur. Women should also be referred to appropriate healthcare providers when issues such as infertility arise.

CASE PRESENTATION

Patient Case: Part 2

Assessment: J.C. has PCOS as evidenced by irregular menstrual periods (oligomenorrhea) and clinical signs of hyperandrogenism (acne, facial hair). In addition, J.C. has impaired fasting glucose, and her glucose tolerance status should be further evaluated. J.C. is using benzoyl peroxide for acne, but her desire for fertility is not being addressed with current regimen.

Recommendation:

- Weight loss of at least 5% through diet and exercise.
- Clomiphene citrate 50 mg orally daily for 5 days, started on day 5 after a spontaneous or progestin-induced menses. Dosage may be increased to 100 mg daily if unresponsive.
- Perform OGTT to determine presence of IGT.

Rationale: Weight loss has been shown to have positive impact on ovulation. Both clomiphene citrate and metformin are viable options in this patient, but clomiphene citrate is preferred based on comparative clinical trial for live-birth rates. OGTT will provide information about the presence of IGT and potential risk for diabetes in the future.

Monitoring: Weight loss; ovulation; side effects of clomiphene citrate, including hot flashes, bloating, nausea, vomiting.

Patient education: Eat diet low in saturated fat and high in fiber from mostly low-glycemic-index carbohydrate foods. Perform physical activity 30–60 minutes daily (with physician approval and guidance). Goal weight loss is at least 5% of body weight (10 lb). For clomiphene citrate, multiple gestation is possible, therapy should not extend beyond four to six cycles, and potential side effects should be reviewed. She should return at least monthly for monitoring of her lifestyle compliance.

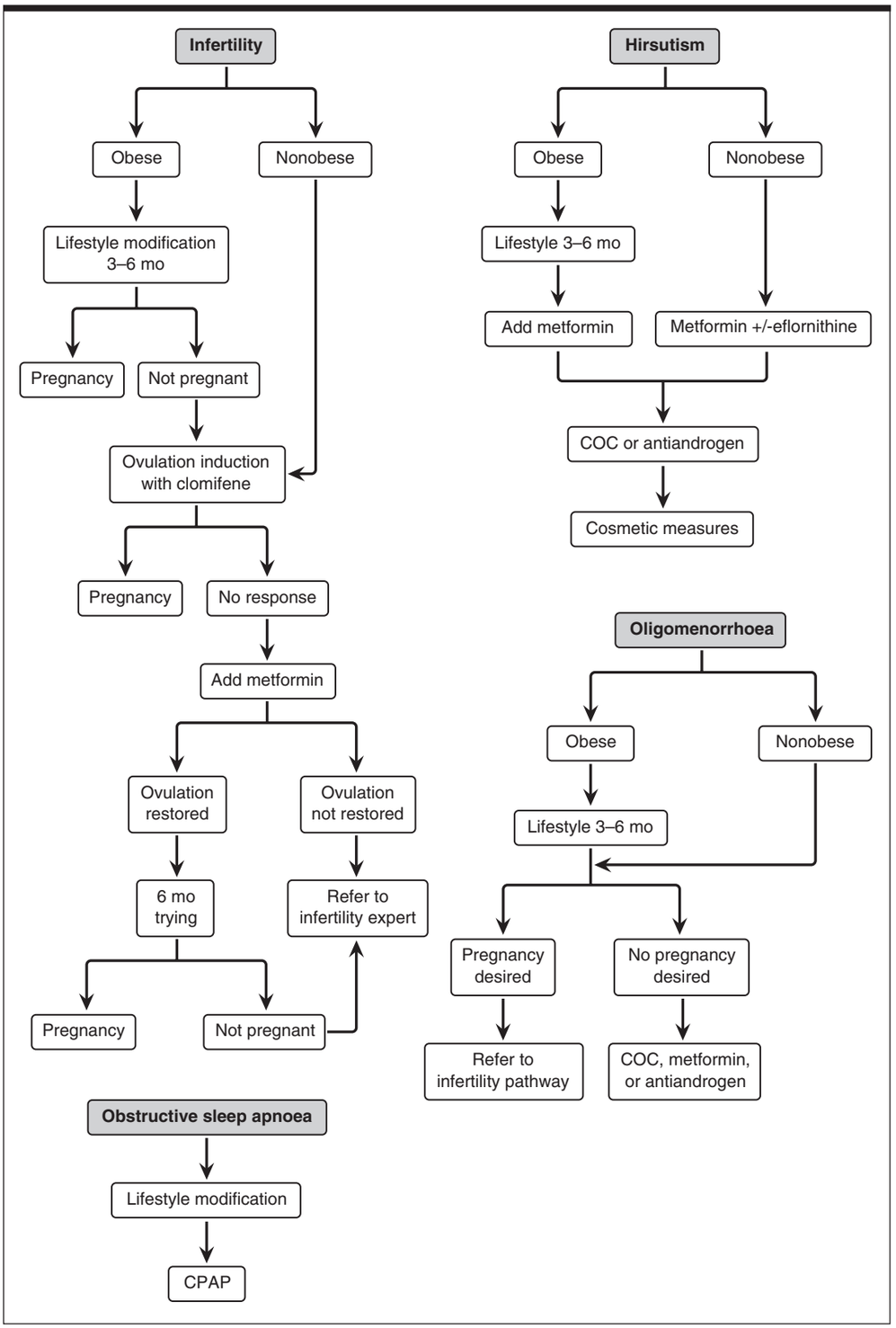
Summary

Women with PCOS exhibit unique clinical features and have individual concerns that should be addressed when making treatment recommendations. Assessment of women with PCOS should include gathering relevant medical information, such as menstrual history, signs and symptoms of hyperandrogenism, time course of symptoms, weight history, previous agents tried, and family history. If PCOS is suspected, laboratory assessments should be performed to rule out any other related disorders. The criteria for PCOS include hyperandrogenism or hyperandrogenemia, anovulation or oligo-ovulation, and/or polycystic ovaries. Once a diagnosis has been made, recommendations about treatment must consider the patient's desires and motivation to attain individualized goals. Figure 16-2 displays various treatment options and preferences when addressing patient priorities.

In any obese patient, weight loss is a first step to improve many of the clinical and biochemical endocrine and metabolic abnormalities in PCOS. If contraception is desired, COCs will improve menstrual cycles and hyperandrogenism. If contraception is not needed, metformin will address menstrual cyclicity, hyperandrogenism, and insulin resistance. Other agents like thiazolidinediones could be used if contraindications or serious side effects were present with metformin or COCs. Hirsutism can be targeted with COCs or antiandrogens. If pregnancy is desired, clomiphene citrate would be a first-line pharmacologic option for ovulation induction. Other alternatives—including metformin, dexamethasone, aromatase inhibitors, gonadotropins, ovarian drilling, or in vitro fertilization—can be considered if clomiphene citrate alone is not effective.

Appropriate follow-up for women with PCOS may include efficacy of current treatment, quality-of-life measures, and medication adherence monitoring.

Figure 16-2. Treatment Algorithms for PCOS.
(Adapted from *Drugs.* 2006;66:910. Copyright ©Adis Data Informations BV.)



Essential routine laboratory monitoring includes fasting lipid profiles and screening for IGT or frank diabetes. Long-term consequences include IGT, type 2 diabetes, hypertension, dyslipidemia, endometrial hyperplasia, and obstructive sleep apnea; however, an increased mortality risk has not been established.

Women with PCOS have a multifaceted disorder that requires individualized attention. Patient history, laboratory assessment, and appropriate therapy selection should be performed while considering the specific needs of patients. Providers should be educators, facilitators, and empathetic listeners to help women with PCOS become informed and actively engaged in their therapy plan.

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12

Pharmacogenetics in the Clinic

Kai I. Cheang

Abstract

Pharmacogenetics is the inherited basis of differences among individuals in their response to drugs. Genetic polymorphisms of drug-metabolizing enzymes may account for as much as 30% of interindividual differences in drug disposition and response. An increasing number of drug target polymorphisms have also been linked to differences in drug response. This chapter reviews some examples of the use of pharmacogenetics in clinical practice. Despite the increasing number of examples of genetic polymorphisms affecting drug response in the literature, pharmacogenetic data are rarely used in current clinical practice. The limitations that have prevented the use of pharmacogenetic testing in clinical practice are reviewed.

Key Words: Pharmacogenetics; pharmacogenomics; adverse drug events; efficacy; individualized therapy; drug–gene interactions; personalized medicine.

1. Introduction

Pharmacogenetics is the study of the genetic basis for interindividual differences in drug response. Genetic polymorphisms, defined as genetic variations occurring in at least 1% of the human population, form the genetic basis for variations in drug response. This field of investigation began half a century ago when hemolysis in some patients taking the antimalarial primaquine was shown to be caused by an inherited deficiency of glucose-6-phosphate dehydrogenase *(1)*. Since the sequencing of the human genome, there have been increasing examples in the literature documenting the association between genetic polymorphisms and drug response. These include reports on the genetic variability for drug-metabolizing enzymes, drug transporters, drug targets, and disease-modifying genes. However, translation of these pharmacogenetic observations into clinical practice has been limited by numerous hurdles. This chapter will first review some examples of how pharmacogenetic testing could aid in tailoring

From: *Biopharmaceutical Drug Design and Development*
Edited by: S. Wu-Pong and Y. Rojanasakul © Humana Press Inc., Totowa, NJ

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
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pharmacotherapy to the individual patient. Limitations of using pharmacogenetic testing in actual clinical practice will also be reviewed.

2. Pharmacogenetics in Clinical Practice

2.1. Dose Adjustment

Genetic polymorphisms may partially account for variability in drug pharmacokinetics or disposition among individuals. In current medical practice, patients are usually treated initially with standard doses of a drug, and the clinician expects an "average" drug exposure and "average" drug response, similar to what would be reported in the clinical literature concerning the drug. Genetic differences in drug-metabolizing enzymes may lead to varying levels of plasma concentrations and drug exposure when the same dose of the drug is administered. As a result, clinical response to the drug may be altered, both in terms of drug efficacy and dose-dependent adverse events. This is especially important for drugs with narrow therapeutic index, such as mercaptopurine, warfarin, and others. More uniform and predictable drug exposure, which may be achieved by dosage adjustment according to the patient's genetic profile of their metabolizing enzymes, may aid in predicting drug response and reducing dose-related side effects.

2.1.1. Pharmacogenetics of Drug-Metabolizing Enzymes

Genetic polymorphisms have been reported for phase I, phase II, and nucleotide base metabolizing enzymes. An important clinical example of how pharmacogenetic differences in drug-metabolizing enzymes affect clinical outcomes is illustrated by the genetic polymorphism of thiopurine methyltransferase (TPMT) *(2)*. TPMT catalyzes the S-methylation of azathioprine and 6-mercaptopurine. In patients treated for acute lymphoblastic leukemia (ALL), mercaptopurine is used as maintenance therapy. Mercaptopurine is metabolized to thioguanine, which is responsible for the bone marrow toxicity, as well as the therapeutic efficacy in ALL. TPMT catalyzes mercaptopurine's metabolism to an inactive metabolite. Patients with TPMT deficiency require a drastic reduction in mercaptopurine dose to prevent severe myelosuppression *(2)*. Certain TPMT genotypes are associated with reduced TPMT activity, and the TPMT genetic polymorphism is an important predictor of myelotoxicity associated with mercaptopurine and is clinically used to prevent myelotoxicity in ALL patients.

Most commercially available drugs are metabolized by cytochrome P450 enzymes. Polymorphisms of these P450 enzymes may also affect therapeutic responses to medications. For example, P450 2C9 is responsible for the metabolism of warfarin and phenytoin, both of which are drugs with narrow therapeutic indices. Clearances of these drugs are decreased in patients with 2C9 variant

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

alleles. For example, certain 2C9 haplotypes are associated with reduced warfarin maintenance therapeutic doses *(3)*. Knowledge of 2C9 phenotypes, therefore, may aid in optimizing titration schedules to achieve therapeutic anticoagulant effect whereas minimizing the risk of bleeding when a patient is initiated on warfarin therapy. Establishment of stable maintenance warfarin doses currently necessitate frequent laboratory monitoring during the first month of therapy. It is estimated that at least 50% of the variability in maintenance warfarin doses is because of genetic polymorphisms of both the 2C9 metabolizing enzyme and the vitamin K epoxide reductase complex 1 (VKORC1), which affects clotting factor synthesis *(4–6)*. Although no genetic polymorphism-based dosing algorithm has been prospectively validated, genetic information on 2C9 and VKORC1 may lead to faster establishment of therapeutic and stable anticoagulation and a decrease in the risk of major bleeding events *(7)*. The change in warfarin's prescribing information to contain such pharmacogenetic information was the subject of discussion during a Food and Drug Administration (FDA) Clinical Pharmacology Subcommittee meeting in November 2005 *(4)*.

Another clinically relevant example of how polymorphisms in drug-metabolizing enzymes affect dosage selection can be illustrated by irinotecan in colorectal cancer. Irinotecan undergoes metabolism by carboxylesterases into an active metabolite, SN-38, which is then conjugated by the enzyme UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite. In individuals with the UGT1A1*28 genetic polymorphism, UGT1A1 enzyme activity is reduced. About 10% of the North American population is homozygous for the UGT1A1*28 polymorphism. Patients homozygous for UGT1A1*28 have a higher exposure to the SN-38 active metabolite than patients without the polymorphism when irinotecan was administered. This increased exposure may increase the risk of neutropenia and diarrhea. This increased drug exposure in patients with the polymorphism is recognized in the labeling of irinotecan, in which recommendations were given to reduce the starting dose by at least one level in patients known to be homozygous for the UGT1A1*28 allele *(8)*. Although a specific recommendation is given, the precise optimal dose modification is unknown and subsequent dose adjustments may be necessary depending on patients' tolerance.

2.1.2. Pharmacogenetics of Drug Transporters

In addition, polymorphisms in genes encoding drug transporters have also been identified. A prominent example of these drug transporters is the P-glycoprotein, a transmembrane efflux pump. P-glycoprotein is found in a wide variety of cells and tissues, including the intestinal enterocytes, renal proximal tubules, hepatocytes, and capillary endothelial cells of the blood–brain barrier. Therefore, P-glycoprotein plays an important role in drug disposition by reducing drug

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

absorption, increasing elimination, and decreasing the ability of drugs to cross the blood–brain barrier. P-glycoproteins are also found in tumor cells. P-glycoproteins mediate an active efflux of chemotherapeutic agents from tumor cells, hence promoting multidrug resistance to anticancer agents. The dispositions of many drugs are affected by P-glycoproteins. These include cancer chemotherapeutic drugs, antiepileptic agents, protease inhibitors (for treating human immunodeficiency virus [HIV] infections), and cardiac drugs such as digoxin and diltiazem. The multidrug resistance 1 gene (MDR1) codes for P-glycoprotein. Numerous single-nucleotide polymorphisms (SNPs) have been identified in the MDR1 gene. These polymorphisms may influence drug pharmacokinetics, and hence drug response.

Numerous genetic polymorphisms exist for other metabolizing enzymes and drug transporters. These genetic polymorphisms may affect the drug's pharmacokinetic parameters in drug absorption, distribution, metabolism and excretion. Table 1 reviews some selected examples of the effect of genetic polymorphisms influencing pharmacokinetics. Interindividual differences in drug response because of variability in drug disposition resulting from genetic polymorphisms may be minimized by appropriate dose adjustment, if a dose-adjustment algorithm based on pharmacogenetic data is available. Importantly, there is now an FDA-approved pharmacogenomic microarray test designed for clinical applications (AmpliChip CYP450 Test®, Roche Diagnostics). The test provides information on 2D6 and 2C19 genes, which are involved in the metabolism of about 25% of prescription drugs. Although not widely used at the time of publication, the test will facilitate individualizing treatment doses for medications metabolized by 2D6 and 2C19.

2.2. Selection of Drug Therapy

Genetic differences may also affect drug selection. Polymorphisms in a gene coding for a drug receptor may render a drug acting via that receptor less (or more) effective. Although not usually considered as examples of pharmacogenetics because they do not involve genetic variations of the host, molecular diagnostics of tumor cells and HIV enable the selection of appropriate therapeutic agents and are important recent advances.

2.2.1. Drug Receptor Polymorphisms

Pharmacogenetic data may affect drug selection. For example, genetic polymorphisms in drug receptors may affect the degree of pharmacologic (either agonistic or antagonistic) actions via these receptors. The beta-1 receptor polymorphisms and antihypertensive response to beta-1 antagonists serve as an example. Beta-antagonists are commonly used for treating hypertension. The beta-1 receptor gene contains two common SNPs at codons 49 and 389. In a

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

Table 1
Selected Examples of Genetic Polymorphisms Affecting Pharmacokinetics

	Medications	Possible drug effects
P450 Drug-metabolizing enzymes		
CYP P450 2C9	Warfarin	Decreased metabolism may lead to supertherapeutic anticoagulant effect
	Phenytoin	Decreased metabolism may lead to toxicity
CYP P450 2D6	Selective serotonin reuptake inhibitors Tricyclic antidepressants	Antidepressant toxicity because of supratherapeutic drug concentrations in poor metabolizer; poor treatment response in extensive metabolizers
	Codeine	Decreased analgesic effect of codeine in patients with reduced 2D6 metabolism of codeine to its active metabolite
	Metoprolol	Potential increased drug concentrations in poor metabolizers which may lead to increased side effects
	Thioridazine	Increased concentration in patients with reduced 2D6 metabolism, which may lead to life-threatening Torsade de pointes arrhythmia
Phase III metabolizing enzymes		
Uridine diphosphate glucuronyltransferase	Irinotecan	Decreased clearance leads to gastrointestinal toxicity
Nucleotide base metabolizing enzymes		
Thiopurine methyl-transferase	Mercaptopurine	Low enzyme activity leads to severe myelosupression
Dihydropyrimidine dehydrogenase	Flurouracil	Deduced enzyme activity may lead to life threatening

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Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

Table 1 (*Continued*)

	Medications	Possible drug effects
		neurological, hematological, and gastrointestinal toxicity
Drug transporters		
P-glycoprotein (MDR-1)	Digoxin	Decreased digoxin bioavailability
	HIV protease inhibitors	Decreased response in CD4 count

clinical study with metoprolol, patients who are homozygous for Ser49 and Arg389 had the greatest blood pressure reduction, suggesting that the beta-1 receptor haplotype may be used as a predictor of response to beta-blockers *(9)*.

2.2.2. Molecular Diagnostics

The field of molecular diagnostics is rapidly advancing. Currently there are already specific clinical examples of how therapeutic agents are chosen based on information obtained with molecular diagnostics, regularly used in clinical practice. Examples of these include transtuzumab (Herceptin®) *(10)*, imatinib (Gleevec®) *(11)*, gefitinib (Iressa®) *(12)*, and HIV genetic testing.

2.2.2.1. Molecular Diagnostics in Oncology

HER2 (human epidermal growth factor receptor) is a protein that is overexpressed in up to 30% of invasive breast cancer cells *(10)*. A humanized monoclonal antibody against HER2, transtuzumab (Herceptin®), is the first of a class of chemotherapeutic agents whose design is directed to specific molecular targets, in this case the HER2 receptors. Transtuzumab has shown efficacy as monotherapy in patients with HER2 overexpression, as well as in combination with taxane-based chemotherapy in metastatic breast cancer overexpressing the HER2 receptor. The molecular diagnostic test for HER2 has received FDA approval.

Another drug that benefits from genetic testing is imatinib (Gleevec®) *(11)*. In chronic myelogenous leukemia, the definitive diagnosis is a cytogenetic analysis of bone marrow specimens for the Philadelphia chromosome, which is a translocation between chromosome 9 and chromosome 22, resulting in the oncogenic fusion protein BCR-ABL (BCR from chromosome 22 and ABL from chromosome 9). The molecular diagnostic test measures the presence of BCR-ABL and the level of overexpression. Imatinib is a tyrosine kinase inhibitor

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

that inhibits BCR-ABL, an abnormal tyrosine kinase. Imatinib inhibits the proliferation and induces programmed cell death in BCR-ABL positive cells. The molecular diagnostic test for BCR-ABL has been approved by FDA.

Gefitinib (Iressa®) is another tyrosine kinase inhibitor used in the treatment of non-small-cell lung cancer (NSCLC), a leading cause of cancer death in the United States in both men and women. Gefitinib targets the epidermal growth factor receptor (EGFR) that is overexpressed in up to 80% of NSCLCs. Most patients with NSCLCs do not respond to gefitinib. However, the drug leads to rapid and dramatic clinical response in 10% of patients. Recently, specific mutations in the EGFR binding site for gefitinib were found to be more prevalent in patients responding to the drug than for nonresponders. It is thought that these mutations in the EGFR binding site mediate increased growth factor signaling which is susceptible to inhibition by gefitinib. Screening for EGFR mutations in NSCLCs may help identify patients who will have clinical response to gefitinib *(12)*. The screening test will also identify those who are not likely to benefit from gefitinib, and help avoid delaying other therapy and the expense of an ineffective drug. An FDA-approved test for EGFR mutations is not yet available at the time of writing, but some medical centers have begun offering molecular diagnostics developed in-house for EGFR mutations, and a clinically relevant test is likely to be developed in the future.

2.2.3. Drug Resistance Testing in HIV

Drug resistance testing for HIV antiretroviral therapy is now considered standard of care. Prospective and retrospective data suggest that the availability of antiretroviral genotypic resistance data is an important factor in achieving response to therapy *(13,14)*. These data have led to expert panels' recommendation of the use of resistance testing in HIV antiretroviral therapy *(15,16)*. The Panel on Clinical Practices of HIV Infection convened by the US Department of Health and Human Services recommends drug resistance testing in cases of virologic failure (to maximize the number of active drugs utilized in a new regimen after a patient has failed combination antiretroviral therapy) or when there is suboptimal suppression of viral load after initiation of antiretroviral therapy.

An FDA-approved test for HIV drug resistance is available. TruGene® (Bayer Diagnostics) involves sequencing of genes encoding the two main drug treatment targets, the protease and reverse transcriptase genes. The genetic information is accompanied by a constantly updated algorithm to guide treatment options. The report is clinician-friendly and points clinicians to resistance or sensitivity of the various antiretroviral therapies. A minimum HIV viral load of 1000 copies per mL is necessary for reliable amplification of the virus and the genotype to be detectable. The goal of HIV resistance testing is to maximize the number of active antiretroviral therapy used against the infection.

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

2.2.4. Drug-Metabolizing Enzyme Polymorphisms Affecting Choice of Therapy

Besides affecting the pharmacokinetic characteristics of drugs and therefore necessitating dosage adjustments, polymorphisms in drug-metabolizing enzymes may also affect the choice of therapeutic agents, when the algorithm for dosage adjustments may not be readily available, such as in the example of tamoxifen. This example also illustrates how genetic polymorphisms in P450 drug-metabolizing enzymes affect therapeutic efficacy by the important role they play in the bioformation of active metabolites, which may have important implications for certain pharmacologic agents. Tamoxifen, an antiestrogen agent used as an adjuvant in the treatment of estrogen-dependent breast cancer, is metabolized by CYP 2D6 to an active metabolite with about 100 times the antiestrogen potency of the parent compound. Breast cancer patients who were homozygous for CYP 2D6 *4 (poor metabolizers) had the highest risk of relapse and worst disease-free survival, even when nodal involvement and tumor size were accounted for, when compared to patients who were heterozygous (*4/wt) or noncarriers of the allele (wt/wt). The poor prognosis of patients who were poor metabolizers was presumably because of a low concentration of the active metabolite with potent antiestrogen effects *(17)*. Hence, differences in response to tamoxifen may be explained by CYP 2D6 genotype. Genotypic information on CYP 2D6 may help clinicians in selecting optimal therapy for estrogen-dependent breast cancer.

Numerous other examples of genetic polymorphisms affecting drug selection exist. Table 2 reviews some selected examples of how genotypic information may aid the clinician in choosing the optimal therapy for different disease states.

3. Hurdles in Translating Pharmacogenetics to the Clinic

Despite great advances in the discovery of how genotypic information could affect drug dosage modifications and initial drug selections, pharmacogenetic information is rarely used in clinical practice. A recent survey suggested that even though knowledge of drug-metabolizing polymorphisms have existed since a half-century ago, pharmacogenetic tests for drug-metabolizing enzymes are rarely used by clinicians *(18)*. This is despite continual claims in the literature that pharmacogenetic testing for drug-metabolizing enzymes may prevent adverse clinical outcomes. Because evidence for some of the drug-metabolizing enzymes have been existent for almost five decades, the reasons for the lack of utilization of pharmacogenetics in the clinic seem to be beyond the natural "lag time" for translation of scientific findings to clinical practice. What are the possible reasons for the low clinical utilization for pharmacogenetic tests?

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

Table 2
Selected Examples of Genetic Polymorphisms Affecting Selection of Drug Therapy

	Medications	Possible drug effects
Beta-1 adrenergic receptor	Beta-1 antagonist (e.g., metoprolol)	Decreased blood pressure and cardiovascular response to beta-1 blockers
Beta-2 adrenergic receptor	Beta-2 agonists (e.g., albuterol)	Decreased bronchodilation effect in asthma
Serotonin transporter	Serotonin reuptake inhibitors (antidepressants)	Decreased antidepressant response
Human major histocompatibility complex (HLA)	Antiretroviral agent, abacavir	Increased risk of hypersensitivity reaction
Prothrombin and factor V	Oral contraceptives	Increased risk of venous thromboembolism (stroke or deep vein thrombosis)

3.1. Inadequacy of Evidence Relevant to the Clinician

3.1.1. Lack of Studies with Clinical Outcomes as Endpoints

With few exceptions, there is a lack of prospective hypothesis-driven clinical studies that demonstrate pharmacogenetic-based individualizations of drug therapy leading to improved clinical outcomes. For example, most pharmacogenetic studies with drug-metabolizing enzymes link genotypic findings to drug concentrations or exposure. Studies with clinical outcomes as endpoints are much more difficult to conduct because nongenetic confounders, such as compliance, health habits (smoking, diet), concomitant drugs, and disease states, are difficult to control. In addition, responses to most drugs are likely to be multigenic in nature. A well-controlled trial with definitive clinical endpoints will require a large number of study subjects and will be very expensive.

In the case of drug-metabolizing enzymes, differences in drug exposure because of metabolizing enzyme genetic polymorphisms do not necessarily translate into differences in therapeutic efficacy or adverse effects. For example, metoprolol, a beta-1 antagonist, is metabolized by CYP 2D6. Individuals who are poor metabolizers of CYP 2D6 have increased exposure to the drug. However, even when CYP 2D6 genotype correlates with variations in pharmacokinetics,

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

these genetic and pharmacokinetic variations are not associated with efficacy or adverse effects because of adrenergic blockade *(19)*. Hence, not all genetic variations associated with a surrogate phenotype will be associated with differences in clinically meaningful outcomes.

In addition to the lack of studies with clinically important outcomes, cost–benefit analyses of pharmacogenetic-based individualizations of pharmacotherapy will also add to the evidence supporting their use.

3.1.2. Lack of Information Regarding Pharmacogenetic-Based Dosing Algorithms

Dosage modifications of 6-mercaptopurin based on TPMT genotypic information serves as an important example of how genotypic information can facilitate optimal dosing of therapeutic agents *(2)*. In this example, patients who are homozygous for the allele resulting in reduced TPMT activity will receive 5–10% of the standard dose of 6-mercaptopurin for ALL maintenance therapy *(2,20)*. However, for many other drugs, information on dosing algorithms that facilitate appropriate and precise pharmacogenetic-based dosage adjustments recommendations does not exist. Most of the studies lack specific explanation on how to translate study findings for use in clinical situations. Specific and straightforward recommendations on how to individualize therapy based on the results of pharmacogenetic tests are critical if current pharmacogenetic knowledge is to be applied in the clinic to assist in rational drug choices and improve the benefit–risk ratio of drug therapy.

3.1.3. Inadequate Pharmacogenetic-Based Prescribing Information

If pharmacogenetics were to be commonly used in clinical therapeutic decision-making, they should be available in prescribing information widely accessible to health-care providers. A recent analysis of drug package inserts (prescribing information) revealed that only 35% of package inserts contained pharmacogenetic data *(21)*. Of these, only about 10% of those contain adequate information to guide therapeutic decisions. Importantly, pharmacogenetic-based dosing recommendations were only available for two drugs. This deficiency in useful prescribing information may reflect the relatively recent widespread interest in the field of pharmacogenetics in the effort to capitalize the human genome. In addition, only recently have pharmacogenetic assays with clinically relevant turnaround time become available. Nonetheless, the lack of clinician-friendly pharmacogenetic information in the labeling of medications will hamper the clinical applicability of pharmacogenetic information.

Clinician-friendly, straightforward, and actionable recommendations can also prevent the misinterpretation of pharmacogenetic information. For example, consider the antidepressant Venlafaxine. Venlafaxine is metabolized by CYP

Job: Wu_Pong
Chapter:12_Cheang_Wu_Pong.qxd
Date: 11.06.2007
Revision: 1st Revises

2D6 to equipotent active metabolite. Given the equipotent nature of the active metabolite, CYP 2D6 genotypic differences do not change the exposure to the active drug moiety because both the parent drug and the metabolite are equally potent. Regular practicing clinicians are likely not aware of a drug's specific pharmacokinetic and metabolite characteristics and will only be able to properly adjust drug dosages if a straightforward recommendation is given for a specific drug.

In addition, because of the lack of molecular genetic training in health-care providers, even when pharmacogenetic information is available, genetic information may be misunderstood even by health-care practitioners. In one study, more than 31% of physicians were reported to misinterpret genetic results *(22)*. This could be because of the lack of systemic education on pharmacogenetics of health-care professionals currently in practice. The re-education of the medical community on these pharmacogenetic relationships to drug response may prove to be challenging. As the complexity of genetic information grew because of a greater understanding of the nature of polygenic drug response, matching a patient's genotype to a specific therapy or dose may be beyond the capability of individual clinicians. Advancement of existing information technology in health-care delivery will be essential for pharmacogenetics to be utilized in clinical practice *(23)*.

3.2. Genotypic Vs Phenotypic Identification

Sometimes, knowledge of a patient's phenotype may render it unnecessary to identify the exact genotype, at least in the clinic. For example, in the above TPMT scenario, one could test for TPMT activity instead of TPMT genotype. The availability of other validated biomarker assays, combined with the lack of third-party reimbursement of genetic tests *(24)*, and the relatively high current cost of these genetic tests may hinder the translation of genetically guided information into clinical practice.

3.3. Polygenic Traits

For the most part, the pharmacogenetic information currently available (such as those in Tables 1 and 2) represents examples of monogenetic determinant of drug response. Many of these polymorphisms are also highly penetrant, with clearly identifiable phenotypes, such as drug concentrations. However, drug treatment outcomes represent a complex phenotype, possibly encoded by dozens to hundreds of genes, influenced by numerous environmental factors, and the interaction between these various gene and environmental variables *(25)*. The term "pharmacogenomics" illustrates these polygenic determinants of drug response. Detailed knowledge to associate specific genetic sequence variants to drug outcome is often not available. Therefore, it will be crucial to screen genotypic markers across

the whole genome systematically and combine them with extensive phenotypic characterization of therapy response. Such studies have yet to be performed.

It has only very recently become technologically possible to perform such whole genome association studies. Although genome-wide association scans offer the potential to discover genetic variants governing drug response, several technological and methodological problems exist. First, in these studies, large samples are necessary to find markers with definite effects on drug response while controlling for false positives. Secondly, currently even with the least expensive genotyping technology and strategy, genome-wide studies are still extremely expensive. The Nation Institutes of Health has issued a request for proposal to encourage the development of appropriate methodologies.

3.4. Standardization and Availability of Genetic Tests with Clinically Relevant Turnaround Time

For pharmacogenetics to be successfully used in the clinic, genetic tests need to be available, with clinically relevant turnaround time. Currently, only few FDA-approved paramagnetic tests are available. Examples of these include BCR-ABL genetic test for imatinib, AmpliChip CYP450® for 2D6 and 2C19 metabolizing enzyme, and TruGene® for testing HIV drug resistance.

Most genetic tests bypass the FDA because they are categorized as services, which are not regulated by the FDA. Laboratories at major academic centers often offer genetic tests that have been developed "in-house." Many of these laboratory-based techniques may be too time intensive for routine clinical use. In addition, although these academic clinical laboratories are Clinical Laboratory Improvement Amendments (CLIA) certified and their "analyte-specific reagents" used in these assays are regulated by the FDA, different laboratories may utilize different test systems such that results may not be generalizable across various platforms *(26)*. In addition, laboratory reporting may also need to be standardized. Andersson et al. *(27)* reported a study evaluating factor V Leiden genetic testing and the clinical utility of different report formats to physicians when interpreting results of the genetic test. It was found that there was a considerable degree of variability in the contents of these reports, with some lacking information deemed critical by the standard of professional guidelines and recommendations.

4. Conclusions

Pharmacogenetics may facilitate rational therapeutics both in dosage adjustments and in initial drug selection for treatment. However, pharmacogenetic-based individualized therapy is yet to be widely translated into clinical practice. Hurdles for translating pharmacogenetics from the laboratory to the clinic include: lack of clinical studies with clinically relevant outcomes, few widely

available standardized genetic assays, lack of information on polygenic drug response, and finally, the lack of straightforward, clinician-friendly pharmacogenetic information at the point of prescribing. The above problems will serve as future investigative directions of translating genetic information into clinical practice. Pharmacogenetics and pharmacogenomics have great potential to facilitate improved therapeutics.

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ACCP White Paper

Research in Women and Special Populations

American College of Clinical Pharmacy
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The American College of Clinical Pharmacy charged a Task Force on Research in Special Populations to review, update, and broaden its 1993 White Paper, Women as Research Subjects. Participants of the task force included pharmacy clinicians and investigators in the field. This resulting White Paper, Research in Women and Special Populations, discusses the current concepts regarding the conduct of research in women, as well as in special populations (children, the elderly, minorities, the cognitively impaired, and other vulnerable populations such as prisoners and refugees). For each specific population, the barriers to research participation, current guidelines and regulations, and available recommendations to address these barriers are discussed. The participation of research by these populations requires addressing special social and ethical challenges. Clinical pharmacy researchers should be cognizant of these guidelines and be an advocate for the inclusion and the rights of women and special populations in research participation.
(Pharmacotherapy 2008;28(9):93e–113e)

In 1993, ACCP published the White Paper, Women as Research Subjects.[1] Since that time, significant changes, including new regulations regarding the conduct of research in women and other special populations, have occurred. In 2004, the ACCP Publications Committee conducted a review of the 1993 White Paper and recommended that it be revised and updated. The Task Force on Research in Special Populations, commissioned by ACCP, was charged to review, update, and broaden the 1993 White Paper, Women as Research Subjects, to include other special populations. This paper, Research in Women and Special Populations, discusses important concepts regarding the conduct of research in these special populations and provides an expanded bibliography for readers interested in more detailed information.

This document was written by the Task Force on Research in Special Populations, commissioned by ACCP: Kai I. Cheang, Pharm.D., BCPS, Chair; Carol Ott, Pharm.D., BCPP; Sandra Garner, Pharm.D., BCPS; Hope Campbell, Pharm.D., BCPS; Laura Hansen, Pharm.D., BCPS, FCCP; Qing Ma, Ph.D.; Elaheh Nazeri, Pharm.D.; Karen Gunning, Pharm.D., BCPS; Daniel Wermeling, Pharm.D. Approved by the American College of Clinical Pharmacy Board of Regents on October 24, 2006; final revision received on February 9, 2007.

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Research in Children

It is well recognized that generalizing results from adult studies to the care of pediatric patients is dangerous, as infants and children are different from adults in many ways.[2] The maturational changes from the newborn period to adolescence results in a striking effect on drug disposition.[3] For example, absorption,

distribution, metabolism, and excretion in neonates are different from adults because of age-specific changes in body composition, function, and/or age-specific patterns of development of phase I and II enzymes and renal function.[4] Knowledge of these developmental changes has recently expanded greatly, resulting in a better understanding of the need for age-dependent drug therapy.[4]

Challenges in Pediatric Research

Inadequate Research Efforts

Until recently, little attention has been paid to the unique issues of medication use in pediatric patients. In fact, only 20% to 30% of drugs approved by the Food and Drug Administration (FDA) are currently labeled for pediatric use.[2] Drugs have historically only been evaluated in children after approval for use in adults, hence use of the phrase "therapeutic orphans" when referring to pediatric patients. This lack of testing is thought to be multifactorial, including a lack of financial incentive and the practical and ethical difficulties in conducting studies in children.[2]

Addressing Research Efforts in Children

Fortunately, recent regulatory and legislative changes have dramatically increased the number of pediatric drug studies. Congress enacted the Food and Drug Administration Modernization Act in 1997, which required the FDA to develop, prioritize, and publish a list of approved drugs for which additional pediatric information may provide health benefits for children.[5] This act included a pediatric exclusivity provision that added 6 months of market exclusivity to any existing patent or exclusivity provided by the Hatch-Waxman Act for a drug for which the FDA requested pediatric studies and the manufacturer satisfactorily complied. In 1998, the FDA issued the Pediatric Rule.[6] Under this regulation, the FDA could require that pediatric studies be conducted for a new drug that would likely be used in a substantial number of pediatric patients or would offer a significant benefit over existing treatments. The manufacturers of marketed drugs may also be required to do the same if either of these conditions applies and inadequate labeling could pose a significant risk.

The FDA in 2000 also published its Guidance for Industry regarding conducting clinical investigations in the pediatric population.[7] In 2002, the Best Pharmaceutical for Children Act became law and continued the pediatric exclusivity incentive until 2007.[8] For those drugs that are off-patent or that industry chooses not to conduct studies, the Best Pharmaceutical for Children Act mandates that the FDA and the National Institutes of Health (NIH) collaborate to assure the generation of pediatric data. The legality of this Act was challenged by the Association of American Physicians and Surgeons, the Competitive Enterprise Institute, and Consumer Alert. Their claim was that the FDA did not have the legal authority to require pharmaceutical companies to conduct studies in the pediatric population. They voiced concerns that the pediatric exclusivity provision would increase the cost of new drug development, delay the availability of new drugs, and possibly cause the exploitation of children as research subjects. In 2003, the Pediatric Research Equity Act was passed that mimics the Pediatric Rule in requiring pediatric studies for certain applications and established a Pediatric Advisory Committee at the FDA.[9]

Ethical Issues of Pediatric Research

Historically, children have been exploited in medical research. A majority involved in early research were poor, institutionalized, mentally ill, or physically disabled.[10] One early example was a study conducted to describe the natural history of hepatitis by deliberately infecting mentally retarded children institutionalized at the Willowbrook State School.[10] Immunization trials for smallpox and pertussis also took advantage of children due to their lack of previous disease exposure and the controlled environment of pediatric institutions.[10] Another more recent example conducted in the 1990s was a comparative study of the effectiveness of lead abatement procedures by the Kennedy Krieger Institute.[10] This study was designed to determine a minimally effective procedure for lead abatement by comparison to the current standard. However, those receiving less than the standard abatement procedure were unnecessarily exposed to potential lead toxicity.[10]

Addressing Ethical Issues of Pediatric Research

In the 1970s, the National Research Act (Pub. L. 93-348) created the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. This group published

various reports, including the Belmont Report (which still serves as the basis of protection of human subjects in research) and the Children in Research report. Based on the National Commission's reports, the Department of Health and Human Services developed regulations for research, including Subpart A,[11] also called the Common Rule, and Subpart D, which provides additional protections for research enrolling children.[12] The National Commission acknowledged the need for research in children but also realized their vulnerability. To minimize these problems, they established strict criteria that research involving children should satisfy:[13] 1) the proposed research is scientifically sound and significant; 2) where appropriate, studies are conducted initially on animals and adult humans, followed by older children and then infants; 3) risks are minimized by using the safest procedures consistent with sound research principles and by using procedures needed for diagnostic or treatment purposes if possible; 4) adequate provisions are in place to maintain confidentiality of data and protect the privacy of children and their parents; 5) subjects are equitably recruited; and 6) adequate provisions for the permission of the parents or guardians and assent of the child are made.[14] Further requirements included evaluation by the local institutional review board (IRB) to determine that the potential risk is: 1) justified by the anticipated benefit to the subjects, 2) the anticipated benefit:risk ratio is as favorable as with any alternative therapies, and 3) adequate permission of the parents or guardians and assent of the child is obtained. These guidelines both protected children from the risks of research and restricted their participation, especially the "children last" requirement. Institutional review boards are also required to categorize research involving children as: 1) research not involving greater than minimal risk, 2) research involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects, 3) research involving no greater than a minor increase over minimal risk and presenting no prospect of direct benefit to individual subjects, but is likely to yield generalizable knowledge about the subject's disorder or condition, and 4) research with greater than a minor increase over minimal risk but presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health and welfare of children. Research belonging to the last category requires approval by the IRB and Secretary of the Department of Health and Human Services after consultation with a panel of experts.[10]

One of the current controversies is what constitutes "minimal risk" and a "minor increase over minimal risk." One point debated is whether minimal risk is relative to that experienced by healthy children in everyday life or by the children with the condition being studied. The National Commission defined minimal risk relative to the healthy child,[13] while Subpart D in the federal regulations did not.[12] Another controversy is that according to the risk categories above, only children with a "disorder or condition" can be involved in nontherapeutic research that potentially has greater than minimal risk. Should healthy children be protected from risks preferentially to those with a disorder or condition? Perhaps it would be more ethical to either allow or prohibit such research for all children. Another recommendation has been proposed that instead of focusing on whether the child is healthy or has a disorder or condition, the definition of minimal risk should be risks and harms to which it would be appropriate to intentionally expose a child, as risks exposed to in the everyday life of a child may or may not be acceptable.[15]

Ideally, to determine age-appropriate dosage regimens for pediatric patients, pharmacokinetic studies enrolling children of all age groups would be conducted. One of the concepts currently being debated is whether healthy children should be allowed to participate in these nontherapeutic pharmacokinetic studies.[16] The purpose for enrolling healthy children would be to isolate the effects of age and development on drug disposition. The risks involved typically include short-term exposure to the medication and the pain of serial blood sampling. In adults, this experience is considered minimal risk. However, blood sampling may be more traumatic in a child. The FDA has published guidelines[17] reiterating the need for such studies and recommending techniques to decrease the risk to subjects (e.g., minimizing blood samples, minimizing blood volume per sample, etc.). Many arguments have been made for and against pharmacokinetic studies in healthy children. Arguments against include a lack of benefit for the subject, lack of true consent, that pharmacokinetics may be affected by disease state, investigator's potential financial gains influencing the need for subject accrual, studies with unnecessary "me too" drugs, and the school of thought that children do not have societal

obligations. Arguments for performing pharmacokinetic studies in healthy children include the fact that a medication may become relevant later if the healthy child becomes ill, availability of procedures to minimize risks (e.g., through the use of local anesthetics and a child-friendly environment), more expeditious generation of knowledge on age-dependent dosing in healthy children, lack of confounding variables to influence the study of age-dependent pharmacokinetics in healthy children, and the school of thought that children are an integral part of society and should be educated to help others.

The FDA provides some guidance on enrolling healthy children in drug trials. The Ethics Working Group of the FDA Pediatric Advisory Subcommittee stated, "In general, pediatric studies should be conducted in subjects who may benefit from participation in the trial. Usually, this implies the subject has or is susceptible to the disease under study. The Advisory Subcommittee utilized a broad definition of potential benefit. For example, any child has the potential to benefit from a treatment for otitis media." This statement makes it permissible to enroll healthy children in a drug trial for specific drugs targeted at specific conditions that commonly occur in pediatric patients. Therefore, it would be ethical if a patient of the appropriate age was enrolled in a study for a drug of clear importance to pediatric therapeutics in which the knowledge to be gained would be generalizable to the treatment of pediatric patients.[16]

Summary of Research in Children

In summary, pediatric drug research is a dilemma in that society wants to spare children from the potential risks of research but also from the inevitable harm of using inadequately studied medications.[18] With the new policies implemented in the 1990s by the FDA, NIH, and Congress, some are concerned that too much focus has shifted from protecting children from research risks to ensuring access.[14] Care must be taken to ensure that children are enrolled in research designed to provide pediatric-specific information and that study subjects receive at least standard of care.[14]

Research in the Elderly

The American population is aging, along with an increase in life expectancy.[19] Currently, there are 38 million seniors in the United States and, by 2030, that number will rise to 75 million.[20] The elderly population represents about 13% of the population and yet they consume about 34% of all prescription drugs,[20] probably due to a higher incidence of disease-related morbidities and therefore multiple medication regimens.[21] Clinical pharmacy practice, research, education, and advocacy in older adults have been reviewed by a separate ACCP White Paper.[22] This section of the current paper focuses on research issues in the elderly.

Given a longer life expectancy and the increased use of medications by the geriatric population, clinical research in the elderly becomes increasingly important. Besides the disproportionate increase in drug use, the elderly population has its particular research needs and gaps in knowledge that are distinct from other populations. Elderly individuals may respond to drugs differently than younger individuals. For example, altered pharmacokinetics and pharmacodynamics have been well documented in the elderly.[23] In addition, certain conditions such as Alzheimer disease and isolated systolic hypertension are prevalent predominantly in the geriatric population. Many drugs in use and in development by pharmaceutical companies are directed toward diseases affecting the elderly. In 2004, there were 800 new medicines targeted to diseases of aging by pharmaceutical companies, including 123 drugs in development for heart disease and stroke, 395 drugs for cancer, 53 drugs for diabetes, 22 drugs for Alzheimer disease, 18 drugs for osteoporosis, and 14 drugs for Parkinson disease.[24]

Challenges in Geriatric Research

Inclusion of the Elderly in Research

Despite the fact that pharmaceutical companies are now required to include a geriatric use section in their product labeling, the information is often insufficient in part because insufficient numbers of elderly are included in the clinical trials.[21] Effective lower doses of many drugs used in the elderly population are not included in product labeling. Cohen reported on 48 major medications where data are available on lower effective doses that is not reflected in the Physicians' Desk Reference (i.e., the product label).[25] Despite the importance of including older persons in clinical trials, under-representation of the elderly in research studies

has been well documented.[26, 27] The lack of participation among the elderly in clinical trials adversely affects patient care ultimately. For example, in an analysis of adverse events reported to the FDA between 1990 and 1996, patients older than 60 years experienced 3 times more adverse events compared with individuals less than 20 years of age.[21]

A reason for lower participation by the elderly in research studies may be due to organ system abnormalities and functional status limitations as these individuals age. It has been estimated that if protocol exclusions for organ system abnormalities (e.g., cardiac function, blood pressure, hematologic, and pulmonary function) and functional status limitations were relaxed, the percentage of elderly participation in cancer trials would be almost 60%.[27] Due to the access barriers to research studies, elderly individuals who are recruited into and continue to be followed in clinical protocols usually are more independent, healthier, and have a higher cognitive function. This recruitment bias may make results in studies involving older individuals not generalizable. For example, patients with dementia included in clinical research are systematically younger than the general dementia patient population by a gap of about 8 years in mean age.[28] In addition, fewer than one-half of the older adults currently prescribed donepezil would have been eligible to participate in the randomized controlled trials that established efficacy of the drug.[29] In particular, discontinuation rates are higher among patient groups not represented in the trials.

In addition, recruitment and attrition may be particularly problematic in elderly individuals. Increased age brings greater inter-individual heterogeneity. Many confounders may also exist in older adults. Such confounders include residence, caregiver type, cognitive function, social support, lifestyle, health status, drug compliance, and health care access, to name a few.[22] These inter-patient variability and confounders increase the sample size necessary in a given study. In addition, the drop-out rates may be higher in elderly individuals. Patients may become lost to follow-up because of relocation, change in health status, family influence, or a decline in cognitive function. The death rate in the elderly population is higher than that of younger populations, and this may be particularly an issue when the follow-up period for a study is long. There also exist regulatory challenges in terms of recruitment. The Health Insurance Portability and Accountability Act (HIPPA) stipulates that authorization from the patient must be obtained before a researcher can collect the exact age of patients older than 89 years.

Addressing Elderly Participation in Research

The FDA published its first guidance document for industry regarding research in elderly individuals in 1989. In this Guidance for Industry, Guideline for the Study of Drugs Likely to be Used in the Elderly, recommendations mainly center on evaluation of age-related difference in pharmacokinetics because age-related pharmacokinetic differences have been documented more frequently than pharmacodynamic differences.[30] The FDA subsequently published another guidance document, Guideline for Industry Studies in Support of Special Populations: Geriatrics, in 1994.[31] This subsequent document specifically stresses the importance of participation of elderly subjects in clinical trials evaluating drugs of clinical significance in the geriatric population. In addition, the guidance document states that arbitrary exclusion criteria based on an upper age limit should not be used and encourages the participation of individuals over 75 years of age.

As mentioned previously, relaxing protocol exclusions for organ system abnormalities in clinical studies can often result in increased elderly participation.[27] Given the inter-patient variability in this age group, sponsors' support would be paramount to expanding the inclusion of elderly individuals as research subjects.

As life expectancy increases, the exact definition of "elderly" may need to be revised. Most consider persons older than 65 years to be "elderly." However, as the population in their 70s, 80s, and 90s increase, research in these age subgroups may become important as well. In addition, measurement tools may need to be validated specifically in the elderly population. For example, in the Geriatric Depression Scale, questions regarding sexual interest and jobs outside the home are eliminated to reflect only items appropriate for older adults.

Cognitive Impairment

Another challenge in research involving older individuals is cognitive impairment and the informed consent process. Research issues in the cognitively impaired are discussed in Section 5.3.

Table 1. Major health disparities in ethnic/minority groups[36]

Ethnic/minority group	Health disparities
African Americans	1. Infant death rates more than double that of whites; 2. Death rates from heart disease and cancer more than 40% and 30% higher than those of whites, respectively; 3. Death rates from HIV/AIDS more than 7 times that for whites; 4. A rate of homicide 6 times that for whites.
Hispanics	1. Are almost twice as likely to die from diabetes as are non-Hispanic whites; 2. Account for 20% of the new cases of tuberculosis; 3. Exhibit higher rates of high blood pressure and obesity than non-Hispanic whites.
Other ethnic/minority groups	1. American Indians and Alaskan Natives have an infant death rate almost double that for whites. 2. The rate of diabetes for this population group is more than twice that for whites. 3. American Indians and Alaska Natives also have disproportionately high death rates from unintentional injuries and suicide. 4. New cases of hepatitis and tuberculosis are higher in Asians and Pacific Islanders than in whites.

The Nuremberg Code states that volunteers participating in research should have "legal capacity to give consent."[32] Some older patients may be excluded from research studies because of cognitive impairment that renders such patients a "vulnerable population" that need protection from exploitation.

Addressing Cognitive Impairment in Elderly Research Subjects

The American Geriatrics Society states that older individuals should not be excluded from participating in research studies solely because of impaired cognitive function.[33] The American Geriatrics Society suggested conditions to be considered in the informed consent procedures by persons with cognitive impairment. Examples of these conditions include existence of advance directive for research, severity of cognitive impairment, existence of health care surrogates, whether the research is ethical and justified, and local laws pertaining to this area. In addition to difficulty in providing informed consent, cognitive function can pose challenges to elderly subjects' ability to follow study protocol appropriately, comply with self-administration of study drugs, and report data accurately.

Summary of Research in the Elderly

As our population ages, inclusion of the elderly in research becomes increasing important. Numerous physiological, social, and ethical barriers to including the elderly in clinical research exist. In addition, elderly participants of clinical trials are often more independent and may not be representative of elderly patients in whom the medication will be used.

Research in Ethnic/ Minority Groups

Although life expectancy and overall health have recently improved for most Americans, blacks or African Americans, Hispanics or Latinos, and Native Americans continue to experience disparities in the burden of illness and death. As these racial and ethnic minorities are expected to grow in proportion to the total United States population, the future health of America as a whole will be influenced substantially by the health of these groups.[34]

The health disparities experienced by these groups compared with the white, non-Hispanic population in the United States have been identified to result from the complex interaction of genetic variations, environmental factors, and specific health behaviors in various clinical and social studies.[35] Table 1 summarizes the major health disparities experienced by African Americans, Hispanics, and other ethnic/minority groups.

Challenges in Research Involving Minorities

Genetic differences among ethnic/minority groups

It has been increasingly recognized that genetic difference among ethnic/minority groups is an important determinant in disease risk, progress, prognosis, and patient's responses to treatment. African Americans have high rates of heart disease, including coronary artery disease, stroke, high blood pressure, and heart failure. A report from the American Heart Association indicates approximately 40% of African Americans have some form of heart disease in comparison to 25% of Caucasians.[37] A recent study has shown that African American women with coronary artery disease are twice as likely to have a heart attack than white women.[38] It has also been reported

that people of African, Hispanic, and Native American heritage are more prone to type 2 diabetes compared with their white counterparts.[39] More recent discoveries have shown that genes are potentially involved in the development of various diseases, such as Alzheimer disease, cystic fibrosis, cancer, hemophilia A, Huntington disease, hemochromatosis, fragile X mental retardation, familial adenomatous polyposis, and thalassemia. Although environmental triggers are necessary for ethnic minorities genetically predisposed to develop these conditions, this information has been or will be used in the prevention, early diagnosis, and treatment of these diseases.[35]

Genetic differences or specific genetic factors found among ethnic/minority groups can affect the way people respond to certain medications. For example, the angiotensin-converting enzyme inhibitor, enalapril, reduces the rates of hospitalization in whites but not in blacks.[40] Furthermore, the β-blocker, carvedilol, is more effective than other agents in the same class in reducing the death rates or hospitalization in black patients.[41] It has also been shown that self-declared black patients with severe heart failure appear to benefit from a combination of hydralazine and isosorbide dinitrate when added to background neurohormonal blockade,[42] and retrospective analyses of heart failure trials suggest that black, but not white, patients have a clinically meaningful response to the isosorbide dinitrate/hydralazine combination.[43, 44] In 2005, FDA granted approval for BiDil® (hydralazine/isosorbide dinitrate) to treat heart failure in black patients, marking the first time that the FDA approved a drug for a specific racial group.[45]

Instead of solely relying on epidemiologic information such as ethnic background, the focus of future studies would shift to individual genes that may influence drug response. As people of the same ethnic background may carry similar genes, studies based on race would provide pharmacogenetic information to some extent. An example is a classical study that evaluated the activity of thiopurine methyltransferase (TPMT) in patients receiving thiopurine drugs such as 6-mercaptopurine.[46] TPMT is an enzyme involved in the biotransformation of many drugs and xenobiotic compounds. The activity of this enzyme is present in the human red blood cell and is controlled by a common genetic polymorphism. Patients with inherited low levels of TPMT activity are at increased risk for thiopurine drug-induced myelotoxicity, whereas subjects with high TPMT activities may be undertreated with these drugs. The genetic polymorphism of this enzyme shows distribution pattern varying with respect to ethnic background. Among Caucasians, 89–94% possess high enzyme activity with *TPMT*3A*, compared with low activity in Chinese and African Americans with *TPMT*3C*.[47]

In recent years, the research on pharmacogenetic differences among ethnic minorities has broadened to include a larger range of targets such as multiple metabolizing enzymes, drug transporters, and receptors.[48] Increasing evidence suggests that drug metabolism alone does not account for the observed inter-racial variability in drug disposition or response but other processes, including drug transport, are important determinants of drug disposition. Among the drug transporters shown to play a key role in drug disposition, P-glycoprotein (P-gp) is one of the most extensively studied. The *MDR1* gene encodes P-gp, a drug efflux pump that decreases gastrointestinal uptake and intracellular concentrations of the antiretroviral protease inhibitors in HIV-infected patients. A recent report indicates that while patients with the homozygous CC genotype had higher plasma protease inhibitor levels when treated with these agents, immune responses significantly increased to a greater degree in patients with the TT genotype at the *MDR1* C3435T locus.[49] The wild type alleles of the *MDR1* gene (CC) are more prevalent in the African American population (75%) than in Caucasians and Hispanics (50%).[50] Such racial differences in the *MDR1* gene polymorphisms may contribute to previously recognized racial differences in the clinical response to protease inhibitors.

With a greater understanding of the genetic differences among ethnic/minority groups, it may be possible to select drugs and doses more precisely using compiled genetic information of a specific ethnic group or, possibly, individual patients. This could lead to more effective drug therapy, with greater safety and fewer adverse effects or treatment failures.

Enhancing Research in Genetic Differences Among Ethnic/Minority Groups

Research in genetic differences among ethnic/minority groups can be enhanced by the following:

a. Expanding funding in the genetic research

infrastructure at institutions to increase the capacity to support ethnic/minority research and increase the number of funded investigators to improve outcomes in this research.

b. Including racial and ethnic minorities in prevention, therapeutic, vaccine, and clinical trials in numbers that reflect the current incidence data and genetic background.

c. Developing, evaluating, and sustaining effective interventions to prevent disease progress among racial and ethnic minorities with respect to genetic background.

Inclusion of Minorities in Research

The inclusion and retention of African Americans and other minority groups as clinical research subjects has become an important goal of contemporary clinical research practitioners. The relatively low participation rates of minority test subjects in clinical trials have slowed progress toward a comprehensive understanding of those emergent diseases that affect minority groups. For example, research has shown that although African Americans are over-represented in many chronic illnesses such as hypertension, diabetes, and cancer, studies of these diseases have often failed to attract and at times include enough African American participants to generate meaningful conclusions concerning these populations.

Within the literature, there is no universally accepted definition of the term "minority." It is a socially defined term, used to identify a group deemed to be occupying a nondominant status position. Minorities are distinguished by age, race, ethnicity, and/or cultural heritage. In American society, minorities include American Indian and Alaskan natives, Asian and Pacific Islanders, blacks, and Hispanics. Accompanying minority status is "vulnerability." Regulatory guidance documents such as DHHS regulation 45 CFR 46.111 (b)[51] and FDA regulation 21 CFR 56.111[52] list children, prisoners, pregnant women, handicapped, mentally disabled, educationally disadvantaged, ethnic minority groups, homeless, impoverished persons, and refugees as groups of persons who are "vulnerable." Vulnerable individuals are prone to coercion and exploitation and may participate in clinical trials as a means to obtaining medical care, thus exposing themselves to the risk of manipulation or undue influence from researchers.

Historically, minorities and impoverished persons have been prone to exploitation in clinical research. The Tuskegee experiment provides an example of how African American minorities were victimized and made vulnerable.[53] Impoverished persons, some of whom are minorities, suffer from discrimination; tend to have less access to education, social services, and health care; and are often behaviorally and politically stigmatized. An example of violations of an impoverished minority group occurred in the San Antonio Contraception study, which enrolled 76 impoverished Mexican-American women with previous multiple pregnancies. Without their knowledge, Mexican-American women who sought oral contraceptives at a clinic were placed in a 2-way, crossover study. In the first phase, one group was given a placebo and the other an oral contraceptive. In the second phase, women initially placed on placebo received oral contraceptive and those initially receiving oral contraceptives were crossed over to placebo. Eleven women became pregnant during the study, 10 while using placebo.[54] These impoverished women sought preventative medical care but were exploited by the existing clinical program. Research occurred without their consent and the risks clearly outweighed any benefit.

In 1990, the NIH established one of the first policies requiring the mandatory inclusion of women and other minorities in clinical research.[55, 56] Despite this policy, minorities were poorly represented in clinical trials because issues barring their participation were quite different than that of women. With minorities, the legacy of the Tuskegee study and mistrust of the medical profession were paramount. These guidelines had been developed but did not require the reporting or analysis of the data regarding race or gender, making it impossible to establish the success of these mandates in attracting and retaining women and minorities. In 1994, after reports indicated that these policies were not being uniformly adhered to, the NIH along with the Office of Research on Women's Health (ORWH) and the NIH office of Minority Health joined forces and issued revised guidelines on the necessity of inclusion of women and minorities in clinical research and emphasized the reporting of analysis of sex and race/ethnicity differences in research results in NIH phase 3 clinical trials.[55] Inclusion of the results of subset

analyses was strongly encouraged in all publication submissions. If the analysis revealed no subset differences, a brief statement to that effect, indicating the subsets analyzed, would suffice.[57]

The barriers to increasing minorities' participation in clinical studies are diverse. Mistrust and fear of being "guinea pigs" have been cited by minority subjects as the major reason for not participating in research studies.[58] In some instances, barriers to their participation in clinical trials reside within organizational structures.[59] Organizational barriers exist where there is a lack of racial diversity in the research team or organization. It has been suggested that if the race of the recruiter and the target group are discordant there is even greater challenge.[60] The same is true when recruiters are viewed as outsiders to the community they wish to study.[61] Basic considerations such as the hours of operation of a facility may adversely impact potential subjects' ability to participate. Minorities or impoverished persons are more likely to have hourly paid jobs and, as such, time away from work for research participation negatively impacts their income.

In addition, bureaucratic processes such as penalizing illegal aliens may deny them from participating in clinical trials even though they live, work, and sometimes die in America. As the United States population becomes more diversified, researchers must be cognizant of the impact of laws and a lack of English proficiency of potential minority subjects[62] on attracting and retaining potential subjects.

Barriers to minority participation in clinical trials can also be resource related. Such barriers occur when there are limited resources available to provide the services needed to meet the needs of the target group. This may include the expense required to make culturally relevant brochures, advertisements, cost of translation services, or staff education. In some cases, the incurred costs of participating are not offset by remuneration received from participating in clinical trials.

Addressing Barriers to Minority Research Participation

In order to improve the health status of underserved populations, including racial and ethnic minorities, several agencies have been created, including the Office of Minority Health in the U.S. Department of Health and Human Services (1985) and the Centers for Disease Control and Prevention (1988). The Disadvantaged Minority Health Act was passed by Congress in 1990.[63] More recently, the initiatives Healthy People 2010 and Racial and Ethnic Approaches to Community Health (REACH) 2010 have been launched to increase quality and years of healthy life and eliminate health disparities. In particular, Racial and Ethnic Approaches to Community Health (REACH) 2010 is designed to eliminate disparities in six priority areas in which racial and ethnic minorities experience serious disparities in health access and outcomes, including infant mortality, deficits in breast and cervical cancer screening and management, cardiovascular diseases, diabetes, HIV infections/AIDS, and child and adult immunizations.[64] In addition, Healthy People 2010 reinforced the need to include enough individuals from different segments of the population in clinical trials.[65] Part of the Healthy People 2010 initiative is to gain comprehensive understanding about diseases that affect minority groups. To achieve this objective, clinical trials must be based on populations that are large enough to eliminate statistical bias.

It has been suggested that the structural, organizational, and economic barriers for recruitment and retention of minorities and impoverished subjects can be achieved through comprehensive planning that considers structural and individual influences negating minority group participation in clinical trials.[59, 66] Among the more important issues to be addressed include the following:

a. Building trust within minority communities through communication about the need for their participation and making participants fully cognizant of their rights as clinical test subjects.
b. Ensuring cultural competency of all clinical researchers.
c. Building diversity among the student population of clinical researchers who are enrolling in relevant college programs of study. In keeping with this thrust to an integrative approach, the Accreditation Council for Pharmacy Education adopted revised Doctor of Pharmacy education standards that included diversity and cultural competency.[67] These standards were released in February 17, 2006 and will become effective July 1, 2007.

d. Ensuring due vigilance in reporting participation rates of clinical research test subjects and analysis of these data with regards to race and gender, such as that required of federally funded programs.

In addition to the above, further outreach approaches on the recruitment and retention of minority research subjects can be found in *Outreach Notebook for the Inclusion, Recruitment and Retention of Women and Minorities in Clinical Research*, published by ORWH.[68]

Summary of Research in Minorities

Numerous barriers exist for including minorities in clinical research. These barriers include language difficulties, financial barriers, mistrust by minority subjects, and researchers' lack of cultural competency. Although federal regulations and guidances encourage the inclusion of minorities in clinical research, improvement of minority participation in research need to be addressed via communities, among clinical researchers, and within education institutions.

Research in Vulnerable Populations

Prisoners, refugees, and the mentally ill are considered to be vulnerable populations for whom measures of protection from possible exploitation and harm need to be taken. There is considerable debate surrounding the use of these persons for clinical research. The principle of "respect for persons" would require that these individuals not be deprived of the opportunity for involvement in research. However, under conditions of incompetence or the institutional setting, protection from coercion or undue influence must be afforded.[69]

Refugee Populations

A refugee has been defined as "a person who is outside his/her country of nationality or habitual residence; has a well-founded fear of persecution because of his/her race, religion, nationality, membership in a particular social group or political opinion; and is unable or unwilling to avail himself/herself of the protection of that country, or to return there, for fear of persecution."[70] Internally displaced people (IDPs) are similar, but still live within the borders of their home country. Refugees are considered to be vulnerable for many reasons. If they are non-citizens of the country in which they are residing, they may not be afforded the same legal rights and regulatory protections of the host country. The United Nations Convention on Refugees in 1951 and the subsequent Protocol in 1967 provided guidance regarding the treatment of refugee populations to include travel documents, unification of families, welfare services, freedom of religion, access to courts, and employment.[70] Host countries can use their own judgment in the application of domestic law to these people. Political upheaval and social destruction are often inherent in the creation of a refugee population. Refugees are the victims of hostility by those who drove them from their home country and may not be entirely welcomed by the citizens of the country to which they fled. Authorities may do little to ensure that ethical conduct of clinical research is maintained in the refugee population.[71] The vast majority of internally displaced peoples are women, children, the elderly, and the disabled. Because IDPs are living within their own country, there is no international agency to protect them; it is assumed that their own government will bear that responsibility. Often, people are transplanted within their own country due to internal conflicts that cause one of the factions to be favored to other factions. Due to this, IDPs are not protected or cared for because of prejudice.[72]

The issue of conducting research in refugee populations is not specifically addressed or mentioned in international regulations that provide direction to those involved in clinical research. This includes the Belmont report, the Nuremberg Code, the Declaration of Helsinki, and the Council for International Organizations of Medical Sciences.[32, 69, 71] Emergency relief personnel may work under conditions in which the interventions provided may lack proven scientific efficacy. There is a need to establish evidence-based emergency practices while protecting the refugee and internally displaced populations from possible exploitation or harm.[73]

Challenges in Research Involving Refugees

There are numerous barriers to ethical clinical research using refugees and internally displaced people as subjects. They are economically impoverished and may be easily influenced to participate in research that they may believe will bring them financial gain, improvements in living conditions, or the semblance of cooperation with

authorities. Effective communication with these persons may be complex due to obstacles created by differing languages and cultural norms. Refugee and IDP populations may be appealing to researchers because of the "captive audience" that they represent. Refugees may be placed in "camps" that will limit their movement and be consistent in population. Follow-up studies may be easier to design and implement.[71] Researchers must consider the psychological and social stressors experienced by refugees that may contribute to varying degrees of mental status changes. Voluntary informed consent of the type that would be expected in other areas of clinical research may be extremely difficult to obtain from this group of subjects. The question of whether consent given in crisis situations meets the test of being freely given must be answered.

The ethical requirements for studies involving this population must be higher than those needed for a less vulnerable population. Refugees participating in clinical research may not receive significant benefit from the study, but may incur risks. Humanitarian researchers believe that studies evaluating the extreme problems experienced by this population will improve knowledge that will benefit future people in crisis situations.[73] Guidelines for research in refugee and IDP populations have been proposed and include the folowing[73]:

a. Undertake only those studies that are urgent and vital to the health and welfare of the study population.
b. Restrict studies to those questions that cannot be addressed in any other context.
c. Restrict studies to those that would provide important direct benefit to the individuals recruited to the study or to the population from which the individuals come.
d. Ensure the study design imposes the absolute minimum of additional risk.
e. Select study participants on the basis of scientific principles without bias introduced by issues of accessibility, cost, or malleability.
f. Establish the highest standards for obtaining informed consent from all individual study participants and, where necessary and culturally appropriate, from heads of household and community leaders (but this consent cannot substitute for the individual consent).
g. Institute procedures to assess, minimize, and monitor the risks to safety and confidentiality for individual subjects, their community, and their future security.
h. Promote the well-being, dignity, and autonomy of all study participants in all phases of the research study.

Prisoner Populations

Prisoners who may be recruited to participate in clinical research are considered vulnerable research subjects for many of the same reasons as refugees and the internally displaced. As opposed to the refugee population, regulatory guidance does exist to aid researchers in developing research protocols that acknowledge these vulnerabilities. The Ethical Principles and Guidelines for the Protection of Human Subjects of Research, or The Belmont Report, defined and summarized basic ethical principles pertaining to general clinical research.[69]

Challenges in Research Involving Prisoners

Socially, prisoners are deprived of many freedoms and are controlled by the environment in which they live. They rely on the prison system for basic needs that include shelter, food, and clothing. A prisoner may believe that he may receive an improved living condition, extra privileges, or a shortened prison sentence secondary to his participation as a research subject. The prisoner may not be able to make sound judgments with respect to voluntary informed consent to participate in a study because of his perceived need to please those in power or receive increased entertainment or exercise time. A prisoner may simply be bored with the regimented life in a prison. Participation as a research subject may relieve boredom or allow the prisoner the opportunity to distinguish himself from other inmates.[71]

The prison population is appealing to researchers for many of the same reasons as the refugee population. Depending on the length of the prison sentence, prisoners will be living in the same place with the same environmental and social conditions throughout the length of the study. Follow-up studies would be less complex to implement in this population. Unfortunately, there is evidence of researchers conducting risky research in this population due to the perception of a reduced societal "value" of prisoners relative to non-incarcerated individuals.[71]

Title 45CFR46, Subpart C, of the U.S. Code of Federal Regulations, entitled Additional Protections Pertaining to Biomedical and Behavioral Research Involving Prisoners as

Subjects, was enacted by the Department of Health and Human Services (DHHS) in 1978 to address many concerns relative to conducting research in the prisoner population.[74] These regulations are applicable to all biomedical and behavioral research that is conducted or supported by the DHHS. Subpart C provides safeguards to prisoners to ensure that a voluntary and uncoerced informed consent is given for participation in clinical research. The membership of the IRB that reviews and approves research protocols in the prisoner population must include a prisoner member or a prisoner representative with appropriate background. Assurances are provided that parole boards will not be aware of a prisoner's participation in a study and that the prisoner is informed of this provision.[74]

Title 45CFR46, Subpart C also defines permitted research in the prisoner population. Proposed research must only include the following[74]:

- Study of the possible causes, effects, and processes of incarceration, and of criminal behavior, provided that the study presents no more than minimal risk and no more than inconvenience to the subjects.
- Study of prisons as institutional structures or of prisoners as incarcerated persons.
- Research on conditions particularly affecting prisoners as a class; and research on social and psychological problems such as alcoholism, drug addiction, and sexual assaults provided that they study may proceed only after the Secretary has consulted with appropriate experts, including experts in penology, medicine, and ethics, and published notice, in the Federal Register, of his intent to approve such research.
- Research on practices, both innovative and accepted, which have the intent and reasonable probability of improving the health or well-being of the subject.
- Except as provided above, biomedical or behavioral research conducted or supported by DHHS shall not involve prisoners as subjects.

The "Mentally Ill" or "Decisionally Impaired"

An accurate term to describe individuals with diminished mental capacity who do not have the consistent ability to provide voluntary informed consent for participation in clinical research studies cannot be found in the guidelines and recommendations put forth by consensus experts and advisory committees. "Mentally ill," "decisionally impaired," "mentally incompetent," and "cognitively impaired" are all descriptors that have been used.[71, 75–77] Although controversy exists as to how to define these individuals, there is agreement that, while persons who are mentally ill are a vulnerable research population, they may be able to be autonomous in participating in clinical research. A statement published by the NIH acknowledged that impaired cognitive ability is not restricted to persons with neurologic, psychiatric, or substance abuse problems, nor should it be assumed that persons with these disorders have questionable capacity.[75]

Challenges in Research Involving Persons with Cognitive Impairment

Disease states that are associated with cognitive impairment include dementia, delirium, schizophrenia, bipolar disorder, and depression. Persons suffering from schizophrenia, bipolar disorder, and depression will have fluctuating courses of illness that may produce periods of impaired capacity to understand the risks and benefits of involvement in research. Even when it is perceived that the subject is able to provide informed consent, it may be difficult for him/her to anticipate the consequences relative to future recurrences of illness.[78] Individuals diagnosed with dementia can be expected to follow a prolonged, consistent decline in cognitive functioning that will continue to impact decision-making abilities. Substance abuse disorders can result in states of intoxication similar to delirium and reduce cognition and attention.[78]

The mentally ill are often stigmatized in many settings by those who assume they are dangerous, impaired, and unable to provide any care for themselves.[76] This compounds the social isolation experienced by persons with severe mental illness who may already feel inadequate. Mentally incompetent individuals may be prone to influence by those close to them such as caregivers or individuals involved in their treatment. They may consent to involvement in clinical research in an attempt to be cooperative or gain acceptance.[71] Living in an institutionalized environment creates concerns similar to those of the prison population, where the belief may be that participating in a study

may allow for special privileges. The consequences of mental illness for those able to live in community settings can be staggering. Unemployment, substance abuse, disability, homelessness, and incarceration are common, contributing to an increased vulnerability in this population.[79] Persons with severe mental illness are often considered to be nonadherent to treatment recommendations, including missing appointments, refusing to follow up on referrals, and noncompliance with medication treatment regimens.[80] Due to these problems, many individuals will be continually hospitalized, both acutely and chronically.

The "deinstitutionalization" of the mentally ill began in the mid-1980s in the United States.[80] Those who were deemed capable of residing in the community were released to group homes, apartments, and shelters. The availability of supervised or structured housing was limited in many areas and the burden on the community mental health centers to provide care was strained. As a result, the number of mentally ill homeless soared and, with it, an increase in comorbid substance abuse and incarcerated persons. Today, jails and prisons can be considered "holding areas" for the mentally ill. It is estimated that approximately 16% of those in state prison facilities have been diagnosed with a mental illness.[80]

Addressing Barriers to Research Participation by Mentally Incompetent Individuals

It is well recognized that research relative to both the underlying pathophysiological processes of psychiatric disorders and the development of new treatments for the illnesses is of paramount importance. It is estimated that 5 to 10 million adults in the United States suffer from severe mental illness and that the annual cost of untreated mental illness is more than $100 billion.[79] It is not a question of whether to engage in clinical research in the mentally ill, but how to ensure that study subjects who may be cognitively impaired are protected from unethical or victimizing research.

The practice of excluding the mentally incompetent from research studies would solve the ethical dilemmas with respect to informed consent and the Nuremberg Code, but would cease advancement of knowledge in the understanding of the physiologic deficits of mental illness and the creation of more effective treatment options. In order to avoid subjecting those with mental illness to unnecessary involvement in research, it has been suggested to limit research to studies that are relevant to the conditions prevalent in the decisionally impaired.[78] Advocacy groups such as the National Alliance for the Mentally Ill support research in severe mental illness, insofar as it is consistent with the highest scientific and ethical standards for protection of research subjects.[79]

The NIH recommend several "points to consider" when developing research involving persons with a questionable capacity to consent.[75] The first is to avoid potential conflicts of interest. Often, the researchers who are recruiting these individuals are clinicians who are also providing care and it may be difficult for the mentally impaired person to differentiate between research and treatment, leading to confusion for the potential participant. Therefore, it is important that the consent process indicate differences between clinician and investigator and between treatment and research. The IRB should include a member who is experienced in working with people with mental illness or a member from an advocacy group. The capacity to consent to participation must be assessed by the investigator, including the individual's understanding of the risks, benefits, and alternatives to participation in the study. The consent process will require ongoing assessment due to fluctuations in the decision-making capacity of the individual. Safeguards should be put in place within the study protocol to account for increases in decisional impairment as the study progresses. The study participant should be reeducated about the study protocol at frequent intervals to assure continued understanding and to provide assessment of cognitive stability. The IRB is advised to appoint an independent monitor to be present when the study investigators interview potential study candidates and/or their caregivers to ensure ethical conduct of research when the study involves greater than minimal risk.[75]

The use of proxy consent was first adopted in the formulation of the Declaration of Helsinki in 1964 as an alternative for those study participants unable to give direct informed consent.[71] The most recognized source for proxy is consent given by one who is legally authorized to do so. A question arises concerning what kind of clinical research is appropriate for proxy consent. Therapeutic research involves direct benefits to the participant, while nontherapeutic research does not. It is generally accepted that

nontherapeutic research can be performed using normal subjects and is, therefore, not appropriate for mentally incompetent individuals. There is concern that use of proxy consent does not adequately protect from exploitative research. Because of this, the NIH suggests that, along with proxy or surrogate consent, the assent of the individual study participant be obtained. The autonomy of the individual should be respected, as well as the right of that individual to withdraw from the study at any time.

An advance directive to participate in research that is executed at a time that the mentally incompetent individual is competent to consent may be considered where the law permits.[75] Another safeguard that may be implemented by the study investigator is a waiting period. Those who are cognitively impaired may need more time to consider the educational information that they are given regarding the study protocol or they may want to consult with family members or trusted caregivers. Information should be provided in small increments over time to allow for improved comprehension by the individual and the greatest likelihood of voluntary informed consent.[75]

The World Medical Association (WMA) adopted a policy statement on ethical issues concerning patients with mental illness in 1995.[81] In it, the WMA states that "the discrimination associated with psychiatry and the mentally ill should be eradicated; this stigma often discourages people in need from seeking psychiatric help, thus aggravating their situation."[81] The inability of the mentally ill patient to exercise autonomy or provide informed consent does not differ from any other legally incompetent patient. The person diagnosed with a mental illness should not automatically be assumed to be legally incompetent and his/her judgment should be respected.[81] The statements of this world body provide the foundation upon which to build ethical and appropriate standards for the conduct on clinical research studies in the mentally ill.

Summary of Research in Vulnerable Populations

Numerous barriers and challenges exist in conducting research in vulnerable populations. Refugees and prisoners are appealing to researchers because of the "captive audience" that they represent. Guidelines for research in refugee and internally displaced populations have been proposed to protect these vulnerable populations from possible exploitation or harm. Similarly, federal regulations were also enacted to protect prisoners participating in biomedical and behavioral research. Mentally ill and decisionally impaired persons constitute another group of vulnerable research subjects. It is recognized that clinical research in subjects with mental illness or dementia is of paramount importance, as the advancement of understanding of these disorders will encourage development of new treatments for these disorders. Recommendations have been put forth by the NIH and WMA for protecting mentally ill and cognitively impaired persons in clinical research.

Research in Women

Sex-related Variability in Pharmacokinetics and Pharmacodynamics

Over the last few decades, it has become increasingly evident that there are sex-related differences in pharmacokinetics and pharmacodynamics.[82, 83] Pharmacokinetics studies focus on the relationship between drug dosage and concentration of drug over time in blood or plasma and preferably in cells and tissues, which may better represent the site(s) of action.[84] Sex-based differences in bioavailability, distribution, metabolism, and elimination exist that contribute to variability in drug response. Bioavailability of oral agents appears to be higher in women than men, while transdermal drug administration does not seem to be affected by sex.[85] Gastric emptying time is slower in women than men, primarily due to the effects of estrogen and transit time may vary throughout pregnancy and the menstrual cycle.[84]

Since body composition, plasma volume, and plasma protein binding vary between women and men, it follows that the rate and extent of distribution may vary. Women have higher body fat, lower average body weight, and smaller average plasma volume than men. Therefore, lipophilic drugs (such as benzodiazepines and neuromuscular blockers) have increased distribution volumes in women.[84, 85] Albumin concentrations do not consistently vary by sex, but exogenous estrogens can increase the levels of serum-binding globulins and may impact sex hormone-binding globulin, corticosteroid-binding globulin, and thyroxine-binding globulin in women.[84] Many believe the most important factor in adjusting medication dosages between women and men may be adjusting for body size,

especially for loading doses or drugs with a narrow therapeutic window.[86, 87] Overall, the extent of clinical differences in distribution has not yet been precisely defined.

Of all pharmacokinetic parameters, drug metabolism seems to play the greatest role in variability between women and men. Sex-based differences exist in both phase I (oxidation, reduction, and hydrolysis mediated through the cytochrome [CYP] P_{450} system) and phase II (glucuronidation, sulfation, acetylation, methylation, or glutathione conjugation of the parent drug or its phase I metabolite) reactions. A classic example of a phase I reaction difference between women and men is shown with drugs metabolized by the isozyme, CYP3A4. Agents such as erythromycin, midazolam, and verapamil are typically metabolized and cleared faster in women. Reasons for this difference are thought to be a variance in CYP content or activity.[84] Although most phase II reactions are primarily due to racial and genetic differences, some data on sex differences exist. A comprehensive discussion of these reactions can be found elsewhere.[85] Conflicting data exist on whether menstrual cycles, menopausal status, or estrogen and progesterone levels in hormone therapy significantly affect drug metabolism in women.[84] In addition, sex-related factors, such as use of hormonal contraceptives, may affect drug metabolizing enzymes.[82]

Drug excretion is usually mediated by the kidney or liver. Glomerular filtration, because it is directly proportional to weight, is higher on average in men than women. This reinforces the importance of sex-adjusted dosage selection for renally excreted drugs with narrow therapeutics windows and/or adverse effects related to concentrations.[85] Further study on sex-based differences in renal excretion is warranted to clearly explain the contribution of this factor.

Pharmacodynamic and behavioral differences between men and women have been well documented. A pharmacodynamic difference exists when similar plasma concentrations of a drug in the two sexes do not produce the same pharmacologic outcomes. Numerous examples in pharmacodynamic differences exit. It is also well documented that women experience drug-related adverse events more frequently than their male counterparts.[88–90] For example, multiple studies have shown that women seem to have more frequent and severe side effects with protease inhibitors and nucleoside reverse transcriptase inhibitors than men.[91–96] Women tend to demonstrate greater analgesic effects with opioid agonists compared with men.[97–100] In clinical trials, women have not always experienced the same benefits as men, especially with regards to cardiovascular outcomes.[101, 102] Negative consequences may result with differences between women and men for delay in presentation and treatment with coronary heart disease.[103] Women also appear to score lower than men on measures of health status and functioning in diabetes.[104, 105] Despite several examples of these pharmacodynamic and behavioral differences between women and men, little is known about the direct interplay of pharmacokinetics and especially genetics with these factors.

Sex-related Genetic Differences

The distinct role of genetics in sex-based differences is much less clear than pharmacokinetics. Findings of differences at the biochemical and cellular level, specifically at the XY (male) and XX (female) sex chromosome level, are beginning to more clearly define how women and men may express individual drug variations.

In 2001, the Committee on Understanding the Biology of Sex and Gender Differences, Institute of Medicine published, Exploring the Biological Contributions to Human Health: Does Sex matter?[87] The report explored sex differences and determinants of these differences at the biological level. There are several mechanisms for the genetic basis of sex differences:

1. Genes on the Y chromosome are expressed only in males, with many having no counterpart on the X chromosome; therefore, expression of Y genes will be limited to males.
2. Some genes on the X chromosome are expressed at higher levels in females than in males. Although there is a process of inactivation of one of the two X chromosomes in females to attempt to equalize the "effective dosage" of the X chromosome, not all genes on the inactivated X chromosome respond to this process. The relatively few genes not equalized can have significant impact on the phenotypes of cells.
3. The expression of many genes is likely to be influenced by hormones. Therefore, some genes may have limited expression in sexually dimorphic tissues or cell types (e.g., the ovary, breast, testis, and prostate).

At this point, only a limited number of genes have been examined for sexual dimorphism. However, some data are known about possible effects between the sexes of any variant in an X-chromosome-linked gene. For example, mosaicism occurs in females where the two X chromosomes express the alleles of the paternally inherited X chromosome and maternally inherited X chromosome, respectively. Therefore, expression of an X-chromosome-linked phenotype is often much more variable in females than in males. With hemizygosity, males have only one X chromosome and functional variants cannot be "masked" by a second X chromosome. Thus, males often demonstrate a more common, clear, or extreme version of the variant phenotypes than females. Scientific discovery of differences in expression through the female and male genome should produce fascinating findings.

The issue of whether there are differences in the biochemistry between female and male cells has also been explored. There is a potential for 1 in 20 biochemical reactions to differentially affect male versus female cells because the sex chromosomes comprise approximately 5% of the total human genome.[87] With this knowledge, it is possible to imagine that male and female cells will differ in at least some aspects of basic biochemistry, especially given the complexity of most biological pathways. Table 2 displays the genetic factors that may differentially affect the basic biochemistry of male and female cells.

Genomic imprinting is the concept that some genes are expressed only from the maternal allele and that others are expressed only from the paternal allele.[87] This concept reinforces the acknowledgment that there are multiple biochemical differences between the gametogenic cells of males and females and that these differences may affect the expression of genetic information in the next generation. For example, since males have only one maternal X chromosome and female have both a maternal and paternal X chromosome, X-chromosome-linked genes that pass through the paternal line

Table 2. Progression of public guidelines regarding women in clinical research

Year	Event
1986:	NIH established New Policy on Women's Health Research to increase participation in women's health research
1988:	FDA issued Guideline for the Format and content of the Clinical and Statistical Sections of New Drug Applications. This guideline emphasized the necessity of including demographic data and their analyses in NDA applications.
1990:	Institute of Medicine Report stated that NIH was not adequately implementing its 1986 policy to increase participation in women's health research.
1990:	General Accounting Office study revealed that implementation of NIH's 1986 policy was slow and gender analysis was not implemented. This subsequently led to media and public reaction.
1990:	Office of Research on Women's Health was established at the NIH to simulate and serve as a central point for women's health research. A scientific workshop and public hearings were held at Hunt Valley, Maryland. These series of scientific meetings produced the report "The National Institutes of Health: Opportunities for Research on Women's Health." The Hunt Valley report served as a guide for women's health research at the NIH.
1993	NIH Revitalization Act was enacted by Congress. This law requires the inclusion of women and members of racial and ethnic minority groups in all federally-funded research studies.
1993	FDA issued "Guidance for Industry. Guideline for the study and evaluation of gender differences in the clinical evaluation of drugs." Under this new guidance, pharmaceutical sponsors were required to include a full range of patients in their clinical studies, and to carry out analysis to evaluate differences in subsets of patients. The guidance also emphasized that women should be included in *all* phases of clinical drug development. Women and minorities must be included in phase III clinical trials *in numbers adequate* to allow for valid analyses of differences in intervention effect. Cost is not allowed as an acceptable reason for excluding these groups.
1994	Offices of Women's Health were established at the FDA and Centers for Disease Control and Prevention
1994	FDA issued guidance lifting the ban (in place since 1977) barring the inclusion of women with childbearing potential from Phase 1 and early Phase 2 clinical studies. This ban had been a significant barrier to women's participation in clinical trials.
1997:	FDA issued "Proposed Rule on Investigational New Drug Applications: Proposed Amendment to Clinical Hold Regulations for Products Intended for Life-Threatening Diseases." This rule allowed the FDA to put protocols under an IND on clinical hold if a pharmaceutical sponsor proposed exclusion of women or men with reproductive potential.
1997:	The Office of Women's Health Research published "An Agenda for Research on Women's Health in the 21st Century." These series of documents expanded the Hunt Valley vision for women's health research.

Table 3. Genetic factors that may differentially affect the basic biochemistry of male and female cells.[87]

Gender	Genetic factors
Male	X-chromosome-linked recessive mutations Expression of Y-chromosome-specific genes Changes in androgen-responsive genes in germ-line or somatic cells
Female	Expression of some genes from both X chromosomes Defect in initiation or maintenance of X-chromosome inactivation Changes in estrogen-responsive genes (e.g., the HER2 gene in breast cancer) in germ-line or somatic cells

have the potential to affect female offspring, but not male offspring. These findings may have more significant clinical implications when applied to behavior or cognitive function in males and females. In addition, recent evidence of differential timing in the establishment of maternal and paternal methylation imprints serve as an example of the sexual dimorphism of imprinted gene expression.[106]

Last, the male produces billions of sperms from a population of stem cells that continue to divide throughout the entire adult life and the female produces a relatively small number of ova (approximately 500) from a limited number of oocytes that form in embryogenesis. This numerical difference indicates that most mutations resulting from DNA replication errors take place in the male germ line. However, the magnitude of this difference and the clinical significance has yet to be determined.

Males and females have partially different genomes. The findings discussed here indicate that there are multiple differences in the genetic information, biochemistry, and pharmacokinetics of males and females that can affect an individual's health and drug response. What we have learned is that many of these differences do not arise solely because of the hormonal environment. Further research in sex-based differences regarding pharmacokinetics and genetics is warranted and important to further our understanding of the human condition.

In summary, sex-related variability and genetic-based sex differences in drug response makes it necessary to include women as research subjects in clinical studies. Despite this fact, women have been historically under-represented in clinical trials.[107, 108]

Challenges in Inclusion of Women in Research

The ethical and social considerations of including women in research have been extensively discussed.[109, 110] The current concern regarding participation of women in clinical studies arises from conflicting public policy positions: protectionism and access. The need for research subject's protection was emphasized in the 1950s and 1960s in response to unethical research conduct. The discovery of adverse outcomes in children who had fetal exposure to certain drugs during pregnancy further reinforced the protectionism emphasis. In the mid-1970s, legislation was passed to protect research subjects from unethical treatment. The regulations resulting from this legislation also were designed to protect against fetal injury by restricting the inclusion of women of childbearing potential and pregnant women and in drug trials. However, women-specific conditions and sexual dimorphism in drug response made researchers recognize the need to recruit women subjects in drug studies. Over the past two decades, the NIH and FDA have switched from a protectionistic to an inclusive policy regarding women participating in clinical trials (Table 3).

Despite these regulatory shifts in public policies to a more inclusive agenda to encourage women participation in clinical studies, recent analyses still show an alarming under-representation in women participants in phase 1, 2, and 3 clinical trials.[110, 111] In an analyses of four high-impact journals, Vidaver et al found that 20% of phase 3 clinical trials excluded women, and only up to 25% of articles included an analysis of results specific to gender differences.[111]

Reasons for a lack of women participation in clinical studies are multifactorial. Inclusion and exclusion criteria, especially in the field of cardiovascular medicine, may favor inclusion of men.[110] Women are usually older and therefore have more comorbidities when they experience their first myocardial infarction. These comorbidities may render them less likely to participate in clinical trials. It has also been documented that women are less likely to provide informed consent,[112] and male physicians are less likely to enroll female than male patients into clinical studies.[113] In addition, phase 1 studies and studies in healthy volunteers have a higher rate of excluding female participants,[110] suggesting that some investigators may still consider it unethical to expose women of childbearing potential to a drug without any benefit other than a small financial gain.

Addressing Challenges in Inclusion of Women in Research

The appropriate inclusion and representation of women and minorities in biomedical research and clinical trials is an explicit criterion evaluated during reviews of such proposals for NIH funding.[114] This criterion also applies to research conducted at NIH-funded General Clinical Research Center units at local institutions. These provide principal investigators incentives to ensure recruitment of balanced and representative mix of gender and minority groups into their research projects.

In addition, the OWRH has published an Outreach Notebook for the Inclusion, Recruitment and Retention of Women and Minorities in Clinical Research.[68] Some strategies that may aid the recruitment and retention of women in clinical research include the following:

a. Involving the community: For example, the support and collaboration of women community physicians who provide care for the targeted population can be solicited.
b. Involving the participants: For example, women participants should be included in the design of the research and preparation of study materials to be sure they meet their needs. In addition, women respond positively to messages of altruism that convey the benefits of research to future generations.
c. Staffing of the research team: For example, women investigators and staff may foster greater trust among female participants.
d. Addressing logistical and financial need: For example, offering childcare, maintaining extended and flexible clinical hours, and other financial incentives may offset some inconveniences due to research participation.
e. Improving communication: Allowing extra time to review study procedures and benefits, questions and answers, for participants with special needs (e.g., parents with young children).

Summary of Research in Women

Sex-based variations in pharmacokinetics and pharmacodynamics have become increasingly evident. In addition, genetics may play a role in defining how men and women respond differently to medications. Sex-based variability in drug response makes it necessary to include women as research subjects in clinical studies. Historically, women were under-represented in clinical trials, in part because they were "protected" from participation due to a concern of fetal exposure to drugs. Over the past two decades, the NIH and FDA have switched to an inclusive policy, encouraging inclusion of women in clinical studies. However, challenges to inclusion of women in research still exist. The OWRH has published strategies that may aid the recruitment and retention of women in clinical research.

Conclusion

Although regulatory policies governing research in women, minorities, elderly, and children have switched from a protectionistic to an inclusive stance, these populations are still under-represented in clinical research. The participation of individuals with cognitive impairment, inmates, and refugees in clinic research requires addressing special social and ethical challenges. The lack of participation in research by women and these other special populations result in a gap of medical knowledge regarding the health and appropriate medication use of these groups. In this paper, current policies from regulatory agencies, expert, and other advocacy groups are presented. Pharmacist-researchers should be cognizant of these guidelines and be an advocate for the inclusion and rights of women and other special populations in research.

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