THE GENETICS OF INSULIN RESISTANCE IN FAMILIES WITH POLYCYSTIC OVARY SYNDROME

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University of Pittsburgh, 2006

Polycystic ovary syndrome (PCOS) is a familial disorder characterized by major metabolic consequences related to insulin resistance (IR), including T2DM and metabolic syndrome (MS). There is mounting evidence, which supports association of each of G-174C variant in IL-6 and Pro12Ala variant in PPARλ genes with PCOS-associated biochemical or metabolic features in hyperandrogenic and PCOS adults/adolescents. The major aim of this study was to demonstrate the ability to enroll PCOS probands, who have one or more clinically PCOS-diagnosed woman, and their multigenerational family members for a total sample size of 100-125 to study IR and inflammation markers in such families. Additional important aims were to test for linkage between the IL6 and PPARλ genes' loci and for associations between the IL6G-174C SNP and the Pro12Ala PPARλ SNP with IR, MS and its components, serum inflammation levels (IL-6 and CRP) and testosterone in this special population of PCOS families. IR was defined by a HOMA-IR value > 3.9 in adults and HOMA-IR values which are age-gender specific in adolescents. MS was defined according to the ATP III diagnostic criteria in adults and the same criteria, modified for age in adolescents. In total, 101 individuals were recruited from 9 multigenerational extended families; eight of the families were Caucasian and the remaining was African American. No evidence for linkage of each of the IL6 and the PPARγ markers to any of the examined phenotypes was found. However, interesting significant SNP-phenotype associations were found in this population of PCOS families. The Ala12 allele was found to be negatively associated with diastolic blood pressure (DBP) and with fasting glucose. Moreover, the G allele of the IL6 SNP was found to be positively associated with DBP, serum IL6 and testosterone levels. These associations are particularly important because they were adjusted for covariates which are known or were found to be significantly associated with the outcome in our population and were the results of the variance components association test, a test which

accounts for family relationships. The findings are of major public health significance, mainly because they are the first to be reported in PCOS extended families.

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1.0 INTRODUCTION AND BACKGROUND

1.1 SIGNIFICANCE OF INSULIN RESISTANCE IN PCOS

Polycystic ovary syndrome (PCOS), a disorder characterized by hyperandrogenism and chronic anovulation, is one of the most common endocrinopathies in premenopausal women (1) and the most common cause of anovulatory infertility. Currently, there is no consensus on the criteria for the diagnosis of PCOS and the differences in the criteria used for the diagnosis of PCOS in the different studies conducted to date is certainly hampering the progress on our knowledge of the pathogenetic mechanisms underlying this prevalent disorder. Most clinicians and researchers in the United States and from Southern Europe have used the diagnostic criteria recommended by the 1990 PCOS conference held at the National Institute of Child Health and Human Development(NICHD): Clinical and/or biochemical hyperandrogenism, menstrual dysfunction, and exclusion of specific diseases of the adrenal, ovary, or pituitary (2). Most researchers in other parts of the world rely mostly on ovarian morphology for this diagnosis, whereas menstrual dysfunction is not required (3). In 2003, a consensus workshop proposed a revision of the criteria for the diagnosis of PCOS, of which two of the following three would be needed: 1) oligoovulation and/or anovulation 2) clinical and/or biochemical signs of hyperandrogenism, and 3) polycystic ovaries, together with the exclusion of other etiologies such as congenital adrenal hyperplasia, androgen secreting tumors, or Cushing's syndrome (4, 5). Most studies on the prevalence of polycystic ovarian syndrome in the United States give unreliable results because of the selection bias that occurs when a referral center for polycystic ovarian syndrome reports on its experience. Based on an unselected sample of 277 white and African-American women between the ages of 18 and 45 years who presented for a University employment physical in Alabama, the overall prevalence of polycystic ovarian syndrome by the NICHD definition was 4–4.7% for white women and 3.4% for African American women (6). Although this is less than

previous estimates of 5%-10%, the 4% figure still implies that approximately 3 million reproductive-aged women have polycystic ovarian syndrome in the United States. In clinical practice, women with PCOS present with infertility (mean incidence, 74%), menstrual irregularity (dysfunctional bleeding, 29%; amenorrhea, 51%), hyperandrogenism (69%), and virilization (21%) (7). The endocrine profile of women with PCOS is characterized by high plasma concentrations of ovarian and adrenal androgens, gonadotropin abnormalities, a relative increase in estrogen levels derived from conversion of androgens, reduced levels of sex hormone binding globulin (SHBG), and often high levels of insulin(8). The etiology of the syndrome is largely unknown, hampering efforts to identify the genetic mechanisms involved in the pathogenesis of this disorder.

The miscarriage rate in PCOS, if pregnancy is achieved, is about 30% of all pregnancies, which is double the rate for early miscarriage in normal women (9, 10). Obesity frequently complicates polycystic ovarian syndrome but is not a defining characteristic. Approximately 60-70% of PCOS patients in the United States are obese (11), with a central body fat distribution pattern described as visceral obesity that is well known to be highly associated with insulin resistance (IR). However, PCOS patients have evidence of insulin resistance independent of obesity (12, 13, 14). Insulin sensitivity is decreased by 35%-40% in women with PCOS, independent of obesity, a decrease similar in magnitude to that seen in type 2 diabetes mellitus (15); still, any degree of obesity further impairs insulin action. About 50% to 70% of all women with polycystic ovary syndrome (PCOS) have some degree of insulin resistance (16). It is now evident that PCOS has major metabolic consequences related to insulin resistance. Insulin resistance in PCOS may be considered a risk factor for gestational diabetes (GD) (17); the prevalence of GD in PCOS patients has been reported to be 40-46%. A link between insulin resistance and hypertensive disorders in pregnancy has been widely reported; preeclampsia is reported to be more frequent in PCOS patients than in normal women (18) and in one case control study, the incidence of this disorder was found to be as high as 28.5%(19). Evidence supporting the possibility of insulin resistance playing a role in the development of endometrial cancer has been provided (20,21); increased risk for endometrial cancer was reported in women with increased serum levels of insulin(22)and lower serum levels of SHBG(23), both prominent features of women with PCOS and of insulin resistance. In addition, an increased prevalence of endometrial cancer among women with PCOS, including young women with the disorder has

been reported (24, 25). Insulin resistance is associated with an increased risk for several disorders, including type 2 diabetes mellitus (T2DM) or , hypertension, dyslipidemia (low high-density lipoprotein cholesterol and high triglycerides), elevated plasminogen activator inhibitor type 1 (PAI-1), elevated endothelin-1, endothelial dysfunction, and heart disease. This clustering of abnormalities with insulin resistance has been termed Syndrome X, the insulin resistance syndrome, or the dysmetabolic syndrome (26, 27).The adverse potential of the dysmetabolic syndrome has recently been highlighted by the National Cholesterol Education Projects Adult Treatment Panel III guidelines, which recognize Syndrome X as a major cardiac risk factor (28). PCOS may truly be considered a component of the dysmetabolic syndrome in women. Evidence that PCOS is associated with a high risk for the development of T2DM and heart disease is mounting. Regarding diabetes risk, prospective clinical trials have demonstrated a 31-35% prevalence of impaired glucose tolerance (IGT) and 7.5-10.0% prevalence of T2DM in women with PCOS (29, 31). Furthermore, studies(31,32) demonstrated that both obese and lean PCOS patients are at increased risk of IGT or overt diabetes during their third or fourth decade; up to 20% of PCOS patients have IGT or T2DM by the third decade(33) and up to 30–50% of obese women with PCOS will develop IGT or T2DM by the age of 30 years(34,35). Regarding cardiovascular risk, PCOS is associated with increased prevalence of several cardiovascular risk factors, including hypertension (36-38) and dyslipidemia (39-42). In addition, women with PCOS display surrogate markers for early atherosclerosis, such as increased PAI-1 (43-45), endothelin-1(46), and C-reactive protein (CRP) concentrations (47). Several studies (48-50) suggest that PCOS is associated with endothelial dysfunction, which is linked to insulin resistance and is a risk factor for cardiovascular disease. PCOS women were shown to have higher mean carotid intima media thickness (IMT) compared with age-matched normal women, a striking illustration of the early atherogenic process in PCOS (51). In addition, several studies reported an increased prevalence of heart disease in PCOS (52, 53, 54-55).Women with PCOS may represent the largest unique female population at high risk for premature atherosclerotic heart disease. The above considerations indicate that PCOS is not only an infertility or cosmetic problem, but perhaps a primary general health problem at whose root lies insulin resistance. Considering that PCOS may affect between 3.5 and 5.0 million young women in the United States, it may be the most important general health issue affecting young women.

While insulin resistance is not part of the diagnostic criteria for PCOS, its importance in the pathogenesis of PCOS cannot be denied. Insulin acts by binding to its receptor, which is a membrane-associated glycoprotein. Ligand binding induces autophosphorylation of the receptor on specific tyrosine residues and an increase in its tyrosine kinase activity (56). The activated insulin receptor initiates signal transduction by tyrosine phosphorylation of intracellular substrates such as insulin receptor substrates (IRSs) (56). IRSs serve as docking proteins for signaling and adaptor molecules, such as phosphatidylinositol 3-kinase (PI3-kinase). Activation of PI3-kinase propagates the signal to regulate several insulin-mediated metabolic functions, such as glucose transport and glycogen synthesis (56, 57) **(See figure 1 below).** Any change in one of these processes could theoretically lead to a reduced cellular response to insulin, causing insulin resistance.

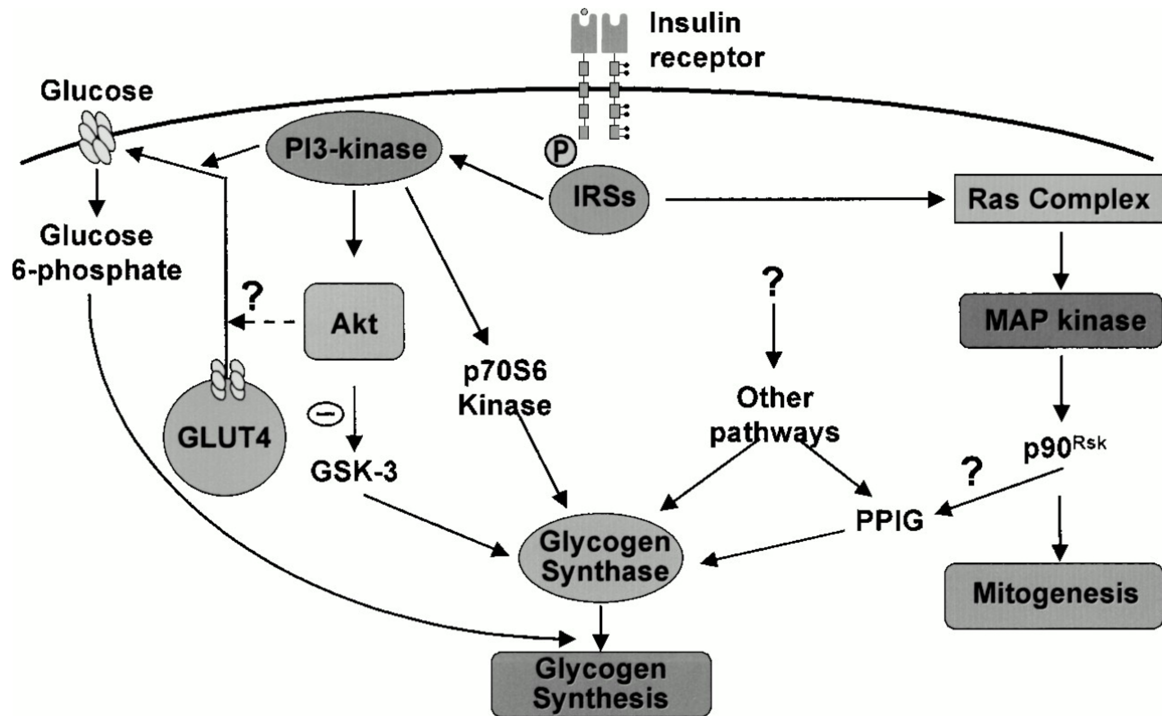


Figure 1: Molecular Mechanism of Insulin Resistance [1]

[1] Source: Dunaif A. Current Concepts in the Polycystic Ovary Syndrome. Annual Review of Medicine; 2001, Vol. 52: 401-419).

Pathogenesis of insulin resistance in PCOS is still a matter of debate. Defects in insulin binding to its receptor or, most probably, defects in downstream effectors of the insulin receptor may be the molecular sites of insulin resistance in PCOS. Insulin resistance, and the associated compensatory hyperinsulinemia, perhaps reflecting an intrinsic beta cell defect, is widely acknowledged to be a common biochemical feature of PCOS. Hyperinsulinemia in PCOS is primarily a result of a compensatory increase in insulin secretion by the pancreatic beta cells secondary to substantial peripheral insulin resistance. Basal insulin secretion is increased and hepatic extraction of insulin is decreased in PCOS (58). Insulin secretion increases as insulin sensitivity decreases to maintain glucose homeostasis. Hyperinsulinemic insulin resistance is characteristic of many, if not all, women with PCOS and is considered to be the cause of the main features of PCOS, namely hyperandrogenism and anovulation. Hyperinsulinism stimulates ovarian and adrenal androgen secretion either directly or indirectly. Indirect mechanisms include stimulation of lutenizing hormone secretion(LH) or Adrenocorticotropic hormone (ACTH) and inhibition of the hepatic production of sex hormone-binding globulin (SHBG) and insulin-like growth factor (IGF)-binding protein-1 (IGFBP-1), thereby increasing the availability of testosterone and IGF-1 to target tissues(59,60) **(See Figure 2 below)**. The intraovarian androgen excess would induce an unfavorable environment for follicle maturation, leading to anovulation and the increased serum concentrations of testosterone and androstenedione, as well as a variable degree of sensitivity of the hair follicle, would be responsible for hirsutism. Functional ovarian hyperandrogenism is the major mechanism leading to PCOS (61) and as many as 50% of PCOS patients also show evidence of functional adrenal hyperandrogenism (62). It is notable that these reproductive actions of insulin appear to be limited to women with PCOS and are not seen in reproductively normal women, which suggests that PCOS itself confers this susceptibility (63, 64).

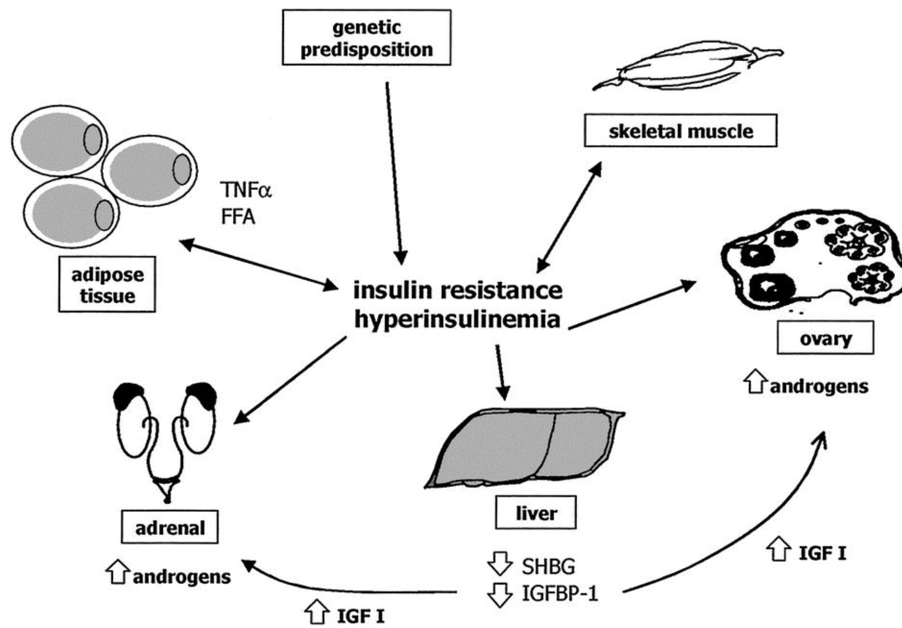


Figure 2: Evidence for Direct Involvement of IR in PCOS: Hyperinsulinemia and Hyperandrogenemia[2]

Similarly, androgens do cause mild insulin resistance in women (65) and lowering circulating androgen levels pharmacologically or by blocking androgen action with receptor antagonists do slightly improve insulin resistance in hyperandrogenemic women (66). However, the magnitude of change is not in the range of the insulin resistance associated with PCOS (66) and therefore, androgens may amplify but do not account for insulin resistance in adult women with PCOS. Most of the evidence on the directionality of the relationship between insulin resistance and hyperandrogenism would suggest that the direction of causation is from insulin to androgen and not the reverse. For example, weight loss and administration of insulin sensitizers, which specifically reduces insulin concentrations, results in a reduction in circulating androgen concentrations(67) However, administration of a gonadotropin-releasing hormone analog, which reduces androgen secretion from the ovary by suppressing gonadotropins, does not result in a reduction in insulin(68).

In addition to contributing to hyperandrogenism and anovulation, the compensatory state of hyperinsulinemia to insulin resistance causes several metabolic abnormalities that occur

[2] Source: De leo, V. Insulin-Lowering Agents in the Management of Polycystic Ovary Syndrome. Endocr Rev;.2003, Volume 24(5) :.633-667

together commonly enough that it can be considered a syndrome. Characteristic abnormalities of the insulin resistance syndrome include obesity, dyslipidemia, high blood pressure, glucose intolerance and a prothrombotic and inflammatory state (69). In view of the fact that over 30% of lean and 75% of obese women with PCOS are hyperinsulinaemic(70), it is not surprising that features of syndrome X are present in PCOS women; Approximately 60-70% of PCOS patients in the United States are obese(11), with a central body fat distribution pattern described as visceral obesity. Independent of obesity, PCOS women have hyperlipidemia characterized by elevated low density lipoprotein (LDL) cholesterol and triglyceride levels and decreased high density lipoprotein (HDL) cholesterol and apolipoprotein A1 levels (71).Women with PCOS commonly have raised systolic blood pressure (72,73), increased prevalence of T2DM when compared with controls (15% versus 2.3%) (74) and up to 40% of women with PCOS develop impaired glucose tolerance or frank diabetes by the age of 40 years (75). Increased levels of tumour necrosis factor- alpha (TNF-α) (76), plasminogen activator inhibitor-1 activity (PAI-1) (77), and fibrinogen (77) in PCOS women relative to controls have been reported. Increased levels of high-sensitivity C-reactive protein(hsCRP) in women with PCOS relative to those age and BMI-matched healthy women, and the correlation of hsCRP with insulin resistance in young and normal-weight women with PCOS has been reported as well(78).

The paradox of insulin promoting androgen production in ovarian and adrenal tissues in the face of insulin resistance in peripheral tissues has been partly explained by tissue differences in insulin sensitivity in PCOS such that the steroidogenic tissues are insulin-sensitive, whereas the major tissues involved in carbohydrate metabolism, namely fat and muscle, are insulin-resistant (79).

As mentioned earlier, insulin resistance is associated with dyslipidemia, hypertension and inflammation. Potential mechanisms by which IR may cause each of these abnormalities have been proposed. In skeletal muscle, insulin resistance leads to decreased rates of glucose uptake. Insulin resistance in the liver leads to increased rates of hepatic glucose production, mainly because of increased gluconeogenesis, but also dyslipidemia (80). The dyslipidemia that is associated with insulin resistant states is characterized by hypertriglyceridemia, an increase in very low-density lipoprotein (VLDL) secretion from the liver, an increase in atherogenic small, dense low-density lipoprotein (LDL), and a decrease in high-density lipoprotein (HDL) cholesterol(81,82). Much of the atherogenic dyslipidemia of the insulin resistance syndrome

begins with hypertriglyceridemia (83); the predominant triglyceride-containing lipoprotein is VLDL, which is synthesized in the liver. Research has shown that in patients with insulin resistance, the chronically high insulin levels make the liver resistant to the inhibitory effects of insulin on VLDL secretion (84). In addition, in insulin resistant states the clearance of VLDL cholesterol appears to be defective, which is primarily due to the decreased activity of tissue lipases, many of which are regulated by insulin (85). The increased plasma levels of VLDL, through the exchange of triglycerides in VLDL for cholesterol in HDL or LDL, results in reduced levels of HDL particles, smaller and denser LDL particles and highly atherogenic VLDL particles. Insulin resistance in adipose tissue causes reduced free fatty acid (FFA) absorption and enhanced lipolysis by adipocytes, both of which cause increased circulating FFA levels. The increased FFA delivery to peripheral tissues (especially liver and intestine), in conjunction with insulin resistance, lead to the overproduction of both hepatically and intestinally derived triglyceride-rich lipoprotein particles. High concentrations of FFAs can also block glucose oxidation, impair glucose transport and lead to impaired glucose metabolism. Furthermore, increased levels of FFAs themselves can also cause insulin resistance (86). This may lead to a vicious cycle of insulin resistance and FFAs potentiating each other. Hypertension and Insulin Resistance Diabetes mellitus is commonly associated with hypertension, and a wealth of epidemiologic data suggests that this association is due to insulin resistance and the resultant hyperinsulinemia (87). Potential suggested mechanisms by which insulin resistance may cause hypertension(87-97) include resistance to insulin-mediated vasodilation(88), abnormal endothelial signaling via nitric oxide dependent pathways(89-91), increased sympathetic nervous system activity (92-94), sodium retention (95), and enhanced growth factor production and activation that leads to proliferation of smooth cells in the vessel wall (96,97). Inflammation is now widely recognized as a central feature of atherogenesis. Several of the metabolic abnormalities that are associated with insulin resistance are proinflammatory and may induce systemic inflammation (98). One of the metabolic abnormalities most commonly associated with insulin resistance and T2DM is obesity (99); obesity is a state of chronic low-level inflammation in which adipose tissue produces numerous inflammatory cytokines and mediators such as C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and fibrinogen(100). Conversely, CRP levels were found to be independently and inversely related to insulin sensitivity (78,101). Fibrinogen levels, as well, were independently associated with

fasting insulin levels in nondiabetic subjects in three other studies (102,103,104). Therefore, the association between insulin resistance, obesity, and inflammation is not well understood. It is not clear whether the relationship is a direct manifestation of insulin resistance, a direct manifestation of excess adipose tissue, or due to metabolic changes that are frequently associated with obesity and insulin resistance. **Figure 3 below** summarizes the lipid abnormalities, hypertension mechanism and inflammation mediators, which are associated with insulin resistant states.

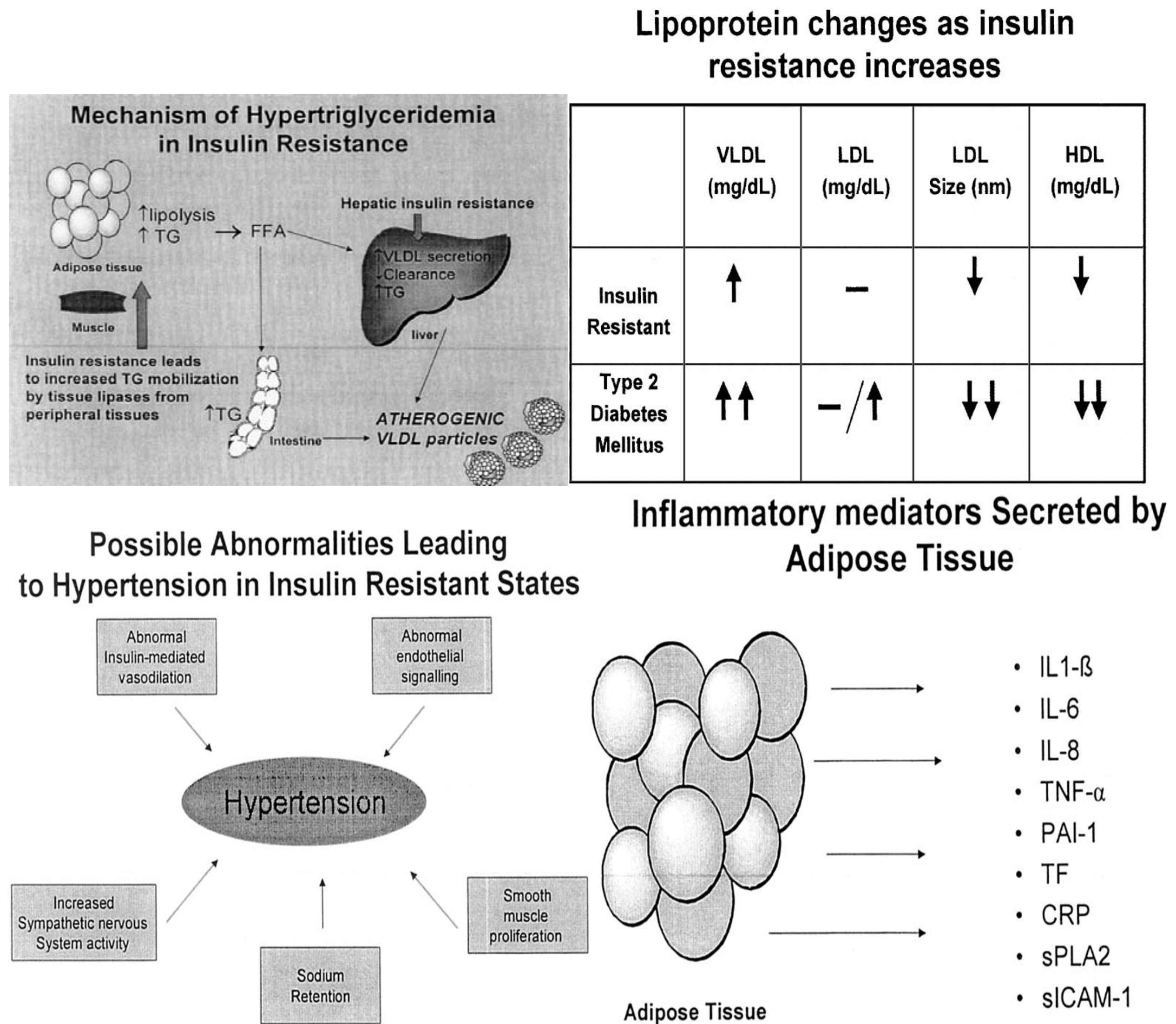


Figure 3: Evidence for Direct Involvement of Insulin Resistance in PCOS: Hyperinsulinemia, Dislypidemia,Hypertension and Inflammation[3]

[3] Source: Watson KE. Atherosclerosis in type 2 diabetes mellitus: the role of insulin resistance. J Cardiovasc Pharmacol Ther; 2003, 8(4):253-260

The metabolic abnormalities associated with insulin resistance syndrome have been associated individually with cardiovascular disease (105) and the syndrome together has been found to greatly increase cardiovascular mortality (106). Based on the prevalence of coronary heart disease risk factors in insulin resistance states, including the relative prothrombotic state of the coagulation cascade (108-110), diabetes mellitus, dyslipidemia, visceral obesity, and hypertension among PCOS women, these women have an estimated 4- to 11-fold increased risk of coronary heart disease (CHD) (107). In fact, insulin resistance has been associated with predictors of cardiac events such as increased IMT and endothelial dysfunction (78,111-112). Compared with age-matched normal women, PCOS women were reported to have thickening of the carotid intima media and increased levels of angioconstrictive peptides (55,113). One case-control study (51) suggested that part of the observed association of PCOS and IMT in middle-aged PCOS women might either be driven by central obesity and hyperinsulinemia or be mediated by factors related to hyperinsulinemia and central obesity, such as plasminogen activator inhibitor-1, C - reactive protein, and TNF-α (114-117). This same study reported a significant difference in the distribution of carotid plaque among PCOS cases compared with controls, with a higher percentage of PCOS cases (7.2%) having a plaque index of >=3 compared with 0.7% of similarly aged controls(51). Significantly increased endothelial dysfunction in women with PCOS relative to those age and BMI-matched healthy women has been reported as well (78); endothelial dysfunction has been considered an early feature of atherosclerosis and might contribute to the increased risk of atherosclerosis in obese and non-obese insulin-resistant subjects, such as those with PCOS (118,119). In addition, PCOS women have a greater prevalence and extent of coronary artery calcification, than either BMI-matched ovulatory women (OR = 2.37) or nonobese community-dwelling women (OR = 5.89) of similar age (120); the extent of coronary artery calcification closely correlates with the atherosclerotic plaque burden (121) and predicts an increased risk of cardiac events (122). Recent data have shown not only increased prevalence of cardiovascular disease (CVD) (51, 52,123) but also higher cardiovascular morbidity even in young and thin women with PCOS (124). Although uncertainty exists, early detection and treatment of insulin resistance in the PCOS population could ultimately reduce the incidence or severity of diabetes mellitus, dyslipidemia, hypertension, and cardiovascular disease, even in nonobese women with PCOS.

Insulin sensitizers are the group of therapeutic agents that hold some promise of helping women with polycystic ovary syndrome (PCOS), since the role of insulin resistance and hyperinsulinemia appear to be major contributors to the pathophysiology of the syndrome. There are studies that suggest that interventions to improve insulin sensitivity can prevent the development of diabetes in individuals at high risk (125,126,127). Women with PCOS are an insulin-resistant group at markedly increased risk for type 2 diabetes. Therefore, it seems reasonable to believe that the demonstrated efficacy of insulin-sensitizing drugs to prevent type 2 diabetes should be applicable to them as well. Evidence for a possible cardioprotective effect of insulin-sensitizing drugs in PCOS is primarily indirect, but nonetheless significant. Insulin-sensitizing drugs have been reported to exert beneficial effects on multiple cardiovascular risk factors in PCOS**.** Some studies have reported that these drugs improve the cardiovascular risk profile by improving lipid profile (128), decreasing serum triglycerides (129-131), blood pressure (129-131), serum PAI-1 (132,133) and endothelin-1 (134) concentrations in women with PCOS. Insulin sensitizers have been shown to decrease inflammation in obese and diabetic subjects (135-137). These drugs also have been shown to reduce carotid intima media thickness, normalize vascular endothelial function, improve fibrinolytic and coagulation parameters (138), reduce MMP-9 (a matrix metalloproteinase, implicated in atherosclerotic plaque rupture) and CRP levels in type 2 diabetics (139). Furthermore, in animal studies, these drugs have also been shown to decrease atherosclerotic plaque area (140)**.** The United Kingdom Prospective Diabetes Study and another recent population-based mortality study from Canada reported a cardioprotective action of metformin in diabetic patients (141,142). Furthermore, studies with insulin-sensitizing drugs suggest that administration of these drugs is associated with substantially increased frequency of ovulation, followed by menstrual bleeding, resulting in at least six ovulatory menses per year in 55-85% of treated women (143-145); this frequency of ovulation would be consistent with the current standard of care for the prevention of endometrial cancer in women with PCOS. Most long-term studies of approximately 6 months duration have shown beneficial effects on hyperandrogenemia and hirsutism. The abundance of evidence from randomized clinical trials supporting the role of insulin sensitizers in alleviating, if not solving, the complicated metabolic and reproductive problems of PCOS women adds further on the crucial role of insulin resistance in the pathogenesis of PCOS.

In view of the discussion above, the common occurrence of insulin resistance in lean and obese PCOS women in the United States; the high prevalence of obesity in U.S. PCOS women, which further impairs insulin sensitivity; the evident role of IR in stimulating steroidogenesis, thus causing the main features of PCOS(namely hyperandrogenism and anovulation), and not otherwise ; the potential role of IR in the etiology of multiple cardiovascular risk factors and thus the major contribution of insulin resistance to the significantly increased risk of developing major diseases such as type 2 diabetes mellitus and cardiovascular diseases in this population, has led us to focus on the role of insulin resistance candidate genes in the development of PCOS/insulin resistance phenotype in PCOS families. Furthermore, the still unidentified association of gene variants, which may play an important role, with the metabolic syndrome has intrigued us to investigate this issue in these families as well (716).

1.2 STUDIES ON FAMILIAL AGGREGATION OF PCOS, FUNCTIONAL HYPERANDROGENISM AND INSULIN RESISTANCE/HYPERINSULINMEMIA

Family studies have indicated a genetic susceptibility to PCOS. Strong evidence indicates that PCOS clusters in families, and the sibling risk ratio (λ_S) for PCOS is 50% to 80% (146,147). Family-based studies reported PCOS to be present in 35% of the mothers and 40% of the sisters of PCOS patients diagnosed according to NICHD (148,149).

Functional hyperandrogenism and PCOS cluster in first-degree relatives of patients (148) and are inherited together with insulin resistance and metabolic disorders (150,151); familial aggregation of hyperandrogenic symptoms (hirsutism and oligomenorrhea) and of metabolic disorders (diabetes mellitus, dyslipidemia, arterial hypertension, and atherosclerosis) in families of patients presenting with hirsutism, oligomenorrhea, and increased ovarian size has been reported in several family-based studies(152-154). One family study reported an increased incidence of oligomenorrhea and polycystic ovaries in female first-degree relatives of patients presenting with polycystic ovaries and clinical/biochemical PCOS-associated traits compared to families of controls (155). Another study reported an increased prevalence of oligomenorrhea, infertility in hirsute women with/without enlarged ovaries and an increased prevalence of hirsutism in their female first-degree relatives compared to controls (156). In one family study, it

was found that 67% of the mothers and 87% of the sisters of patients presenting with ultrasonographic polycystic ovaries and hyperandrogenic symptoms were affected (157). In families of Norwegian women with polycystic ovaries previously treated by ovarian wedge resection, who also had at least two of the following symptoms: menstrual irregularity, hirsutism, infertility, and/or obesity and a control group of women and their families ,clinical manifestations of hyperandrogenism were approximately ten times more prevalent among female relatives of the patients as compared with the female relatives of the controls (31.4% vs. 3.2% respectively)(158). In this same study, among male relatives the prevalence of premature balding was approximately 3 times more among the relatives of the patients as compared to the relatives of the controls (19.7% vs. 6.5% respectively)(158). A study of monozygotic and dizygotic twins has also demonstrated that androgen levels and androgen production rates in humans are under genetic control (159-161). In addition to hyperandrogenism, insulin resistance clusters in the families of hyperandrogenic women. The heritability of insulin sensitivity, evaluated by the minimal model technique, has been relatively constant, in the 30%-40% range (162). One family study of PCOS women diagnosed according to NICHD criteria reported that 22% of the sisters of these women had PCOS, whereas an additional 24% of the sisters of these patients presented with hyperandrogenemia and regular menstrual cycles. The adrenal androgen, dehydroepiandrosterone sulfate (DHEAS), was also elevated in affected sisters(163).The same authors of this study reported that insulin resistance is associated with hyperandrogenemia rather than with menstrual dysfunction in these families suggesting that insulin resistance and hyperandrogenemia share the same pathogenic mechanisms (164). In addition, this study reported that the brothers of women with PCOS show evidence of insulin resistance and elevated DHEAS levels, which suggests that their reproductive and metabolic phenotype resembles that of their sisters with PCOS (164). Another study supporting the increased prevalence of insulin resistance in families of PCOS patients was conducted in the Turkish population; PCOS patients were defined by NICHD criteria and relatives of PCOS patients were matched for sex, age, and pre- or postmenopausal status with different population-based control groups. Insulin resistance and disorders of carbohydrate metabolism were more frequent and serum androgen levels were increased in the mothers and sisters of PCOS women compared with the controls. Brothers of PCOS patients had increased frequency of insulin resistance and disorders of carbohydrate metabolism compared with controls as well (165). One study reported that a positive family

history of T2DM is observed in a high percentage (>80%) of women with PCOS and T2DM compared with 30% among women with only PCOS (166). Hyperinsulinaemia is also common in family members of women with PCOS. One family study studied siblings and parents of PCOS individuals in five families and found that hyperinsulinaemia (69%) and hypertriglyceridaemia (56%) were common in family members, as were PCOS in 79% and premature baldness in men (88%). These authors concluded that hyperinsulinaemia is a potential metabolic and genetic marker for individuals who may be carriers of a familial tendency for PCOS (167). There are very few data regarding PCOS in twins; in one twin study, the authors suggested that fasting insulin level, serum androstanediol glucuronide, body mass index and unfavorable lipid profile in twin pairs concordant for polycystic ovaries were significantly influenced by genetic factors (168,169). Evidence also suggests that cardiovascular factors cluster in PCOS patients (170-172). The familial clustering of women with PCOS suggests that heredity is implicated in the origin of the syndrome. However, genetic approaches to its pathogenesis have been hampered by the heterogeneity of phenotypic features within families, and the lack of uniform criteria for diagnosis.

The mode of inheritance and the genetic defect responsible for PCOS remain to be established, despite significant efforts. None of the existing family studies of PCOS convincingly establishes a mode of inheritance, whether because the number of families studied was too small; the parental phenotypes could not be firmly established; the male phenotype is uncertain or the diagnostic criteria used to assign affected status and the methods used to ascertain the status of first- and second-degree relatives differed among the studies. Different studies suggested a simple Mendelian pattern of PCOS inheritance consistent with an autosomal dominant (173,177,164,178) or X-linked pattern of inheritance (179). Two studies using the NICHD criteria for diagnosis (180,149) also suggested a genetic origin for PCOS, with autosomal dominance as the most likely pattern of inheritance. Although the majority of studies suggest autosomal dominance, the limitations of the investigative designs and the lack of clear uniformity in the results suggest that PCOS may be best suited to genetic analyses without any presumption of the mode of inheritance.

Given the large number of genetic variants found in association with PCOS and the marked phenotypic heterogeneity of the patients, even within a single family (181), PCOS appears to be a complex polygenic trait in which environmental influences play an important

role. Past research included candidate genes for PCOS related to androgenic pathways and metabolic associations of the syndrome. More recently, genes encoding inflammatory cytokines have been identified as target genes for PCOS, as proinflammatory genotypes and phenotypes are also associated with obesity, insulin resistance, type 2 diabetes, PCOS, and increased cardiovascular risk. Despite a significant amount of research, none of the genes studied so far has been identified as the PCOS susceptibility gene for the majority of cases. The exploration of the genetics of PCOS has been hampered by the heterogeneity in the diagnostic criteria used to define PCOS, the infertility and low fecundity associated with PCOS which limits the pedigrees and generations studied to two (mothers and daughters) in most of the linkage analyses performed to date, the complexity of assigning phenotypes to pre-menarchal girls and post-menopausal women, the lack of rigorous established clinical or biochemical features that can be used to identify PCOS males, the increased complexity of the genetic analysis associated with the increased number of distinct phenotypes within the affected category, and the lack of precision in the identification of ethnic and environmental factors that trigger the development of hyperandrogenic disorders.

1.3 FINDINGS OF STUDIES ON CANDIDATE GENES IN PCOS AND FUNCTIONAL HYPERANDROGENISM

Functional hyperandrogenism and hyperinsulinism may be detected early in life in affected women, even before pubertal development. To date, several genomic variants related to insulin resistance and genes involved in androgen biosynthesis, metabolism and action have been studied in children, adolescents and adults. Lately, genes involved in chronic inflammation and atherosclerosis have been studied in adults as well.

1.3.1 Studies on Candidate Genes Related to androgen biosynthesis, metabolism and action in functional hyperandrogenism and PCOS

Table 1: Candidate genes that were studied in Children & adolescent girls with Premature Pubarche (PP) and Hyperandrogenic adults

Candidate Gene	Reference	Population	Findings
CYP21	183-184; 185	Children with PP & Hyperandrogenic adolescent girls **AND** Non-Hyperandrogenic Controls.	• Increased heterozygosity for mutations in CYP21 in cases. • Increased prevalence of common variants and mutations in CYP21, HSD3B2, IRS1, β_3-adrenergic receptor gene (ADRB3) and Glucocorticoid receptor in Hyperandrogenic patients.
	186	Hyperandrogenic women **AND** Non-Hyperandrogenic Controls.	• No association between the CYP21 genotype and functional origin of androgen excess.
	176	PCO and Congenital Adrenal Hyperplasia Patients **AND** Controls.	• Association between the presence of PCO with increased frequency of DRW6 and decreased DR7 human leukocyte antigen haplotypes. • 21-hydroxylase deficiency associated with Bw47, B14 or DR1 haplotype.
HSD3B2	182;185;189	Children with PP & Hyperandrogenic adolescent girls **AND**Non-Hyperandrogenic Controls.	• Increased heterozygosity for mutations in HSD3B2 in cases. • Increased prevalence of common variants and mutations in CYP21, HSD3B2, IRS1, β_3-adrenergic receptor gene (ADRB3) and Glucocorticoid receptor in Hyperandrogenic patients. • Moderately decreased adrenal 3 beta-HSD activity is not caused by a mutation in the type II *HSD3B2*.
	188	Families with Hyperandrogenemia or PCOS.	• No evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the HSD3B2 locus.
Androgen Receptor	190	Children and adolescent girls with PP **AND** control girls	• Shorter AR gene CAG repeat numbers associated with premature pubarche and ovarian hyperandrogenism.
	191;192; 193-194; 195	Hyperandrogenic adults and healthy Controls	• Decreased number of CAG repeats is associated with hirsutism in women. • Decreased number of CAG repeats is associated with androgen-dependent skin disorders in both men and women. • Distorted inactivation of X chromosome (chr) in every cell of a woman with the larger CAG repeat has been proposed to play a role for idiopathic hirsutism and PCOS. • Distorted inactivation of X chr in every cell of a woman with the larger CAG repeat doesn't associate with idiopathic hirsutism and PCOS.

Table 1 **(Cont'd)**

Androgen Receptor (cont'd)	196	Patients with ultrasound diagnosis of polycystic ovaries, irregular menstrual cycles, and anovulatory infertility	• Decreased number of AR gene CAG repeats explain the normal serum androgen levels found in some women with polycystic ovaries, infertility, and oligomenorrhea, in whom the hyperandrogenic symptoms would result from the intrinsic increase in the AR activity.
	188	Families with Hyperandrogenemia or PCOS.	• No evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the AR locus.
CYP17	197	Children with premature pubarche and adolescent girls	• No association between *CYP17* variants with hyperandrogenism
	199	Families with 81 affected individuals have been assessed in which polycystic ovaries/male pattern baldness	• T/C SNP at –34 bp associated with the presence of polycystic ovaries on ultrasound
	200-201	Patients with PCOS and healthy women.	• PCOS patients homozygous for C alleles of the T/C SNP at –34 bp presented with increased serum testosterone levels
	202-205	Patients with PCOS/ hyperandrogenism and healthy controls	• No association between T/C SNP at -34 bp & functional consequences for development of PCO & hyperandrogenism.
	188	Families with Hyperandrogenemia or PCOS.	• No evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the *CYP17* locus.

Table 2: Candidate genes that were studied in Children with Premature Pubarche (PP) & adolescent girls only

Candidate Gene	Reference	Findings
ß₃-adrenergic receptor	185	• Increased prevalence of common variants and mutations in CYP21, *HSD3B2, IRS1,* $ß_3$-adrenergic receptor gene (ADRB3) *and Glucocorticoid receptor Hyperandrogenic patients* than healthy controls.
***UDP-* glucuronyltransferase 2B15**	198	• No association between D85 variant andhyperandrogenism
Glucocorticoid receptor	185	• Increased prevalence of common variants and mutations in CYP21, *HSD3B2, IRS1,* $ß_3$-adrenergic receptor gene (ADRB3) *and Glucocorticoid receptor in Hyperandrogen c patients.*

Table 3: Candidate genes that were studied in hyperandrogenic/PCOS adults only

Candidate Gene	Reference	Findings
CYP11A	206	• Evidence for linkage with the *CYP11A* locus was found in 20 pedigrees presenting with PCOS, based mostly on the presence of polycystic ovaries or male pattern premature balding. • Absence of the more common four-repeat allele was associated with hirsute PCOS patients and with higher serum testosterone levels.
	207	• Absence of the more common four-repeat allele was associated with PCOS patients and with higher serum testosterone levels, using NICHD criteria for the definition of PCOS.
	208	• Nine-repeat alleles were more frequent in PCOS patients, defined by oligomenorrhea and polycystic ovaries and four- and six- allele repeats were more frequent in controls
	209	• No association of *CYP11A* VNTR alleles with functional hyperandrogenism
	210	• No association of *CYP11A* VNTR alleles with polycystic ovaries & serum testosterone levels
	211	• No consistent genomic abnormalities have been found in the entire *CYP11A* coding region. • No consistent genomic abnormalities found in genes encoding steroidogenic acute regulatory protein, steroidogenic factor-1, & dosage-sensitive sex reversal-adrenal hypoplasia gene on X Chr gene-1
	188	• No evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the *CYP11A* locus or the steroidogenic acute regulatory protein
CYP11B2	212	• Increased frequency of the C alleles of -344T/C SNP than in hyperandrogenic patients. • Increased levels of plasma rennin activity, serum angiotensin II, aldosterone and testosterone levels in women homozygous for C alleles compared with women homozygous for T alleles
LHβ	213	• Association between Trp^{8}Arg & Ile^{15} Thr variants and increased serum testosterone, estrogen and SHBG levels in healthy women, but not in PCOS patients.
	214,215	• Reduced prevalence of Trp^{8}Arg & Ile^{15} Thr variants in obese PCOS patients.
	216,217	• Association between the Gly^{102}Ser variant and menstrual disorders.
	218	• Increased frequency of the –894C/T, –1018G/C, –1036C/A, –1098C/T, and –1423C/T variants in patients with ovulatory disorders.
	216,217,219	• No association between LHβ variants &PCOS, hyperandrogenism, serum androgen or estrogen levels
FSHβ	220a	• Increased frequency of homozygosity of the T/C SNP at codon 76 in PCOS patients, as defined by oligomenorrhea and polycystic ovaries, compared with nonhyperandrogenic women and correlation with higher serum androgen concentrations
FSH receptor	220-221;188	• Negative associations between variants in FSHβ gene with PCOS.
Dopamine receptor	222	• Association between the MscI variant with hyperandrogenic chronic anovulation and resistance to clomiphene citrate in women of Hispanic ancestry.
	223	• No association between MscI variant with hyperandrogenic chronic anovulation in non-Hispanic women.

Table3 **(Cont'd)**

SHBG	224	• Detection of missense Pro^{156}Leu mutation in women presenting with severe hyperandrogenism during pregnancy.
	225	• Association between a (TAAAA) n polymorphism in the promoter of the SHBG gene and PCOS. • Increased frequency of longer (TAAAA) n alleles (more than eight repeats) in PCOS patients, defined by NICHD criteria, compared to non-hyperandrogenic women. • Decreased SHBG levels in carriers of longer (TAAAA) n allele genotypes in PCOS women
	226	• Association between longer (TAAAA)n alleles with Decreased serum SHBG levels. • Strong linkage disequilibrium between (TAAAA)n polymorphism in hirsute women and an Asp^{327}Asn SNP in SHBG, 327Asn alleles being associated with eight-repeat (TAAAA)n alleles, and resulting in increased serum SHBG levels when compared with subjects homozygous for 327Asp alleles.
	188	• No evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the *SHBG* locus
HSD17 β	188	• No evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the HSD17β locus
	228	• No association between the G289A variant and PCOS
CYP19	188	• No evidence for linkage or association was found between PCOS, defined by NICHD criteria, and the CYP19 locus
	206	• No association between the (ttta)n variant and PCOS
GnRH Receptor	229	• No mutations found in the gene encoding GnRH receptor in PCOS patients.

Table 4: SUMMARY TABLE on Genes Related to Androgen Biosynthesis and Action and their Regulation in hyperandrogenic/PCOS populations

Candidate Genes	Reference	Population	Findings
CYP21,*HSD3B2*, *AR*, *CYP17*	176,183-186; 182,185,188-189;185 188,190-196 188,197,199-205;	Children with PP & adolescent girls; Hyperandrogenic adults.	*CYP21, *HSD3B2* & *CYP17* ***(Not consistent)***
			****AR (CAG repeat variant) (Most Consistent)***
β3 adrenergic receptor Glucocorticoid receptor UDP-glucuronyltransferase 2B15	185 185 198	Children with PP & adolescent girls	+ve (??-based on one case control study) -ve findings
CYP11A, CYP11B2, LHβ, FSHβ ,FSHR, Dopamine R, SHBG, HSD17B; CYP19, GnRHr	188, 206-211; 212; 213-219; 220a; 188, 220-221; 222-223;188, 224-226; 188,228;188,206; 229	PCOS/ Hyperandrogenic adults	*CYP11A.LHβ&DopamineR **(Not Consistent)**
			*HSD17B,CYP19,FSHR, GnRH R**(-ve)**
			*FSHβ, CYP11B2 **(+ve ???-Based only on one case control Study)**
			****SHBG((TAAAA) n variant in its promoter (Most Consistent)***

1.3.2 Studies on Candidate Genes Related to Insulin Resistance and associated disorders in functional hyperandrogenism and PCOS

1.3.2.1 Candidate genes that were studied in hyperandrogenic Adults and Adolescents

These include insulin gene (INS), insulin receptor substarte-1 (IRS-1), Human homolog for the sorbin and SH3-domain-containing-1 gene (SORBS1), and PPAR-γ2.

Insulin gene (INS): The presence of pancreatic ß-cell dysfunction in women presenting with PCOS appears to have a genetic origin (233). Therefore, INS has been studied in women with PCOS and functional hyperandrogenism. Four out of seven studies have reported positive results. In girls with a history of premature pubarche, a positive association was reported between carriers of class I alleles of the VNTR locus at the insulin gene (*INS)* and insulin resistance (234). In hyperandrogenic adults, a family based study/case control study reported a positive evidence for linkage to INS VNTR locus in a group of PCOS/male pattern baldness families. The authors also found that women with menstrual disturbances and/or hirsutism and polycystic ovaries, who were homozygous for class III alleles, were more frequently anovulatory and had increased body mass index and fasting insulin compared with women homozygous for class I alleles (235). Moreover, class III alleles predisposed these avovulatory PCOS patients to both PCOS and type 2 diabetes mellitus (235-237). However, later case-control studies in European Caucasian women, conducted outside the United Kingdom, have failed to replicate these results (238,239), and the INS locus was not associated with PCOS in a linkage study in American PCOS patients (188).

Insulin Receptor Substrate-1 (IRS-1): After insulin binding to its receptor, autophosphorylation of tyrosine residues results in the activation of the insulin receptor (INSR), and tyrosine kinase activity phosphorylates intracellular substrates such as IRS-1 and IRS-2 (240). Insulin receptor substrates (IRSs) serve as docking proteins for signaling and adaptor molecules, such as phosphatidylinositol 3-kinase (PI3-kinase). Activation of PI3-kinase propagates the signal to regulate several insulin-mediated metabolic functions, such as glucose transport and glycogen synthesis. PCOS women present a defect in insulin receptor signaling characterized by a decreased IRS-1-associated PI3-kinase activity (241). One common SNP in the gene encoding insulin-receptor substrate $Gly^{972}Arg$ in IRS-1 is a susceptibility gene for type

2 diabetes mellitus (242).Two case control studies (one involving hyperandrogenic adults, the other involving adolescent girls with a history of precocious pubarche) , one case series and a family based study(TDT) considered IRS-1 gene in relation to functional hyperandrogenism and PCOS. The two case control studies, studying Gly^{972}Arg variant in the gene IRS-1, reported a positive result. In the case control study studying this variant in adolescent girls with a history of precocious pubarche and healthy adolescent female control subjects, it was found that the frequency of heterozygosity for the Gly972 allele were 31% among girls with a history of premature pubarche, 40% among girls with hyperinsulinemic ovarian hyperandrogenism, and only 19% among healthy control subjects. Moreover, carriers of Gly972Arg variant presented with decreased sex hormone-binding levels, leading the authors to suggest that the Gly972Arg variant of the *IRS-1* gene may be an additional minor locus associated with the development of hyperinsulinemic insulin resistance and ovarian androgen excess in girls with a history of PP (243). The other case control study involving hyperandrogenic adults, reported gene-dosage effects on fasting insulin for the Gly972Arg IRS-1 variant, with heterozygous carriers having higher fasting insulin than carriers of the Gly972 wild type variant of IRS-1. Moreover, significantly more prevalence of the Gly972Arg IRS-1 variant was in insulin-resistant patients compared with non-insulin-resistant individuals or control subjects (39.3 vs. 4.0 and 16.6%, $P < 0.0031$, respectively) (244). The other two studies were a family based study and a case series study and both involved hyperandrogenic adults. In the family-based study conducted in the United States, no evidence for linkage or association with PCOS was found with IRS-1 (188). This family-based study studied 150 nuclear families, 148 were of European origin and 2 were of Caribbean origin, and tested a collection of 37 candidate genes for linkage and association with PCOS or hyperandrogenemia in data from these families. The case series study, involving nondiabetic white and African American PCOS subjects, found no evidence for an effect of the IRS-1 Gly972Arg polymorphism on glucose or insulin levels during an OGTT or on androgen levels in either white or African-American women with PCOS (245).

Human homolog for the sorbin and SH3-domain-containing-1 gene (*SORBS1*): The Ala228 allele of the Thr^{228}Ala polymorphism of SORBS1 is known to be a protective factor for both obesity and diabetes (246).Two case control studies, one involving girls presenting with premature pubarche and/or functional hyperandrogenism and the other involving hyperandrogenic adults have considered the Thr^{228}Ala polymorphism SORBS1 gene. The case

control study, which involved girls presenting with premature pubarche and/or functional hyperandrogenism and healthy adolescents, reported no differences in allelic distributions of the $Thr^{228}Ala$ polymorphism SORBS1 gene between the two groups *(247)*.In hyperandrogenic **adults**, a case control study reported that allele frequencies of $Thr^{228}Ala$ polymorphism were similar in PCOS patients and nonhyperandrogenic women. However, carriers of Ala228 alleles of SORBS1 presented with increased body mass index compared with subjects homozygous for 228T alleles (248).

PPAR-γ2: Five case control studies and two case series studies considered the common $Pro^{12}Ala$ polymorphism in the gene encoding the peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) in relation to functional hyperandrogenism and PCOS. One family based study (TDT) considered the PPAR-γ2 gene as well**.** Three case control studies and the two case series studies reported a positive result. Two case control studies, one involving children with precocious pubarche and adolescent hyperandrogenic girls and the other involving adults, reported that Ala12 alleles favor weight gain in obese hyperandrogenic girls and adolescents (249) and obese adults (250). However, no association was found with any of the alleles of the $Pro^{12}Ala$ polymorphism PPAR-γ2 gene and hyperandrogenism in the former study (249). Another case control study in Finland reported a marginally significant decrease in the frequency of the Ala12 allele in women with polycystic ovaries compared to controls (12.6% vs.19.1% respectively, $P = 0.045$); the authors concluded that this PPARγ gene polymorphism plays a role in the pathogenesis of PCOS with the presence of the Ala allele being protective against the development of PCOS (251). The two case series studies reported that the Ala12 allele is associated with improved insulin sensitivity in Caucasian men (252) and in Caucasian PCOS women defined by NICHD criteria (253). However, other two case control studies, one involving PCOS patients from Italy defined by NICHD criteria and ultrasonography (254) and the other involving PCOS patients from Spain(248), have not confirmed a difference in frequency of the Ala12 allele in women with polycystic ovaries compared to controls. This latter case control study considered a silent C to T substitution at position 142 in exon 6 as well and found that T alleles are more frequent in women with PCOS than controls (254). In a family-based study conducted in the United States, no evidence for linkage or association with PCOS was found for a marker close to the PPAR-γ2 gene (188). This family-based study studied 150 nuclear families, 148 were of European origin and 2 were of Caribbean origin, and tested a collection of

37 candidate genes for linkage and association with PCOS or hyperandrogenemia in data from these families.

1.3.2.2 Additional candidate genes that were studied in hyperandrogenic adults only

These include insulin receptor gene (INSR), insulin receptor substarte-2 (IRS-2), Insulin Growth Factor system (IGF), Paraoxonase (PON1), Caplain-10, Glycogen Synthetase, Leptin and its receptor, Apolipoprotein E, PC-1, PTP1B, and adiponectin.

INSR: Three case controls, three case series and a family-based study (TDT) considered the INSR gene in relation to functional hyperandrogenism and PCOS. Three out of these seven studies have reported positive results. The family-based study using the transmission disequilibrium test (188) and one case control study (255), both conducted in the United States, found an association of D19S884 with PCOS defined by NICHD criteria (188,255). However, this association was not validated in either a case control study involving PCOS patients from Spain and Italy (257) or in a case series which sequenced the 22 exons of INSR in PCOS patients (258). Furthermore, a molecular scanning of the insulin receptor gene found no difference in frequency of polymorphisms between patients with polycystic ovaries and hyperandrogenism and controls (259). Another case control study found an association of a C/T SNP at the tyrosine kinase domain of INSR with PCOS, defined by NICHD criteria (256). However, search for mutations in the tyrosine kinase domain of the INSR gene did not show any abnormalities, which were associated with insulin resistance (260).

IRS-2: One case control study and another case series study considered the $Gly^{1057}Asp$ variant, a common SNP in the gene encoding insulin-receptor substrate-2 (IRS-2) and a susceptibility gene for type 2 diabetes mellitus (261), in relation to functional hyperandrogenism and PCOS. These two studies provided conflicting results. The case control study reported that carriers of Asp1057 IRS-2 alleles presented with increased glucose and insulin levels 2 h after an oral glucose load and had an increased prevalence of glucose intolerance compared with subjects homozygous for Gly1057 alleles (244). However, the case series study in a larger series of PCOS patients showed just the opposite to what was found previously; the 2-h glucose values were actually increased in subjects homozygous for Gly1057 alleles when compared with carriers of Asp1057 alleles (245).

IGF System: One case control study and another family-based study (TDT) considered the IGF system in relation to functional hyperandrogenism and PCOS. The case control study reported associations of PCOS with homozygosity for G alleles of the ApaI polymorphism in IGF-II and of increased fasting glucose levels and fasting insulin resistance index with homozygosity for 90-bp alleles of a trinucleotide repeat polymorphism in the gene encoding IGF-1 receptor compared with subjects carrying 93-bp alleles (248). The family based study, conducted in the United States, found no evidence for linkage with PCOS for markers close to the genes encoding IGF-I and IGF-binding proteins 1 and 3 (188).

PON1: One case control study considered the PON1 gene in relation to functional hyperandrogenism and PCOS. This case control study explored the –108C/T, $Leu^{55}Met$, and $Gln^{192}Arg$ polymorphisms in the gene encoding serum PON1 in PCOS patients defined by NICHD criteria. This study reported that homozygosity for T alleles of the –108C/T polymorphism in PON1 was more frequent in patients compared with nonhyperandrogenic women (248). The same study reported that subjects homozygous for Met55 alleles presented with increased body mass index and indexes of insulin resistance compared with carriers of Leu55 alleles. However, the $Leu^{55}Met$ and $Gln^{192}Arg$ polymorphisms in PON1 were not associated with PCOS.

Calpain-10: Two case control studies and two family based studies (case-control) considered the Caplain-10 gene in relation to functional hyperandrogenism and PCOS. The two case control studies and one family based study reported positive results. The family based study found a significant association between 112/121-haplotype [(UCSNP)-43, UCSNP-19, and UCSNP-63 polymorphisms)] and higher insulin levels in response to an oral glucose tolerance test in African-American, nondiabetic PCOS women. This haplotype was also found to be associated with a 2-fold increase in susceptibility to PCOS, when considering Caucasian and African-American, nondiabetic PCOS patients as a whole (262). In one case control study conducted in Spain, C alleles at the UCSNP-45 locus were found to be associated with idiopathic hirsutism but neither the UCSNP-43 nor the UCSNP-44 was associated with hyperandrogenism or PCOS (263). However, in a different Spanish population, another case control study reported an association between PCOS and USCNP-44 (264-265). In the other family-based study, the association of calpain-10 SNPs with PCOS, as defined by polycystic ovaries, hyperandrogenism, and/or anovulation, was not confirmed in PCOS patients from the United Kingdom (266).

In summary, No consistent findings were reported for a specific variant at each of INSR, IRS-2, INS, IGF system, PON1, SORBS1 and Caplain-10 genes. Among other genes tested, no association has been reported between genomic variants in the genes encoding β3 adrenergic receptor (108), glycogen synthetase (267), resistin (268), leptin and its receptor (269), apoprotein E (270), or with variants in the genes of plasma cell differentiation antigen glycoprotein, protein tyrosine phosphatase 1B, and adiponectin (248) with PCOS. The most consistent findings were reported for the $Pro^{12}Ala$ polymorphism in the gene encoding PPAR-γ2 and the $Gly^{972}Arg$ variant in the gene IRS-1.

1.3.3 Studies on Candidate Genes Related to Inflammation in functional hyperandrogenism and PCOS

It is now well established that chronic inflammation is involved in the pathogenesis of metabolic syndrome and cardiovascular disease. Evidence supports clustering of serum inflammatory markers in patients with cardiovascular disease, suggesting a role in the pathogenesis of atherosclerosis (271-272). Positive correlations have been reported between indices of insulin resistance and inflammatory markers such as circulating serum levels of TNF-α (273), IL-6 (275) and C-reactive protein (CRP) (275). The directionality of the relationship between inflammation and insulin resisatnce is still equivocal. There is evidence, which shows that inflammatory cytokines may induce insulin resistance by direct actions on insulin-signaling postreceptor molecules (279) or by inducing central obesity through activation of the hypothalamic-pituitary-adrenal axis (280). On the other hand, several of the metabolic abnormalities that are associated with insulin resistance, such as obesity, are proinflammatory and may induce systemic inflammation (98). Adipose tissue plays a major role in the relationship between cytokines and insulin resistance; the expression of TNF-α and IL-6 in adipose tissue is increased in obesity (276-278), correlated with indexes of insulin resistance and decreased with weight loss along with the improvement in insulin sensitivity (276). Because obesity and insulin resistance are common findings in hyperandrogenic women (240), chronic inflammation might be involved in the pathogenesis of functional hyperandrogenism and PCOS. Increased CRP levels have been reported in PCOS patients defined by NICHD criteria (282-283). Similarly, increased serum IL-6 (284) and TNF-α (285-287) concentrations have been reported in women with PCOS or

functional hyperandrogenism. Given that proinflammatory genotypes influence obesity, type 2 diabetes mellitus, and insulin resistance-related disorders (272), several genomic variants in the genes encoding inflammatory markers have been studied. These were Tumor necrosis factor alpha (TNF-α), soluble type 2 TNF receptor TNFR2 (TNFRSF1B), Interleukin 6(IL-6), and IL-6 receptor genes.

TNF-α : Four case control studies have considered TNF-α, with three of these studies studying the -308G/A variant of TNF-α gene, in relation to functional hyperandrogenism and PCOS. One out of these four case control studies reported a positive result. In this study which considered nine common polymorphisms of TNF-α gene (–1196C/T, –1125G/C, –1031T/C, –863C/A, –857C/T, –316G/A, –308G/A, –238G/A, and –163G/A) in hyperandrogenic women and BMI-matched healthy controls, it was found that carriers of –308A alleles presented with increased serum androgen and 17-hydroxyprogesterone levels before and after stimulation with a GnRH analog when considering patients and controls as a whole. However, no differences in allele frequencies of any of the polymorphisms studied between patients and controls has been reported (291). Similar equal distribution of –308G/A alleles between patients with polycystic ovaries and hyperandrogenic symptoms and controls in other two case control studies were reported (292-293). Equal frequency of alleles for another variant, the –805C/T of TNF-α gene, in PCOS patients and controls has been reported as well (294).

TNFRSF1B: One case control study has considered this gene in relation to functional hyperandrogenism and PCOS. This case control study considered several polymorphisms in the TNFRSF1B in women with functional hyperandrogenism, including PCOS defined by NICHD criteria. The authors reported that the uncommon 196Arg allele of the Met^{196}Arg (676T/G) polymorphism in exon 6 of this gene is more frequent in patients with PCOS compared with healthy controls (295). However, when studying patients and controls separately, this polymorphism did not influence any phenotypic trait associated with hyperandrogenism, insulin resistance, or obesity (295). In addition, the three other studied SNPs in TNFRSF1B, 1663G/A, 1668T/G, and 1690T/C, were found to be not associated with hyperandrogenism (295).

IL-6: Among cytokines, IL-6 circulates in plasma and acts in distant tissues (296). TNF-α stimulates IL-6 secretion by adipocytes, and mounting evidence suggests that IL-6 is also implicated in insulin resistance and associated syndromes (272, 297-299). IL-6 concentrations are increased in peritoneal fluid in clomiphene-resistant, anovulatory PCOS patients, suggesting

a role in the pathogenesis of hyperandrogenic disorders (302). To date, two case control studies have considered IL-6 in relation to functional hyperandrogenism and PCOS. It is noteworthy to mention that no linkage or association studies have considered variants in IL-6 gene in relation to PCOS. Both case-control studies considered the –174G/C polymorphism in IL6 and reported that the G allele is associated with hyperandrogenism or androgen-related phenotypic trait. In one case control study involving hyperandrogenic patients and healthy controls, it was found that the G alleles were more frequent in patients compared to controls, and in controls G alleles at -174 were associated with statistically significant higher circulating levels of IL-6 and basal cortisol, 11-deoxycortisol, and 17-hydroxyprogesterone and a tendency for higher total testosterone concentrations compared with -174C alleles(300). In conceptual agreement, the other case control study involving PCOS women and age-matched healthy controls reported that the heterozygous –174G/C genotype in PCOS patients was associated with lower serum androstenedione levels (289). As for the other studied common polymorphisms in the promoter of the IL-6 gene (–597G/A, –572G/C, –373A (n) T (n)), it was found that the G alleles of the -597 G/A polymorphism were more frequent in patients compared to controls. Homozygosity and heterozygosity for –597G alleles were more frequent in controls, and in controls G alleles at -597 were associated with statistically significant higher circulating levels of IL-6 and basal cortisol, 11-deoxycortisol, and 17-hydroxyprogesterone and a tendency for higher total testosterone concentrations compared with -597A alleles(300). The –572G/C and –373A (n) T (n) were not associated with hyperandrogenism or with any androgen-related phenotypic trait (300).

IL-6 receptor: One case control study has considered IL6 receptor in relation to functional hyperandrogenism and PCOS. In one case control study of hyperandrogenic women and controls from Spain, the uncommon Arg148 allele of the $Gly^{148}Arg$ polymorphism in the gp130 gene was found to be more frequent in controls compared to hyperandrogenic patients suggesting a protective effect of this uncommon allele against androgen excess and adrenal hyperactivity (286). The same study reported association of a microsatellite CA-repeat polymorphism in the 80-kDa IL-6 binding unit locus with obesity, when considering patients and controls as a whole.

In summary, No consistent findings were reported for any specific variant at each of TNF-α and TNFRSF1B. Despite the reported positive findings for each of $Gly^{148}Arg$ polymorphism in the gp130 gene and microsatellite CA-repeat polymorphism in the 80-kDa IL-6

binding unit, each of these findings were based on one case control study and require further confirmation in future studies. The most consistent finding for proinflammtory genotypes in relation to functional hyperandrogenism and PCOS was that for –174G/C polymorphism in IL6 gene.

1.3.4 Studies on Other Candidate Genes in Functional hyperandrogenism and PCOS

Table 5: Other Candidate Genes that were studied in hyperandrogenic/PCOS populations

Candidate Gene	Reference	Study Design	Findings
Follistatin	188	Family Study	• Evidence for linkage between the follistatin locus and PCOS
	304	Family Study	• Follistatin gene contributes minimally, if any, to etiology of PCOS
	305	Case Series	• No mutations in the follistatin gene have been found in Chinese PCOS patients, defined by menstrual dysfunction, hyperandrogenism, and polycystic ovaries.
	306	Case Control	• No mutations in the coding regions of the follistatin gene appear to be related to PCOS in patients from Spain and healthy controls.
Thrombophilic factors	248, 308	Case Control	• Homozygosity for 4G alleles of the –675 4G/5G, a common and functional –675 4G/5G promoter polymorphism in the PAI-1 gene, has been reported in association with PCOS (308).
	309-310	Case Control	• No difference in the prevalence of other mutations associated with thrombophilia (antithrombin III, protein S and protein C deficiencies, factor V Leiden, prothrombin G20210A factor, and methylene tetrahydrofolate reductase 677C/T mutations)between patients with polycystic ovaries, menstrual dysfunction, and hyperandrogenism and nonhyperandrogenic controls.
Microsomal Epoxide Hydrolase	311	Case Control	• No association between the two SNPs, $Tyr^{113}His$ and $His^{139}Arg$, in the gene encoding the detoxifying enzyme microsomal epoxide hydrolase and PCOS , defined by the presence of polycystic ovaries and hyperandrogenic symptoms. • Significant association between the presence of the His113-Arg139 haplotype and PCOS .
Bone morphogenetic proteins	312	Case Control	• No missense mutations in the genes encoding the growth differentiation factor 9 and bone morphogenetic protein 15 have been found in Japanese women, with premature ovarian failure, women with PCOS(defined as amenorrhea or oligomenorrhea with or without hirsutism, a high plasma LH level and a high LH:FSH ratio, and bilaterally enlarged ovaries with multiple small cysts as assessed by transvaginal ultrasonography) and normal fertile controls.

In summary, no consistent associations were reported for any specific variant at each of Follistatin, thrombophilic factors, microsomal epoxide hydrolase genes with PCOS. Moreover, negative findings were reported for Bone morphogenetic proteins gene.

In conclusion, studies conducted to date suggest a polygenic etiology for functional hyperandrogenism and PCOS in which a complex interaction between predisposing and protective genomic variants and various environmental factors takes place. As far as candidate genes involved in insulin resistance or inflammatory pathways, studies in hyperandrogenic adults/adolescents and PCOS most consistently support association of each of Gly972Arg variant of IRS-1, Pro12 Ala variant of PPARλ and G-174C variant of IL-6 with biochemical or metabolic features with PCOS.

1.3.5 Findings on Gly972Arg variant of IRS-1, Pro12 Ala variant of PPARλ and G-174C variant of IL-6 in in-vitro Studies, PCOS/Hyperandrogenic and non-diabetic populations

1.3.5.1 Insulin Receptor Substrate 1(IRS-1)

The association of PCOS and hyperinsulinemia has been documented for decades. PCOS women have marked peripheral resistance that is independent of obesity and defects in insulin secretion, suggesting that this syndrome is characterized by a unique abnormality in the insulin pathway (313). Recent studies suggest that 50% of obese PCOS women are insulin resistant when compared with appropriately age- and weight-matched controls (314). The role of IR remains elusive in complex diseases however; most of the defects in insulin action are expected to be at the post receptor level (315). The insulin receptor substrate proteins function immediately downstream of the insulin receptor and are key proteins in insulin transduction; the IR substrate (IRS) genes encoding for these proteins are thus attractive candidates to study IR. Insulin receptor substrate-1 (IRS-1) is the major substrate of the insulin receptor and insulin-like growth factor (IGF)-1 receptor tyrosine kinase (316-317). The gene encoding the insulin receptor substrate-1 (IRS-1) protein is located on chromosome 2q35-q36.1. The IRS-1 protein is a cytoplasm molecule expressed in most insulin-sensitive tissues and has been demonstrated to play a pivotal role in modulating the cellular effects of insulin (318-319). After the binding of insulin to its receptor, the intrinsic tyrosine kinase activity of the receptor [beta]-subunit is activated, thus catalyzing the phosphorylation of specific tyrosine residues on the IRS-1 protein.

Thereby, phosphorylated IRS-1 binds with high affinity to several cellular signal proteins, thus functioning as a multisite “docking” protein linking the receptor kinase to the variety of cell functions regulated by insulin (320-321). The genetic analysis of the IRS-1 gene has revealed several base-pair changes that result in amino acid substitutions(322-324).The most common amino acid change is a glycine to arginine substitution at codon 972 (G972R), which has an overall frequency of approximately 6% in the general population(325). The Insulin receptor substrate-1 (IRS-1) plays a key role in tissue insulin sensitivity (326) and the common mutation (G972R) of the IRS-1 gene has been shown to impair IRS-1 function. Women with PCOS present a defect in insulin receptor signaling characterized by a decrease in insulin receptor substrate protein (IRS)-1-associated phosphatidylinositol 3-kinase (PI-3K) activity(327). One case control study involving PCOS women reported that carriers of Arg972(R972) IRS1 alleles presented with increased fasting insulin levels compared with women homozygous for Gly972(G972) alleles and that the Gly972Arg (G972R)IRS-1 variant was more prevalent in insulin-resistant patients compared with non-insulin-resistant individuals or control subjects (39.3 vs. 4.0 and 16.6%, $P<.0031$, respectively) (244). Recent investigations, assessing the functional significance of the R972 allele of G972R variant of IRS-1, have consistently demonstrated that the R972 allele is associated with impaired glucose-stimulated insulin secretion by the pancreatic beta cells, decreased insulin-stimulated glucose transport in skeletal muscle cells (329-331) and thus reduced insulin sensitivity. Another case control study reported that the Gly972Arg variant of the IRS1 gene has also been associated with lower SHBG levels in adolescent girls with a history of precocious pubarche (243).

A case control study, involving predisposed individuals to coronary artery disease (CAD) defined as angiographically documented coronary atherosclerosis (>50% stenosis) and population control subjects, found that the G972R IRS-1 gene variant may predispose to proatherogenic alterations in plasma lipids, even after adjustment for CAD status(332). This finding suggests a potential role of the IRS-1 gene in the pathogenesis of lipid abnormalities associated with CAD. In this study, it was also found that the G972R polymorphism significantly contributes to increasing the risk of CAD in subjects with obesity or insulin resistance syndrome (IRS), two prominent features of PCOS women. After controlling for other coronary risk factors, the relative risk of CAD associated with the G972R polymorphism was 2.93 (95% CI 1.30 to 6.60; $P<0.02$) in the entire cohort. In the subgroups of obese subjects and subjects with clinical

features of IRS, this risk was found to be even higher (OR= 6.97, 95% CI 2.24 to 21.4; *P*<0.001) (OR 27.3, 95% CI 7.19 to 104.0; *P*<0.001) respectively; these findings suggest that the G972R mutation in the IRS-1 gene may worsen or induce these abnormalities. The frequency of the G972R variant as well as the R972 allele was found to be almost 3 times more in CAD patients than in controls and the difference was highly significant. Independent of CAD status, carrier individuals (G972R) showed significantly higher values of plasma total cholesterol (*P*<0.001) and triglycerides (*P*<0.001), lower levels of HDL cholesterol, significantly higher ratio of total cholesterol to HDL cholesterol (*P*<0.001) and significantly higher frequency of diabetes when compared with wild-type carriers (17% versus 7.1%, respectively; *P*<0.01). In conceptual agreement with this study, other observations have indicated that the presence of a mutated IRS-1 gene is associated with dyslipidemia (333-335). Significantly higher triglyceride levels in heterozygous carriers of the G972R mutation has been described in other studies as well (336).This mutation has been reported to significantly impair IRS-1 function in experimental models (337). In one experimental study, the authors used the cuff placement model as an atherosclerotic model, which they believed to be close to the initial lesion of human atherosclerosis (type 1 lesion). The findings of this study are: insulin receptor substrate (IRS)-1 deficient (IRS-$1^{-/-}$) mice showed insulin resistance, significantly impaired endothelium-dependent relaxation by acetylcholine (Ach), higher Plasma free fatty acid levels, systolic blood pressure and more enhanced neointima formation than the wild-type mice. The authors concluded that the increased neointima formation in the IRS-$1^{-/-}$ mice is likely to be related to abnormalities induced by the altered metabolic environment in insulin-resistant states (340). More recently, hypertriglyceridemia has been shown in homozygous IRS-1–deficient mice, suggesting a direct role of the IRS-1 gene in modulating triglyceride levels (341). In this respect, the IRS-1–mediated activation of phosphatidylinositol-3 kinase has been reported to be involved in the antilipolytic effect of insulin (342). Because it has been demonstrated that the G972R substitution significantly reduces the IRS-1–mediated phosphatidylinositol-3 kinase activation (343), it is conceivable that mutation carriers may have impaired antilipolysis. As a consequence, an increased efflux of free fatty acid from adipose tissue would provide more substrate available for VLDL-triglyceride synthesis by the liver (344). In the Atherosclerosis Risk in Communities Study, it was reported that there was a modest association of the IRS-1 Arg972 allele with higher BMI in middle age African-Americans and BMI increase since age 25 years (345). In humans,

the role of IRS genes was suggested by the identification of several more prevalent allelic variants in type 2 diabetes (346-351). PCOS is another good model to study influent genes, because in this complex disease, characterized by chronic anovulation and hyperandrogenism in women, insulin resistance is a major component. It is noteworthy that in a study addressing Pro12Ala variant of PPARλ and the Gly972Arg variant of IRS-1 interaction, significant differences were found in insulin sensitivity between the X/Ala and Pro/Pro genotypes within the Arg 972 background that was not present in the whole population or against the Gly 972 background. They concluded that this suggests that the Ala allele of PPAR λ becomes particularly advantageous against the background of an additional, possibly disadvantageous genetic polymorphism (353).

1.3.5.2 Peroxisome proliferator-activated receptor-γ (PPARγ)

The Peroxisome proliferator-activated receptor-γ (PPARγ) gene is located in chromosome 3q25. PPARγ, which belongs to the family of PPARs which also includes the isoforms PPARα and PPARδ (354), plays a pivotal role in the regulation of energy storage, adipocyte differentiation, insulin sensitivity, and lipoprotein metabolism (355-356); hence, variation in the PPARγ gene may be a risk factor for the development of diabetes and the metabolic syndrome (357-359). PPARγ is a nuclear hormone receptor that functions as a transcriptional regulator in a variety of tissues; it has the highest expression levels in adipose tissue compared with other metabolic organs, such as skeletal muscle, liver, and pancreas (360). PPARγ comprises an agonist-dependent activation domain, DNA binding domain, and agonist-independent activation domain. Patients with a dominant-negative mutation in the PPAR-γ gene show severe hyperglycemia, which provides a genetic link between PPAR-γ and type 2 diabetes (361). PPARγ activation, through binding of the synthetic Thiazolidinediones(TZDs) or multiple endogenous ligands including fatty acids and fatty acid derivatives (362) results in a marked improvement in type 2 diabetic patients of insulin and glucose parameters resulting from an improvement of whole body insulin sensitivity(363-366). The binding affinity between TZDs and PPAR-λ correlates well with their insulin-sensitizing activity (367).Moreover, the fact that non-TZD PPAR-λ ligands also lower glucose provides additional evidence that glucose lowering through TZDs occurs because of PPAR-λ activation and not some other response (368).Therefore, it is generally accepted that TZDs exert their action through PPAR-λ (369-370). Upon the binding of the

agonists, PPAR-γ heterodimerizes with retinoid X receptor-α and activates the transcription of target genes through the binding of the PPAR response element (PPRE); target genes include those involved in glucose disposal such as GLUT2 and ß-glucokinase. Evidence supporting the direct action of PPAR-γ on glucose metabolism has been reported; TZDs increase the expression of insulin receptor substrate (IRS)-1 (371), IRS-2 (372), the p85 subunit of phosphatidylinositol 3-kinase (373), and the Cbl-associated protein (374-375). In conceptual agreement, TZDs increase insulin-stimulated glucose uptake in L6 myotubes (376) and in cultured human skeletal muscle cells (377-378). Adipose PPARγ has been identified as an essential mediator for the maintenance of whole body insulin sensitivity. Major mechanisms include: 1) Adipose PPARγ protects non-adipose tissue against excessive lipid overload - through sequestering lipids into fat stores through the induction of genes such as CD36, LPL, and aP2-, thus maintaining normal organ function (liver, skeletal muscle) 2) Adipose PPARγ guarantees a balanced and adequate production of secretion from adipose tissue of adipocytokines such as adiponectin , leptin, TNFα and IL-6, which are important mediators of regular insulin action in peripheral tissues (379-383). Gene expression profiling by microarray suggests that the detectable changes in expression by TZDs are mostly in the adipocyte (384). These include genes involved in glucose uptake [c-Cbl-associated protein (CAP) and glucose transporter 4 (GLUT4)], lipid uptake and storage (CD36, aP2, LPL, FATP, and acyl-CoA synthetase), and energy expenditure [glycerol kinase (GyK), uncoupling protein (UCP) 2 and UCP 3](384-391). Another relevant gene that has been found to be down regulated in white adipose tissue by PPARγ agonists (392-393) is that which codes for 11β-hydroxysteroid dehydrogenase 1 (11βHSD1), an enzyme expressed in the brain, liver, and adipose tissue that locally converts inactive glucocorticoids into bioactive forms such as cortisol in humans (394). This enzyme is particularly relevant to the metabolic effects of PPARγ agonism because excess glucocorticoids promote visceral obesity, hyperlipidemia, and insulin resistance (395-396). PPARγ is the master regulator of adipogenesis, thereby stimulating the production of small insulin-sensitive adipocytes (397,398) which produce less free fatty acids, tumor necrosis factor-α, and leptin. The induction of adipogenesis associated with the capability for fatty acid trapping has been shown to be an important contributor to the maintainance of systemic insulin sensitivity (399). Metabolic measurements in adipose PPARγ-deficient mice demonstrated marked hyperlipidemia with elevated free fatty acid (FFA) and trigylceride levels, and a significant decrease in plasma adipocytokines (leptin, adiponectin). Hyperlipidemia subsequently

resulted in hepatic lipid accumulation, which in turn, led to liver insulin resistance. However, in this study, despite a marked accumulation of lipids, intact insulin sensitivity in skeletal muscle of adipose PPARγ-deficient mice was maintained (400). On the other hand, other studies have shown that lypodystrophic animals develop severe insulin resistance and diabetes, mainly because of impaired muscular glucose disposal due to lipid accumulation (401,402). When adipocyte PPARγ-deficient and control mice were treated with TZDs, TZD treatment lowered FFA levels in control mice, which was not observed in adipocyte PPARγ- deficient mice, indicating that TZD-mediated regulation of plasma FFAs is dependent on PPARγ function in intact adipose tissue(400). PPARγ deficiency in adipocytes also demonstrated that adipose tissue is a major mediator of TZD effects to decrease circulating FFAs by inhibiting lipolysis in fat. This mechanism is likely a major contributor to TZD insulin-sensitizing activity (403). In addition, TZDs have been postulated to improve insulin sensitivity by redistributing fat from visceral to subcutaneous adipose tissue (404-405) and by increasing blood levels of adiponectin (406-407). Studies in fatless animal models revealed the development of systemic insulin resistance and diabetes. The diabetes state was reversed upon surgical reimplantation of adipose tissue in those animals, highlighting the importance of adipose tissue in maintaining insulin and glucose homeostasis (408-409). Lack of PPARγ in mature adipocytes did impair insulin sensitivity under high-fat diet conditions, a nutritional environment prevalent in industrialized countries and rising in developing nations (400). Against the background of this epidemic in over nutrition, the importance of adipose PPARγ in maintaining intact systemic insulin sensitivity is evident. These studies pinpoint adipose tissue as the major target of TZD-mediated improvement of hyperlipidemia and insulin sensitization (400,410), despite the important contribution of PPARγ expressed in liver and skeletal muscle to glucose and lipid metabolism (411-412). With respect to the pharmacological treatment of insulin resistance and type 2 diabetes mellitus, all studies in tissue-specific PPARγ knockout mice in which only PPARγ2 was knocked out, have demonstrated that adipose tissue is the major primary target of TZD-induced insulin sensitization (400,410-412). In addition to the insulin-sensitizing effects in peripheral tissues, PPAR-γ is known to increase the glucose-sensing ability of pancreatic ß-cells (413) through protecting the ß-cells from apoptosis and restoring the function of ß-cells. TZDs therapy appears to result in a variety of effects independent of blood glucose lowering which may have the potential to revolutionize cardiovascular risk management in Diabetes Mellitus type 2(T2D). PPAR-λ

agonists have been shown to reduce endothelial expression of vascular cell adhesion molecule-1 VCAM-1 (414) and intercellular adhesion molecule-1 (415), both resulting from the inflammatory response initiated by modified lipids in context of insulin resistance and T2D. It was recently demonstrated TZD has direct anti-inflammatory effects in a rat model of atherosclerosis via interference with monocyte chemo attractant protein-1, and its monocyte receptor, CCR2 (416),chemokines which are overexpressed in developing atheromatous plaques, and plays a role in leukocyte recruitment into the intima (417). PPAR-λ ligands have been also demonstrated to down regulate major histocompatibility complex class II expression, involved in antigen presentation to T lymphocytes thus provoking an immune response, in atheroma-associated cells (418); this may result in suppression of CD4+T lymphocyte activation and proliferation in the atherosclerotic plaque, thus attenuating the immune response to modified lipids in the arterial intima. PPAR-λ is up regulated in activated macrophages (419-420), while PPAR-λ agonists have been shown to attenuate the inflammatory response in activated monocytes and macrophages; thus activation of PPAR-λ receptors in macrophages within the arterial intima may reduce cytokine production, limiting the local inflammatory response, hence arresting atherogenesis. In monocytes/macrophages, PPAR-λ activation induces expression of CD36, a receptor-mediating uptake of oxidized low-density lipoprotein and potentially inducing foam cell formation (421]. However, any untoward increased uptake in lipids may be offset by cholesterol efflux through concomitant induction of the cholesterol transporter ABCA1 (422-423). PPAR-λ agonists have been shown to inhibit tube formation induced by vascular endothelial growth factor in vitro and in vivo (424); thus, PPAR-λ activation may inhibit plaque progression. PPAR-λ receptors have been identified in VSMCs at the site of atherosclerotic plaques, and current evidence suggests that PPAR-λ agonists may inhibit VSMC migration and proliferation (425). Clinical studies of TZD treatment in T2D patients have demonstrated a reduction in serum concentrations of MMP-9, which is known to impair atherosclerotic plaque stability, a reduction of serum levels CRP(426), which is now recognized in association with increased cardiovascular risk (427), a significant reduction of carotid intima thickness (428),a decrease in microalbuminuria (429) ,blood pressure (430) and PAI-1 levels (431) and reverse(432-433) or improve (434) endothelial dysfunction in T2D (432-433). Furthermore, one clinical trial reported that treatment with TZD has reversed the endothelial dysfunction associated with the polycystic ovarian syndrome (435), an insulin resistant state. Clinical trials

reported a lowering of 9.6% in triglyceride (436), an HDL increase of 12% (436-437) and a triglyceride depletion of lipoprotein resulting in the generation of larger less dense particles (437-438). Only a small number of studies on the effect of TZDs on insulin resistance in PCOS were conducted in the United States. Trials reported a reduction in fasting glucose, integrated insulin response to glucose load and improvement in insulin sensitivity (439-441) in PCOS patients receiving TZD. All studies that examined the effects of troglitazone, a TZD, on PCOS-associated hyperandrogenism have reported positive results; after 3 months of therapy with 400 mg troglitazone/d, free T dropped 25-35% and SHBG increased by 25-66% (435-436). The therapeutic effect of the drug is probably mediated by the reduction in insulin resistance and hyperinsulinemia. However, the drug could also have a direct effect on the ovaries (442-443). PPAR-λ receptors were recently demonstrated in pig ovaries. Addition of troglitazone to cultures of porcine theca cells resulted in a 53-69% decrease in LH- and/or insulin-stimulated A and T production (444). PPAR-γ2 was reported to influence insulin sensitivity in Caucasians (252) and activation of PPAR-γ by using the insulin sensitizer drugs, thiazolidinediones, has proved to increase insulin sensitivity, hyperandrogenism, and ovulation in women with PCOS (445-447).

The PPARλ gene contains a common missense mutation that results in a substitution of proline by alanine in codon 12 (Pro12Ala) (448) and consistently has been associated with improved insulin sensitivity and decreased risk of type 2 diabetes in many studies(252,451,453) including a meta-analysis (454). In addition, the Pro12Ala polymorphism is associated with increased insulin sensitivity of glucose disposal and suppression of lipolysis (452). Ala12 alleles of PPARλ have been shown to favor weight gain in obese adults (250) and in obese hyperandrogenic girls and adolescents (249) and to preserve insulin sensitivity in Caucasian men (252) and Caucasian women presenting with PCOS defined by NICHD (253). Recently, a marginally significant decrease in the frequency of the Ala12 allele in Finnish PCOS patients has been reported (251). In a community-based sample of white young adults and children and a sub sample of a cohort who participated both as children and adults, with an average follow-up period of 13.4 years, it was found that ,independent of sex, age, and BMI, the Ala12 allele beneficially influences insulin resistance status(455). The finding of the association between Pro12Ala polymorphism and measures of insulin resistance after adjusting for BMI has been supported in other studies as well(252,453). Furthermore, studies (455-456) reported that the Ala12 allele attenuates the adverse association between adiposity and insulin resistance measures

and the persistence of high levels of insulin and HOMA-IR from childhood to adulthood in whites.

Consistent with these findings in Caucasians, in a population-based sample of 1,441 middle-aged African-American individuals, a significant association between the Pro/Ala genotype of PPAR-λ2 and markers of insulin sensitivity and perhaps protection from the development of type 2 diabetes among nonobese African-American individuals has been reported (457). In a large and representative Asian population from Singapore (Chinese Malays and Indians), it was found that carriers of the Ala12 allele have a statistically significant 3.5% higher mean HDL than Pro12 homozygotes. The Ala12 allele was also found to be significantly associated with a decreased risk of IGT(458), consistent with another study (459) in Japanese-American subjects, which reported a frequency of the Ala12 allele among IGT subjects intermediate between that of normal and diabetic subjects, with the association of the Pro12Pro genotype with a higher risk of IGT. The Ala12 allele was associated with the lower triglyceride concentrations in Spanish women from the general population (461), and in Ala12Ala homozygotes in the Danish MONICA cohort (462). Given that insulin resistance is a well-established risk factor for CVD and type 2 diabetes, carriers of the variant Ala12 allele may have a reduced risk for developing these diseases.

1.3.5.3 Interleukin-6 (IL-6)

From current available evidence, it is not still clear whether inflammatory parameters are markers or mediators of insulin resistance and/or cardiovascular disease. Initially, active chronic inflammatory disease was found to lead to peripheral insulin resistance (463) and subsequent epidemiological evidence that inflammatory markers predict the development of diabetes and glucose disorders emerged (464-466). In both insulin resistance and atherosclerosis, studies implicated cytokines and growth factors in the pathophysiology of insulin resistance and atherosclerosis and in their complications. On the other hand, insulin resistance has also been increasingly recognized as having an important role in inflammatory pathways (467-470). Insulin seems to be one of the main regulators of the cytokine-associated acute-phase reaction (471-472). PCOS women have increased C-reactive protein levels as compared to healthy weight-matched controls.However when adjusted for insulin sensitivity, C-RP was no longer significantly different between groups, suggesting the key mediating role of insulin resistance to

increased inflammatory markers levels (473-474). Furthermore, CRP and IL-6 decreased significantly after improvement of metabolic control in type 2 diabetic patients, indicating that the inflammatory pathways are modulated by insulin (475). Atherosclerosis and insulin resistance share similar pathophysiological mechanisms, mainly due to the actions of the two major proinflammatory cytokines, TNF-α and Interleukin-6 (IL-6) (476). TNF-α functions locally at the level of the adipocyte in a paracrine fashion. IL-6 circulates in plasma at high concentrations. In this sense, IL-6 may be more important systemically and perhaps represents a hormonal factor that induces muscle insulin resistance. In fact, IL-6 is named the endocrine cytokine (477).

The human IL-6 gene (IL6) is mapped to chromosome 7p21-24. IL-6 is a phosphorylated glycoprotein containing 185 amino acids and is involved in different physiologic and pathophysiologic processes. Interleukin-6 is known to play a role in the development of atherosclerosis pathway (478), coronary heart disease (479), and in the increased induction of hepatic C-reactive protein (CRP), which is an independent risk factor for type 2 diabetes (480-482). IL-6 has been recently implicated in the development of insulin resistance and type 2 diabetes (483). A recent prospective study showed that circulating IL-6 levels correlate with risk for developing Type 2 diabetes irrespective of the amount of body fat (484).Actually, in insulin-resistant patients the degree of correlation between the IL-6 concentration and the severity of insulin resistance was actually found to be higher than that found than that with TNF α(483). Recent evidence suggests that some of the diminishing effects of TNFα on insulin action may be mediated by its ability to induce IL-6 and IL-6 receptor expression in tissues such as the liver and muscle (485). Down regulation of adiponectin expression by IL-6 (486) has also been suggested as contributing to insulin resistance induced by IL-6. As to heart disease association with IL-6, IL-6 has been hypothesized to be responsible for the lipid abnormalities occurring in subjects with the insulin resistance syndrome (466-467). IL-6 inhibits adipocyte Lipoprotein lipase (LPL) activity (487) and induces increases in hepatic triglyceride secretion in rats (488). In man, IL-6 infusion leads to increased free fatty acid concentration (489), and fasting triglycerides, VLDL triglycerides, and post-glucose load free fatty acids are linked to serum IL-6 concentration (490). Both IL6 and IL6 gene transcripts have been localized within atherosclerotic plaques (491-492) supporting a possible local role of inflammation in the initiation and progression of atherosclerosis (493). Furthermore, prospective clinical studies have

shown that elevated levels of IL6 are predictive of future myocardial infarction in healthy men [6] and useful for the prediction of cardiovascular disease in women (494). IL-6, through stimulating the central nervous system and the sympathetic nervous system (495-496) inducing fibrinogen (497), or inducing expression of angiotensin II (498) may result in hypertension. Significant correlations of IL-6 levels with BMI, percent fat mass, systolic and diastolic blood pressure and fasting insulin levels have been reported (478,499-500).Circulating protein markers of inflammation such as IL-6 and C-reactive protein (CRP) have been found to be predictive for the risk of future coronary events in apparently healthy men and women (501-503). IL-6 is a central mediator of the acute phase response and may therefore play a causal role in atherosclerotic disease. It is expressed in macrophages within human atheroma, (504) has a stimulatory effect on smooth muscle cell proliferation (505) and has the ability to accelerate atherosclerosis in murine models. (506-507). Raised plasma concentrations have been found in patients with unstable angina, (508) and in healthy subjects at risk of future cardiovascular events. (509).As to reproductive processes, IL-6 is believed to affect the processes of fertilization and implantation (510). In a mouse model, follicular cystic ovaries showed increased production of IL-6. Consequently, it has been hypothesized that increased IL-6 production might alter vital steps in follicular maturation, ultimately contributing to ovarian dysfunction (511).

In an in vitro study evaluating the chronic effect of IL-6 on insulin signaling in adipocytes, it was found that IL-6 is not only produced by the fat cells but it is also capable of inducing insulin resistance in these cells. IL-6 had adverse effects at both the receptor (IR-β and IRS-1 tyrosine phosphorylation) and post-receptor levels (such as glucose transport, and lipogenesis); IL-6 suppressed the insulin-induced lipogenesis and glucose transport consistent with a diminished expression of GLUT4, and induced the expression of SOCS-3, a potential inhibitor of insulin signaling. IL-6-treated adipocytes failed to maintain their adipocyte phenotype as shown by the down regulation of the adipogenic markers, one of which is PPAR-γ, which play a role in insulin sensitivity. These results show that IL-6, through effects on gene transcription, is capable of impairing insulin signaling and action. Finally, this study also found that the negative effects of IL-6 on insulin signaling could be prevented by TZD (rosiglitazone), an insulin-sensitizing agent. TZD treatment of adipocytes negatively affected basal IL-6 secretion and SOCS-3 expression, suggesting that one mechanism for insulin sensitizing by TZD may be through a direct effect on IL-6 signaling and reinforcing the potential role of IL-6 as a

mediator of insulin resistance (512). In another in vitro study in which adipocytes from non-obese insulin resistant subjects and controls were cultured with IL-6, it was found that IL-6 is not only produced by the fat cells but it is also capable of inducing insulin resistance in these cell. The authors concluded that the potential role of IL-6 in whole body insulin resistance in man is further supported by the observation that non-obese insulin-resistant individuals showed evidence of a marked up-regulation of the IL-6 gene as compared to non-obese controls (513).

Genetic predisposition to increased transcription rates of these cytokines is associated with metabolic derangement and simultaneously with coronary heart disease. Dysregulation of the inflammatory axis predicts the development of insulin resistance and type 2 diabetes mellitus. A common G/C single nucleotide polymorphism of the IL6 promoter at position 174(G-174C) has been found to influence the transcription rate of this multifunctional cytokine (514). This IL-6 gene polymorphism has been suggested to be involved in the regulation of insulin sensitivity (516, 522), type 2 diabetes (467), energy expenditure (523), hyperandrogenism (300,515) and lipid abnormalities (490). Studies involving the G-174C variant of the *IL-6* gene in relation to insulin sensitivity and T2D have reported conflicting results. Caucasian subjects homozygous for the C allele at position -174 of the IL-6 gene have been found to have lower plasma IL-6 levels, significantly lower integrated area under the curve of serum glucose concentrations ($AUC_{glucose}$) after an oral glucose tolerance test, lower fasting insulin levels, lower total and differential white blood cell count, and an increased insulin sensitivity index than carriers of the G allele, despite similar age and body composition (516). However, in a cross sectional study of Caucasians; subjects homozygous for the C allele of the G-174C IL-6 polymorphism were found to have decreased insulin sensitivity than carriers of the G allele (520). Studies, which have addressed this variant in IL-6 gene in relation to hyperandrogenism, have reported conflicting results as well. In a recent case control study involving hyperandrogenic patients and healthy women from Spain, it was found that that the common G allele of G-174C IL6 gene polymorphism is associated with hyperandrogenism. Moreover, when studying controls alone, carriers of G alleles presented higher serum IL-6, 17-hydroxyprogesterone and 11-deoxycortisol levels, and a near-significant increase in serum total testosterone levels, compared with subjects homozygous for the uncommon -174C alleles, suggesting a protective role for these uncommon alleles against adrenal hyperactivity and hyperandrogenism(300). On the other hand, another case control study of Caucasian patients with PCOS and healthy controls found conflicting results. Among PCOS

women, there was a significant difference in BMI>27 kg/m^2 , total serum testosterone level> 0.86ng/ml and a pathological OGTT result between women carrying at least one C allele of the G-174C(GC and CC)variant of IL-6 and women with the G-174G genotype. Women carrying at least one C allele of the G-174C(GC and CC)variant of IL-6 were more likely to present with a body mass index >27 kg/m^2, elevated total T serum levels, and a pathological OGTT result(515). Studies, which have addressed this variant in IL-6 in relation to lipid abnormalities, have reported inconsistent results as well. In one study involving healthy Caucasian subjects, it was found that carriers of the G allele, though similar in age, sex, body mass index, and waist to hip ratio to carriers of the C allele, had significantly almost twice plasma triglycerides, very low-density lipoprotein (VLDL)-triglycerides, higher fasting insulin and post glucose load free fatty acids. In addition, the G allele carriers showed slightly lower high-density lipoprotein-2 cholesterol than carriers of the C allele (490). On the contrary, another cross sectional study involving Australian subjects found that subjects carrying the CC genotype have 12% higher triglyceride levels than subjects with the GG or GC genotype combined (521). This discrepancy could be explained by genotype-environment interaction, diet for example.

Other studies have focused on the relation between G-174C variant in IL-6 and predictors of cardiovascular disease. In one cross sectional study (521) of healthy Australian subjects, it was found that the -174C allele of the IL-6 G-174C variant is independently associated with carotid plaque formation in the whole population and an increased carotid IMT in older subjects. However, this finding has not been supported in other two studies, which they have shown the GG genotype, and not the CC genotype to be associated with thicker carotid IMT (524-525). In a case-control study of men and women within the Cardiovascular Health Study, the -174C allele was associated with higher IL-6, C-reactive protein and fibrinogen levels. Compared with its absence, presence of the -174C allele was associated with risk of MRI infarcts (odds ratio 1.5), higher blood pressure and conveyed a modestly higher relative risk (RR) for coronary heart disease (529). On the other hand, another study found that the GG genotype of the -174 G/C IL-6 polymorphism to be significantly more common in peripheral artery occlusive disease cases. The CC genotype was also found to be significantly more prevalent in controls (534). Furthermore, the -174 G allele of the -174 G/C IL-6-promoter polymorphism has been found to be associated with higher circulating levels of CRP (533). The -174 G/C polymorphism of the interleukin-6 gene promoter has been found to show divergent associations with blood pressure as well (530-

531). In general, fewer studies have reported negative impact of the uncommon C allele of the G-174C variant of IL-6 as compared to the G allele.

1.3.6 Linkage and Association Studies of G972R IRS1, G-174C IL6 and P12Ala PPARγ in T2Diabetic populations

1.3.6.1 Linkage & Association Studies of G972R IRS1 in T2Diabetic Populations

Non-Insulin Dependent Diabetes Mellitus (T2DM or Type II Diabetes Mellitus -T2DM), characterized by insulin resistance and progressive pancreatic β-cell failure, is the most common of all metabolic disorders. T2DM currently affects about 6–7% of the US population, with a cumulative risk of 17% by age 80 (535). The metabolic abnormalities created by chronic hyperglycemia, together with the strong association between T2DM, obesity, hypertension, and hyperlipidemia, lead to a broad list of long-term complications, including a high rate of cardiovascular death and amputation due to accelerated atherosclerosis, as well as the typical complications of diabetes such as retinopathy, nephropathy, and neuropathy. As with most common medical disorders, T2DM is heterogeneous and the result of an interaction between genetic and environmental factors (535,315). A strong genetic component is suggested by the remarkable clustering of T2DM in families (536), by the high prevalence of T2DM in certain ethnic groups (537-538), and especially by the twin studies and the high concordance rate (50–95%) for T2DM between monozygotic twins (539,540-543,361). The genetic component of T2DM appears to be complex, involving multiple genes (544).

Insulin resistance is a well established component of the pathogenesis of T2DM (545), and in prospective studies in various populations with a high prevalence of T2DM such as Pima Indians and Caucasian offspring of two diabetic parents, insulin resistance both precedes and predicts the onset of T2DM (546-549). Insulin resistance is prevalent among family members of T2DM patients who are at risk for future diabetes (550-551) and is heritable as an autosomal (dominant/recessive) disease (552-558). Moreover, excess concordance rates in monozygotic versus dizygotic twins clearly suggest a contribution of genetic factors to insulin resistance (10). These data suggest the hypothesis that genetic defects at loci that directly or indirectly control insulin action contribute to the inherited predisposition to T2DM and might reside in the direct

pathway of insulin action and peripheral glucose uptake, the glycolytic pathway, or triglyceride and free fatty acid (FFA) metabolism (26).

Much progress has been made in the rare, autosomal dominant forms of T2DM. In contrast, the genes for typical T2DM, which shows a complex pattern of inheritance, have been elusive. A large number of genes have been examined based on their function in the pathways of insulin action or insulin secretion; however despite the apparently pivotal role for inherited defects in these pathways in the pathogenesis of T2DM, the variants described in the candidate genes in these pathways have been inconsistently associated with T2DM or traits that lead to T2DM. The association has been difficult to confirm due to the variable age of onset, excess mortality, unknown mode of inheritance, and genetic heterogeneity (559-561). Some evidence of involvement has been produced for several genes including insulin-receptor substrate-1 (IRS1), but the contribution of these genes to T2DM is probably small (562-565). IRS1, located on chromosome 2q36, is a critical element in insulin-signaling pathways (566) and is therefore an attractive candidate gene to harbor genetic variation that might influence insulin resistance and/or T2DM in humans. Amino acid substitutions, one of which is the G972R variant, have been identified in IRS-1 among various populations such as Caucasian and Japanese individuals (322-324;567-569) and have been reported to have a role in determining susceptibility to traits related to T2DM. Although the pathophysiology and the relevance of the G972R variant in T2DM etiology is unclear, studies of the 972 variant suggest that it impairs insulin-stimulated signaling and contribute to insulin resistance in normal and diabetic populations (337). In one study from Japan, both diabetics and nondiabetics with mutations in IRS-1, including G972R, had about a 30% lower value for insulin-stimulated glucose uptake as compared to those in individuals with a normal IRS-1 sequence (567). The G972R IRS1 variant has inconsistently been shown to associate with T2DM (322-324; 567; 570,325,242). G972R has been found to be significantly associated with late-onset T2DM in case control studies of Danish, Italian, German and UK white patients (322,324;571-572) as well as in three meta-analyses (242,349,584). In the former two European studies, the proportion of R972 carriers was ~12-23% among T2DM patients as compared to 4-12.5% among controls respectively (322,324). In one meta analysis, which was based on seven studies published up to 1996 (322-324; 570,325; 349), a combined significant odds ratio of 1.49 has been reported (349). A larger more recent meta-analysis, which examined 27(322-324;567; 571,325; 571-572;573,574;575-583;349;332;345) studies comprising

8,827 subjects, reported a modest but significant association with T2DM (OR=1.25) in favor of carriers of the minor R972 allele (242). Two following meta-analyses attempted to replicate the findings of this latter meta-analysis. The first meta-analysis combining data in this same study (584) with data from this latter meta-analysis and from a recent report of two G972R association studies (31 studies total) (242;585) supported the previous finding of a significant positive association of the R 972 allele IRS1 variant with T2DM, although with a diminished OR than that reported in previous meta-analyses (OR=1.15) (584). However, the second adequately powered large meta-analysis of T2diabetic case and control subjects, involving Scandinavian, Swedish, Canadian, Finnish, US and Polish case-control samples failed to replicate this previously reported association (586). Moreover, Combining this data with the diabetic trios reported in a previous study (454) and all the studies included in this latter meta-analysis (242), the G972R association was still found not to exist.

In several populations, including Caucasian, Chinese, Mexican American and Indian populations, an increased frequency of R972 allele of the IRS-1 gene was found in T2DM patients compared with the control subjects however, the association between T2DM and IRS-1 G972R variant was not significant. This would suggest the potential role of G972R IRS1 variant in the pathogenesis of T2DM (323, 570; 573; 454,587; 325). In the latter study however, the result became significant when data from the white populations involved in this study namely, Finnish and South Indian were combined together with data from two previously published studies in other two white populations, specifically Danish and French populations (325).

Some studies reported no evidence for an association or for a positive association of the R972 allele of the G972R IRS1 variant with T2DM in several populations including Caucasian UK, Mexican, Caucasian Dutch, African American, Japanese and Taiwanese populations (567; 349; 574,345; 578; 581; 583-585; 588-589; 572). The latter study(the UKPDS cohort) however, despite showing no association of the IRS-1 G972R variant with common Type 2 diabetes, reported a higher prevalence of this variant in diabetes characterized by strong insulin resistance (572). Other findings which were reported regarding the association of the G972R IRS-1 variant with T2DM are the following. One study involving two large population-based studies of Caucasian Dutch T2 diabetic cases and control subjects (the general population sample and the high-risk sample –defined as predominantly BMI ≥ 25 kg/m2 and/or a family history of T2DM) reported that the association between the R972 allele and glucose intolerance (defined as

impaired fasting glucose, impaired glucose tolerance or T2DM) in the high risk population or T2DM in the general population did not differ appreciably by obesity, a finding supported in several earlier studies (574). In this same study, the R972 allele was not associated with detrimental values for cardiovascular risk factors (waist circumference, plasma HDL-and total cholesterol or hypertension) among cases or persons with high BMI (≥ 27 kg/m2) in both samples, even after adjusting for age, sex, study centre(585). This latter finding was supported in another study involving French T2 diabetic individuals with a family history of T2DM and unrelated normoglycaemic individuals (570). In conceptual disagreement, a study conducted in the UK found that T2 diabetic patients with the R972 allele had significantly lower levels of cholesterol as well as lower levels of triglyceride, factor VII: C activity and PAI-1 antigen compared with homozygotes for the wild type (590). This same study also reported that there were no differences in BMI, indices of glycaemic control, fasting insulin or the prevalence of hypertension in the T2DM subjects with the R972 allele compared with homozygotes for the wild type. In a study involving Chinese patients with T2DM and control subjects, the G972R IRS1 variant was found in T2DM cases but not in controls (591).

Several family-based studies have involved IRS1 in relation to T2DM. One study involving Chinese T2DM and unrelated normal control subjects and multiplex Chinese families living in Taiwan, the prevalence of the G972R variant was not increased in the T2DM population but increased in the probands of the multiplex families however, this increase was not significant. More importantly, the G972R variant of the IRS-1 gene did not cosegregate with BMI and T2DM in these families (583). This finding was supported in other studies. One study showed no co-segregation between the G972R variant and the onset of T2DM in Japanese individuals (576). In another study screening all T2diabetic and non-diabetic members of the 31 French families of subjects carrying the G972R IRS1 mutation patients and assuming a dominant mode of inheritance, variation of the codon 972 of IRS-1 was found not to co-segregate with T2DM in these families. In this same study screening 233 unrelated T2 diabetic individuals with a family history of T2DM and 130 unrelated normoglycaemic individuals for the IRS1 G972R variant resulted in no effect of this variant on clinical and biological indices in diabetic and normoglycaemic individuals(570). Another study (454), which evaluated 16 single nucleotide polymorphisms (SNPs) that had been previously reported to be associated with T2DM or related sub-phenotypes using a family-based multi-layered design (333 Scandinavian parent-offspring

trios with T2DM or abnormal glucose homeostasis) and replication samples(1,130 individuals from Scandinavian sibships discordant for T2DM, 608 case-control pairs from Scandinavia and Canada), found non-significant deviation of the G972R IRS1 variant from 50:50 transmission from heterozygous parents in this stringent multi-layer analysis. Two linkage studies involving the IRS1 locus and T2DM have excluded IRS1 as a major T2DM susceptibility locus in Caucasians. The first study was a linkage analysis of candidate regions chosen for their association with insulin resistance, known effects on lipid metabolism, or effects on glucose metabolism or insulin action. The authors, however, concluded that the possibility remains that any of the studied loci could play a major role in the intermediate steps in T2DM pathogenesis, such as insulin resistance, and not be linked to T2DM; that they could play a small role in T2DM, which was not detectable in their study; or that they are major loci in populations other than their studied Caucasian population (592). The second linkage analyses was between four candidate genes for insulin resistance(glycogen synthase (GSY), insulin receptor substrate-1 (IRS-1) and apolipoprotein C-II (APOC-II) and phosphoenolpyruvate carboxykinase gene (PCK1)genes) and T2DM in a set of 55 multigenerational French Caucasian families, no significant results were obtained with IRS-1(593). Another linkage study, involving the IRS1 locus and a putative major gene for age of T2DM onset, has suggested IRS1 as a potential susceptibility locus in Mexican Americans. In this study, two independent family studies (family heart data set and the family diabetes-FD-data set), were carried out in Mexican-Americans from San Antonio, Texas. In both sets of families, segregation analyses revealed support for a major gene with an autosomal dominant mode of inheritance influencing early age of onset of T2DM. Linkage analyses, to this putative major gene for age of diabetes onset in the FD data set, were performed with 11 candidate genes. The IRS1 marker gave a faintly positive significant LOD score (LOD score= 0.92). The authors performed simulation studies which showed that there was only a 3 percent chance of obtaining a LOD score of 0.5 or greater with an unlinked marker and thus, considering the known role of IRS1 in insulin signaling, this locus may deserve further exploration (594).

1.3.6.2 Linkage & Association Studies of G-174C IL6 in T2Diabetic Populations

Increasing evidence suggests that chronic activation of the innate immune system reduces insulin sensitivity and hence may precede the development of T2DM. Support for an effect of low-grade activation of the immune system on T2DM derives mostly from cross-sectional findings of associations between increased markers of inflammation, which indicate immune system activation, and insulin resistance and/or T2DM (101;468;595-596). In addition, there is some evidence from prospective studies in Pima Indians and other populations supporting this hypothesis (465;484;597]. Subgroups of the population have also been identified who are at risk of T2DM and have elevated inflammatory markers. These include overweight adults and children, women with polycystic ovary syndrome, Pima Indians and subjects with a family history of T2DM (598). Molecular markers of inflammation in T2DM have included the acute-phase response proteins such as C-reactive protein and the cytokine IL-6 (467,499;599). IL6 levels are increased in T2DM and insulin-resistant states (600-602;603-606;467-468) and are correlated with measures of insulin sensitivity (600; 278-299). Moreover, high circulating IL6 concentrations have been found to predict the development of T2DM (484; 607). Recently, a G/C variant in the IL6 promoter region at position –174 that regulates transcription of the IL6 gene was described and is associated with plasma IL6 levels in healthy Caucasians (514). In fact, the G–174C IL-6 gene variant has been proposed as a risk factor for and associated with T2DM based on studies of unrelated individuals however, these results have been conflicting. In one study involving Caucasian subjects, the −174C allele was found to be associated with higher BMI in T2DM, but not amongst healthy subjects. In the same study, it was also found that the frequency of the −174C allele was significantly lower in type 2 diabetes compared to the non-diabetic men. This study also found no significant association between the G-174C IL6 gene variant and plasma CRP (608). This finding was supported in several other studies. One population-based sample (KORA S4 1999/2001) involving 704 German Caucasian elderly subjects : 230 T2D patients, 235 patients with IGT, and 239 normoglycemic controls frequency-matched for age and sex , found that the -174G allele of the G-174C IL6 variant was significantly positively associated with T2DM but not associated with impaired glucose tolerance. Moreover, no association of the IL6 variant and other key parameters characterizing the metabolic syndrome, such as waist circumference, total cholesterol, LDL cholesterol, HDL

cholesterol, leukocyte count, insulin, insulin resistance (HOMA), fasting triglycerides and hypertension has been observed (604). Another study involving a case-control sample and a family-based association of sibships of Native Americans and another case-control sample consisting of Spanish Caucasians reported similar results. It was found that among both the Spanish Caucasian and the Native American case-control samples the GG genotype was significantly more common in diabetic than in non-diabetic subjects. When the Native American sample population was stratified according to ethnic heritage, all subjects who were of full Pima Indian heritage had the GG genotype, whereas in the American Indian subjects with non-Pima admixture, T2DM was significantly associated with IL6 genotype with the GG genotype significantly more common in diabetic than in non-diabetic subjects. Among the 175 individuals selected in the family based association study, there were 149 sibling pairs discordant for T2DM with only 17 discordant for IL6 genotype; the odds ratio for T2DM in the GG compared with GC sibs was 2.23 but this association was not significant (517). The finding of a protective effect of the -174C allele on T2DM, however, was not supported in one study. One family-based analysis evaluated associations between the G–174C IL-6 promoter variant and fasting plasma glucose (FPG) and T2DM in a sample of 670 individuals from the largest 182 families in the National Heart, Lung and Blood Institute's Framingham Heart Study population. In this study and in the subset of informative families (n=144), the risk of T2DM associated with the GG genotype was significantly lower relative to the GC and CC genotypes combined. Moreover, the GG genotype was found to be associated with significantly lower fasting plasma glucose (FPG) values than either the GC or CC genotypes further indicating a protective role for the –174 IL-6 G allele against T2DM (609). Other findings which were reported regarding the association of the G-174C IL6 variant with T2DM are the following. In a nested case-control study within the European Prospective Investigation into Cancer and Nutrition-Potsdam cohort (n= 27,548) involving 188 T2DM cases and 376 controls, the G-174C IL6 polymorhism was found to be an effect modifier for the impact of BMI regarding T2DM; obese individuals (BMI $\geq$ 28 kg/m2)carrying the CC genotype showed a more than 5-fold increased risk of developing T2DM compared with the remaining genotypes (610). In another case control study involving 101 Taiwanese T2 diabetic subjects and 112, non-diabetic, healthy individuals, the C allele of the G-174C IL6 polymorphism was not found; the authors concluded that the IL-6 C-174G

polymorphism is unlikely to play a role in the development of type 2 diabetes Taiwanese population (611).

1.3.6.3 Linkage & Association Studies of Pro12Ala PPARγ in T2Diabetic Populations

The PPARγ gene is located on chromosome 3(612). PPARγ, on chromosome 3p25, encodes the nuclear receptor peroxisome proliferator-activated receptor [gamma] PPARγ. PPARγ is an attractive candidate gene for susceptibility to T2DM and related phenotypes because its products play a key role in the modulation of insulin sensitivity, inflammation, and in adipocyte differentiation and proliferation, through regulation of the expression of adipocyte-specific developmental genes (613; 356,374). A number of genetic variants in the PPARγ gene have been identified; these include the highly prevalent Pro12Ala variant in PPARγ 2. This variant confers a loss-of-function phenotype to individuals carrying the less common Ala12.

Mutation analyses of selected 'candidate' T2DM susceptibility genes in various populations have identified the widespread Pro12Ala variant of the PPARγ gene (614). An initial report (359) of the association of the Pro12Ala variant to T2DM in Finnish and second-generation Japanese populations showed that the less common Ala12 allele led to increased insulin sensitivity and was protective against T2DM. In this study, the magnitude of the effect was reported to be a 70% reduction in T2DM risk associated with the Ala12 allele (358). Four of five subsequent publications (451;615;616-618) failed to confirm the association (615; 616-618). However, the association of the Pro12Ala variant to T2DM became clearer as larger and more numerous studies were published (561; 454; 615;451;616-620;460;621-626). Although some of these individual published studies did not reach statistical significance, these were mainly studies, which examined smaller samples. Yet, these studies still generally obtained results that were consistent with the global estimate of risk (454). All the large studies (which examined >1,000 individuals), however, showed similar and statistically convincing associations (451; 619-620;460; 624). Generally, therefore, robust and consistently reproducible associations have been obtained for the Pro12Ala variant to T2DM. Individuals from different populations- Caucasian (Scandinavian, Canadian, Polish, US, Finnish, Scottish, Danish, French and Swedish), Pima Indians, Chez Republicans, Japanese and Japanese Americans- with the variant Ala12 allele of the Pro12Ala variant enjoy protection against T2DM (586,454; 358; 451; 620;460; 624;

627-635). The Ala12 allele has also been associated, in studies involving T2diabetic populations, with better insulin sensitivity (358; 626; 634; 636), lower BMI (358; 634; 638) and better lipid profile as well (358; 460; 637). Subsequent studies either did not support an association of the Ala12 allele of the Pro12Ala variant with T2DM (600; 615; 616-618; 622; 625-626; 639-641;642-644;457), insulin sensitivity (451;616; 621; 624; 638; 647-648), insulin secretion (451), BMI (454; 451;616; 618; 624; 643; 645; 647-649; 633] dyslipidemia (451;616; 618; 643; 645; 647-648) and blood pressure (615-616; 624; 643; 645-646; 648) in T2 diabetic populations, or reported opposite effects of this genetic variant on T2DM (626; 652), insulin sensitivity (624), BMI (460; 642; 650-651) and lipid profile (624; 643; 653) in T2 diabetic populations. Other association studies reported original findings from that in the above studies. Three studies examined the association of the Ala12 variant with blood pressure in T2diabetic populations. One study found that the Ala12 allele was significantly associated with a high level of systolic blood pressure in T2 Tunisian diabetic patients (639). A second study found that among Finnish grossly obese (BMI >40 kg/m2) T2diabetics and compared to subjects without the Ala12 allele, subjects carrying this allele had higher Diastolic blood pressure (460). A third study found that the Pro12Ala variant was associated with lower diastolic blood pressure in male, but not female subjects with T2DM (647). Two studies reported a differential effect of the Ala12 allele on risk of T2DM across gender or populations. One study showed a highly significant protective effect of Ala12 allele on risk of T2DM in Polish whites, but not in US whites (620). Another study involving PPARγ in the entire adult Oji-Cree population of northern Ontario, found that the PPARγ Ala12 to be strongly and significantly associated with T2DM in women, but not men; among women, the odds of being affected for carriers of PPARγ Ala12 compared with noncarriers was 2.3. Furthermore, affected female carriers of PPARγ Ala12 had a significantly earlier age-of-onset and/or age-at-diagnosis compared with noncarriers (621). Two studies reported a differential effect of the Ala12 allele on cholesterol levels across BMI levels or gender in T2 diabetic populations. One study found that in obese UK T2 diabetic patients (BMI > 29 kg/m2), the Ala12 substitution was associated with elevated total and non-HDL cholesterol levels (649). Another study involving German T2diabetic patients found that in contrast to females, total cholesterol and LDL-cholesterol were significantly higher in males in the presence of the Ala12 allele as compared to the wild type subgroup (643). Two studies evaluated the association of the Ala12 allele to plasma glucose in T2diabetic populations. One study involving

139 sedentary T2 diabetic patients who completed 3 months of supervised exercise training found that the Ala12 carriers had a bigger significant change in fasting plasma glucose as compared to Pro12Pro carriers even after adjusting for statistically significant predictors (age, changes in insulin and BMI) (654). Another study involving T2 diabetic Korean patients who were treated with rosiglitazone (4 mg/d) for 12 weeks, found that the decrease in fasting plasma glucose and in HbA1c levels was significantly greater in subjects with the Ala12 allele than in those without the allele (655). Three studies evaluated the association of the Ala12 allele with diabetic retinopathy in T2diabetic populations. One study involving Slovenian T2 diabetic subjects with diabetic retinopathy and without diabetic retinopathy found that the Pro12Ala variant failed to yield an association with diabetic retinopathy (656). This finding was supported in other two studies (624; 643). Three studies evaluated the association of the Ala12 allele with diabetic nephropathy in T2 diabetic populations. One study involving Brazilian T2D patients with chronic renal insufficiency and normoalbuminuric T2diabetic patients found that the Pro12Ala carriers have a decreased risk of diabetic nephropathy compared to wild-type carriers in the two groups (648). This finding was supported in another study (657) but not in another (624). Four studies examined the association of the Ala12 allele to cardiovascular disease in T2 diabetic populations. One study has considered the impact of both the Ala12 allele of the Pro12Ala variant and the linked T allele of the C1431T variant, which was shown to influence Ala12-associated T2DM and to have opposing associations with body weight, on coronary artery disease in a German T2 diabetic population. This study found, however, no association of these or variants with atherosclerotic vascular disease (651).Three other studies did not support this finding. One study involving T2diabetic Scottish individuals found that the hazard for a first nonfatal event associated with the Ala12 allele was significantly lower than that associated with the Pro/Pro genotype. This is after adjusting for age, sex, and other conventional cardiovascular risk factors (smoking status, log10 mean BMI, first-recorded HDL-cholesterol, total cholesterol, log10 triglycerides, and mean arterial blood pressure)) and the linked T allele of the C1431T polymorphism (658). Another study of Japanese T2 diabetic patients found that the group with the Ala12 allele had a significantly lower value of carotid artery intima-media thickness (IMT) than that without it. This is although there was no difference between two groups in sex, age or other clinical variables (659). A prospective study also supported this finding. This study found that at both baseline and 10-year follow-up, type 2 diabetic patients having the Ala12 allele had

higher levels of ox-LDL autoantibodies than did type 2 diabetic patients with the Pro12Pro genotype. For the 10-year follow-up, this association was adjusted for age, gender, BMI, and hemoglobin A(1c) [HbA(1c)] at 5 years(660). Two studies evaluated the association of the Ala12 allele with leptin levels In T2 diabetic populations. One study, examining the relationship between the PPARγ Pro12Ala variant, T2DM, and its correlation with some cytokine determinants of insulin resistance such as tumor necrosis factor (TNF)-alpha and leptin in Spanish type-2 diabetic patients and healthy controls, found that women carriers of the Pro12Ala mutation exhibited significantly higher leptin levels than women non-carriers (661). In contrast, another study of German T2 diabetic patients found no association between the Pro12Ala PPAR g variant and serum levels of leptin (643]. Other findings from the above, which were reported by other association studies are the following. One study including T2 diabetic German patients who were treated with pioglitazone during a course of ≥26 weeks found that the Pro12Ala variant was not associated with the response rate to pioglitazone treatment in patients with type 2 diabetes (662). Another study, involving Japanese normotensive diabetic and non- diabetic subjects, hypertensive diabetic and non-diabetic subjects, found that the Ala12 phenotype frequency was lowest in patients with both T2DM and hypertension, followed by patients with either one of these conditions, and highest in subjects without these conditions. When stratified by hypertensive/diabetic status, the Ala phenotype was negatively associated with diabetes/ hypertension, giving an odds ratio of 0.53 and 0.75 respectively (663). A third study, involving Polish T2DM patients and non-diabetic control subjects, found that the only association between the Pro12Ala genotype and T2DM was that in T2Diabetics > 50years; the Pro/Ala and Ala/Ala genotypes was found to be significantly more frequent in T2DM cases with age of diagnosis >50 years than in controls(641).

One study conducted a family-based association strategy in familial T2DM to examine the association of the Pro12Ala variant with the insulin resistance syndrome by genotyping members of 52 Caucasian familial T2DM kindreds. No association of Pro12Ala with direct measures of insulin sensitivity was found. However, a significant association of the Ala 12 allele with traits commonly attributed to the insulin resistance syndrome was found; in a multivariate analysis, the Ala12 allele was found to be significantly associated with higher BMI, systolic and diastolic blood pressures, triglyceride levels, and glucose concentration. Despite no significant effect of Pro12Ala on liability for diabetes and no increase in the overall frequency of the Ala12

allele in this population characterized by a strong family history of T2DM, as compared to randomly selected Caucasian samples from the general population (12.1 ± 1.7% vs. 11-15% respectively (618;448;664-665) the proportion of individuals with T2DM increased with the number of Ala12 alleles (561). On the other hand, another family-based study [454] reported an opposing result to this latter finding. This study evaluated 16 single nucleotide polymorphisms (SNPs) that had been previously reported to be associated with T2DM or related sub-phenotypes using a family-based design(333 Scandinavian parent-offspring trios with T2DM or abnormal glucose homeostasis) and replication samples(1,130 individuals from Scandinavian sibships discordant for T2DM, 608 case-control pairs from Scandinavia and Canada). Of the 16 genetic associations with T2DM, only one, corresponding to the Pro12Ala polymorphism in PPARγ gene, was confirmed in this stringent multi-layer analysis with the proline allele associated with a modest (RR=1.25) but significant increase in T2DM risk. This finding was supported in a recent meta-analysis and a recent review. The meta-analysis of 13 different Pro12Ala association studies yielded statistically significant replication of this finding (OR=1.21-1.22) in favor of the Pro12 allele, thereby confirming effects, although modest ones, on susceptibility to T2DM (619). The review also reported that the most consistent findings in non-mendelian T2DM is the common coding variant Pro12Ala of the PPAR-γ gene, where the Pro12 allele confers a modest increased risk of T2DM (odds ratio about 1.2)(108). Because the risk allele is the more common allele occurring at a frequency of 83–87%, its modest effect translates into a large population attributable risk influencing as much as 25% of T2D in the general population [454].

Below is a **Summary Table** of the association and linkage studies in T2 diabetic populations.

Table 6: Summary Table of the association and linkage studies in T2 diabetic populations

SNP	Study Design	Population	Findings	Reference
G972R IRS1	Case control study	Danish, Italian, German, UK white	Significant +ve association of the R 972 allele with T2DM	322,324,571-572
	Meta analysis	7 studies	Significant +ve association of the R 972 allele with T2DM (OR=1.49)	349(322-324;570,325;349)
	Meta analysis	27 studies	Significant +ve association of the R 972 allele with T2DM (OR=1.25)	242(322-324; 567; 570;325; 571-583;349;332;345)
	Meta analysis	31 studies	Significant +ve association of the R 972 allele with T2DM (OR=1.15)	584(242,584-585)
	Meta analysis	Caucasians- 3 studies	Significant +ve association of the R 972 allele with T2DM	325
	Meta analysis	Scandinavian sibships + 6 case-control samples(Scandinavian, Swedish, Canadian, Finnish, US and Polish)	No association of the G972R with T2DM	586
		Above Data +2 meta-analysis(1st-16 studies;2nd-27 studies)	No association of the G972R with T2DM	586
	Case Control study	Caucasian, Chinese, Mexican American and Indian	Non-significant increased frequency of R972 allele in T2DM patients.	323, 570; 576; 454;587; 325
	Case Control study	Caucasian UK, Dutch, Mexican, African American, Japanese & Taiwanese	*No evidence for an association. *No evidence for a positive association of the R972 allele with T2DM	567; 349; 574;345; 578; 581; 583-585; 588-589; 572
	Case Control	Chinese patients	G972R IRS1 variant was found in T2DM cases but not in controls.	591
	Family-based study/case control	Chinese	*Prevalence of G972R variant was not increased in the T2DM population. *Non-significant increase in prevalence of this variant in probands of multiplex families. *No cosegregation of IRS1 variant with T2DM.	583
	Family Based study	Chinese, Japanese, French	*No cosegregation of IRS1 variant with T2DM.	591, 576,570

Table 6 **(Cont'd)**

SNP	Study Design	Population	Findings	Reference
G972R IRS1	Family Based study/case control pairs	Scandinavia and Canadian	Non-significant deviation of the G972R IRS1 variant from 50:50 transmission from heterozygous parents	454
	Linkage Study	Caucasians	*Exclusion of IRS1 a major T2DM susceptibility locus	592-593
	Linkage Study	Mexican Americans	* Suggestion of IRS1 as a potential susceptibility locus to a gene for age of onset for T2DM	594
G-174C IL6	Case control Study	Caucasian, Caucasian, Native Americans+ caucasians	Protective effect of the −174C allele on T2DM. *Significantly decreased frequency of the −174C allele in T2DM *Significant +ve association of the -174G allele with T2DM * Significant increased frequency of the GG genotype in T2 diabetics.	608,604 517
	Family-based study	Native Americans	Non-significant +ve association between the GG genotype and T2DM	100
	Family-based Study	Caucasian	Protective effect of the G allele on T2DM. *Significantly decreased risk of T2DM associated with the GG genotype	609
	Case Control study	Taiwanese	*Non-existence of the C allele of the G-174C IL6 polymorphism.	611
P12A PPARλ	Case control Study	Caucasian, Japanese, Pima Indians, Japanese Americans, Chez Republicans	Protective effect of Ala12 allele on T2DM	586;454,358,451, 620;460, 624, 627-635
	Family-based study	Caucasian	Significant positive association of the Proline allele with T2DM	454
	Meta-analysis	13 studies	Significant positive association of the Proline allele with T2DM	619
	Review		Significant positive association of the Proline allele with T2DM	614
	Family-based study	Caucasian familial T2DM kindreds	No increase in the overall frequency of the Ala12 allele in this population.	561

In summary, as for the G972R IRS1, there was an almost equal number of evidence which supports or negates the association of R972 allele of the G972R IRS1 variant with T2DM in Caucasians. In Other populations including Asian, Mexicans, Mexican American, Indians, most of the evidence supported no association of R972 allele of the G972R IRS1 variant with T2DM. The findings of linkage studies, however, were more consistent; IRS1 locus was not found to be a major T2DM susceptibility locus and no cosegregation was found of the IRS1 variant with T2DM in Caucasian and Asian populations. One linkage study, which cannot stand as enough evidence by itself, supported a potential role of IRS1 as a susceptibility locus to a gene for age of onset for T2DM in Mexican Americans.

As for the IL6 -174 SNP, the findings in different populations were as follows: In Caucasians, 3 case control studies supported a protective effect of the 174 C allele on T2DM. However, one family-based study supported a protective effect of the 174G allele on T2DM. In Native Americans, 1 case control study supported a protective effect of the 174 C allele on T2DM and one family-based study supported a Non-significant +ve association between the GG genotype and T2DM. In Taiwanese population, the C allele of the G-174C IL6 polymorphism did not exist, which suggests that the IL-6 C-174G polymorphism is unlikely to play a role in the development of type 2 diabetes in this population. In summary, the evidence is more towards a protective effect of the -174 C allele on T2DM in Caucasians, the evidence is not enough to come up with a consistent association between a specific allele of the IL6 SNP and T2DM in Native Americans and the existing evidence does not support a role for the IL6-174 SNP in the development of T2DM in Japanese population.

As for the Pro12Ala variant of the PPARλ gene, generally robust and consistently reproducible associations have been obtained for this variant to T2DM. Most of the existing literature on the association between the P12A PPARλ SNP and T2DM support a protective role of the Ala12 allele on T2DM in different populations including, Caucasian, Japanese, Pima Indians, Japanese Americans and Chez Republicans.

The above discussion emphasizes the importance of testing for linkage and association of each of the Pro12Ala variant of PPARγ, Gly972Arg variant of IRS-1 and G-174C variant of IL-6 with PCOS/insulin resistance and metabolic syndrome in PCOS families. In brief, there is mounting evidence, which supports association of each of Gly972Arg variant in IRS-1, G-174C variant in IL-6 and Pro12Ala genotype in PPAR-λ2 genes with insulin resistance and several

traits associated with metabolic syndrome. Moreover, the Gly972Arg variant of IRS-1 gene has a potential for interaction with the Pro12Ala genotype of the PPAR-λ 2 (353). Given these findings, it is important to examine how these molecules interact with each other, and with metabolic abnormalities associated with atherosclerosis, such as insulin resistance.

Additional support for selecting these variants is demonstrated in the following reasons. To date there has been no studies that tested for linkage or association of IL-6 locus/SNPs with PCOS or any other phenotype in PCOS families. As for IRS-1 and PPAR-λ, there has been only one family-based study, which assessed any evidence for linkage or association of IRS-1 and PPAR-λ genes with PCOS or hyperandrogenemia. This family-based study studied nuclear families; the majority of which were of European origin and the rest were of Caribbean origin. Our study will be the first linkage and association study, which tests for linkage and association of each of the above-mentioned variants with novel phenotypes, PCOS/insulin resistance, Metabolic Syndrome (MS) and its components, and serum inflammatory markers' levels (IL6/CRP) in African American (AA) and Caucasian extended families. Therefore, our study is going to be the first to address different phenotypes (PCOS/IR) and MS in different populations (AA and Caucasians) of PCOS families, which are multigenerational extended families.

2.0 PRIMARY HYPOTHESES AND SPECIFIC AIMS

2.1 HYPOTHESES

The primary hypotheses of this proposal are that **1)** It is feasible to recruit multigenerational, multiplex family members of women with PCOS, given the association of PCOS with infertility and low fecundity and thus the inherent difficulty in finding large extended families with multiple PCOS-affected individuals.**2)** There is a genetic component of PCOS related to insulin resistance (IR), inflammation status and metabolic syndrome (MS) that can be detected and tracked in families of women with PCOS.**3)** There is a linkage between the disease locus, defined as PCOS/IR phenotype, IR status/severity, MS or its components and serum inflammation levels and Gly972Arg, Pro12Ala, G-174C variant's locus at each of IRS-1, PPAR-λ and IL-6 genes respectively.**4)** The phenotype status of IR status/severity and/or metabolic syndrome and/or MS components and/or serum inflammation levels (IL6/CRP) and/or serum testosterone levels found in women with PCOS is due, at least partially, to genotype effects - defined as variation at PPARγ, IRS-1 and IL-6 genes, either known or suspected to be associated with IR-, environment effects (age, race, BMI, W/H ratio, physical activity), genotype/genotype interactions and genotype/environment interactions. **5**) There is a significant difference in the allele frequencies of each of Pro12Ala variant at PPARγ, Gly972Arg variant at IRS-1 and G-174C variant at IL-6 between PCOS families and the general population. We expect increased frequency of the Arg972 allele and decreased frequencies of Ala12 allele and C-174 allele in PCOS families relative to the frequencies of these alleles in the general population.

2.2 SPECIFIC AIMS

1) To demonstrate our ability to enroll 10 PCOS probands and their multigenerational, multiplex family members, for a total sample size of 100-125, to study insulin resistance and inflammation markers in families with PCOS .To identify and recruit the 10 families in which at least two clinically PCOS-diagnosed women and ten of their relatives are willing to participate in this feasibility study, we will use the following recruitment sources:
    a. Our current cohort of PCOS women in the ongoing prospective "Cardiovascular Health and Risk Measurement (CHARM)" study.
    b. The local chapter of the polycystic ovarian syndrome Association (PCOSA).
    c. Advertisements through the Pittsburgh Post Gazette and other local newspapers, University of Pittsburgh Audix service and/or selected reproductive endocrinologist offices.
2) To genotype 10 PCOS probands & their multigenerational,multiplex family members for:
    a. Single Nucleotide polymorphisms (SNPs) at three-insulin resistance candidate gene Pro12Ala variant of PPARγ, Gly972Arg variant of IRS-1 and G-174C variant of IL-6.
3) To test for linkage between the variants' loci at the above-selected insulin resistance candidate genes with various putative disease loci: PCOS/insulin resistance, Insulin Resistance status/severity, Metabolic Syndrome and its components and serum inflammation levels in multigenerational, multiplex PCOS families. Linkage will be assessed using a non-parametric linkage analysis method (variance components).
4) Since this research study is exploratory, associations between the alleles of the above-selected variants and the putative disease loci (Insulin resistance severity/status, Metabolic Syndrome and its components and CRP or IL-6 or total testosterone serum levels) were evaluated in two ways:
    a. We used a family-based association test (FBAT) for dichotomous traits and a variance components association test for quantitative traits. FBAT is a variation of the transmission disequilibrium test (TDT). The advantage of these tests over

linear/logistic regression models is that they take dependency between observations into account.

b. We used logistic regression model analyses for dichotomous outcomes (metabolic syndrome status and Insulin resistance status). On the other hand, we used linear regression analyses for quantitative outcomes (insulin resistance severity, components of MS, serum inflammatory markers' levels (CRP or IL-6) and total testosterone levels). These two types of models consider observations as independent events.

In each of the variance components association test and linear/logistic regression models, we assessed the effects of several environmental covariates (age, race, BMI, W/H ratio, physical activity, smoking, alcohol consumption,..) and other covariates expected to affect the outcome under study (serum CRP and IL-6 inflammation marker levels as covariates in case of IR as an outcome, or IR as one covariate in case of serum inflammatory level as the outcome) and the impact of variation at the candidate genes on IR status/severity, metabolic Syndrome status and its components, serum inflammatory markers' levels and total testosterone levels through evaluating genotype effects, environment effects, genotype/genotype interactions and genotype / environment interactions.

5) To compare allele frequencies of the above-selected variants in the studied PCOS families with population frequencies using NCBI dbSNP or NCI databases.

3.0 RESEARCH DESIGN AND METHODS

3.1 RECRUITMENT SOURCES

The enrollement plan was to recruit our PCOS families from four major recruitment sources; the Cardiovascular Health and Risk Measurement (CHARM) study population, the Pittsburgh chapter of the nationally based Polycystic Ovarian Syndrome Association (PCOSA) organization, responses received through an advertisement in the Pittsburgh Post Gazette, other newspapers or magazines, University of Pittsburgh AUDIX service, or from selected reproductive endocrinologists offices. The current CHARM population was our major target recruitment source because of the good long-standing relationship, which we established with the CHARM PCOS women and thus our subsequent expectation of a high cooperation on the behalf of these women. This population has demonstrated ongoing interest in the CHARM study and individuals have stated their interest in any other studies that may be planned. Moreover, in this population there are families not only with existing multigenerational members but also with several of those members having PCOS both across and within generations. A second population that was explored for recruitment is the local chapter of the PCOSA. PCOSA is a national nonprofit support organization operated by and for women with PCOS, centrally operated out of Colorado. Pittsburgh has a local chapter of this organization that our PCOS research group has worked closely with to plan a PCOS symposium (PCOS: The Keys to a Healthier You, 2002). We Chose this population as a recruitment source on the basis that: 1) they have indicated a possible high level of interest in a genetics study from their membership or themselves, 2) they are a population that is highly motivated to learn about and be active with their disease, as demonstrated by their active involvement with PCOSA (i.e., instead of being ashamed or neutral about their disease), and 3) they were, at the time of our recruitment process, not enrolled in any other studies that we know of; this being important due to increased interest in this study from a

general desire to be involved in a study that may be otherwise unfulfilled. In addition, we decided on two other sources to identify potential probands. These included advertisements through the Pittsburgh Post Gazette and /or selected reproductive endocrinologist offices and /or University of Pittsburgh AUDIX Serivce. Recruiting multiplex families from these populations, and subsequently performing linkage analyses on these families, may allow identification of genes that affect susceptibility to insulin resistance or metabolic sybdrome in PCOS families.

3.2 INCLUSION/EXCLUSION CRITERIA

The two major inclusion criteria for a family in this study were: 1) each family should have at least two or more women with a clinical diagnosis of PCOS AND provide us with a proof of this diagnosis. 2) At least 10 family members of each interested family(first-degree, second-degree, third-degree etc.. family members for e.g., parents, siblings, children, aunts, uncles, nieces, nephews, grandparents, and grandchildren) should indicate interest in participation in this genetics study, be willing to come to our clinic or be within 60 miles from Pittsburgh so that we arrange for a home visit from us. Subjects enrolled in this study should be 14 years and older, regardless of gender, race, ethnicity, pregnancy or presence of any disease or illness; children aged 14 to 17 years were included in this study on the basis that they are old enough to display the PCOS phenotype. Mentally disabled persons were excluded from this study on the basis that this study was above minimal risk, did not offer direct or significant individual benefit to participants, did not provide knowledge toward understanding or alleviating the subject's disorder or condition, and did not present an opportunity to gain knowledge of any significance to the prevention, diagnosis or treatment of mental disorders. In an attempt to recruit subjects in respective proportion to the demographics of Pittsburgh and the surrounding area and/or the patient population of the University of Pittsburgh Medical Center, the plan was that 20 % of the total number of recruited individuals will be African American. Since we were enrolling entire families and not selecting on basis of gender and given the estimated national ratio of men: women is approximately 1:1, this study assumed enrollment of 50-62.5 men and 50-62.5 women aged 14 years and older with a total sample size of 100-125 people. The racial, gender, and ethnic characteristics of the proposed subject population reflects these demographics.

3.3 RECRUITMENT PROCEDURES

Only the probands of the recruited families underwent any screening procedures to determine eligibility. The recruitment procedures are listed in order of completion.

3.3.1 PROBAND

1. Initial Contact/Pre-Screening: We contacted each proband by letter (the CHARM Proband's Initial Contact Letter , the PCOSA Proband's Initial Contact Letter or the Proband's Initial Contact Letter responding to ad) and enclosed a self-addressed, stamped postcard (University of Pittsburgh: GeneIRP Study postcard) as well as a "fancy" GeneIRP newsletter playing up the great free things this study would give them [i.e., complete cholesterol screening, heart health report (based on assessment of body mass index and waist: hip ratio), and insulin resistance screening]. This newsletter was intended to increase the number of positive responses to the mailing. We requested that the postcard be returned to indicate a desire to be either contacted or not contacted in regards to the study. If the postcard was returned indicating that she does not wish to be contacted, we would eliminate the proband from our mailing list. If the postcard was not returned, we assumed she was not interested in being contacted and would not contact her again.
2. If the postcard was returned indicating that she wishes to be contacted, we would call her to give her more information about the study and screen her for eligibility. We would use the CHARM/PCOSA Proband Telephone Interview Script and the Proband Eligibility Screening Questions – Telephone Interview to screen probands. If she did not have 10 to 15 family members, then we would thank her for her time, tell her she was not eligible and destroy all the information collected during the screening process. We would also offer to keep her in mind for any future genetics studies if she was interested. If she had 10 to 15 family members, then we would indicate our interest in enrolling her family in this study and ask the proband if she would be willing to contact her family members to determine each person's interest in this study. We would then offer to visit the proband in her home to give her more information and more incentive to speak with her family

members about what this study will ask them to do and what this study has to offer them that might otherwise be unavailable to them. This was expected to increase interest and incentive to participate. Arrangements for these introductory visits were planned to be made on an individual basis, depending on participant preference. The most likely scenario for the introductory home visit was that the Principal Investigator, together with the study coordinator would go into the participant's home to inform them about details of the study, discuss study protocol, answer any concerns and questions, and brainstorm about different methods for increasing family interest and participation in this study. This home visit would allow the investigators to determine and directly address individual concerns of the proband and any family members that also wish to be present during this visit. Family members would be encouraged to come to these introductory home visits as their concerns may also be addressed at this time. We hoped that this direct communication would increase comfort that people feel with this study and this would help them when speaking with/recruiting their family members that did not attend this initial visit.

3. If the participant were still interested and eligible after the phone call, we would mail her a Confirmation of Interest letter, a postage-paid self –adressed envelope along with a Family Member Address form that could be used to organize and track family members as the proband contacted them. The confirmation of interest letter would be used only to reiterate the probands stated interest in the study and their stated willingness to contact their family members to determine the overall level of family interest. The fill-in family member address form might help to motivate people to talk with their family members about the study and to organize the family members that have stated their interest.

3.3.2 Family Member

Once we received the completed family address form by the proband, we would contact the interested family member in two ways:

1. Provide the project office number to the proband for direct contact of the study staff by the interested family member or
2. Send an introductory letter (CHARM Family Member Initial Contact Letter, PCOSA Family Member Initial Contact Letter or Family Member Initial Contact Letter of proband responding to ad) to the interested relative at the address provided by the proband.

We would then use the CHARM/PCOSA Family Member Telephone Interview Script to determine if they were taking medication for diabetes. We would ask this question only to avoid possible complications from overnight fasting that may affect diabetics. If they indicated they were taking diabetic medication, we would ask them to not fast, but to be aware that some blood parameters we are measuring (i.e., lipids, insulin, and glucose) may not be accurate. We would offer to visit the family members in their homes to recruit them prior to getting their participation, just to give them information about what this study had to offer them. This was intended to increase interest and incentive to participate. Arrangements for these introductory visits would be made on an individual basis, depending on participant preference. The most likely scenario for the introductory home visit was that the Principal Investigator, together with the study coordinator would go into the family member's home to inform them about details of the study, discuss study protocol, answer any concerns and questions, and brainstorm about different methods for increasing even more family interest and participation in this study. This home visit would allow the investigators to determine and directly address individual concerns of any family members that wish to be present during this visit. All family members would be encouraged to come to these introductory home visits as their concerns might also be addressed at this time. We hoped that this direct communication would increase the comfort level that each family feels with this study and this would help them when speaking with/recruiting more family members that did not attend this visit.

We would speak with family members by the process outlined above until we have verified interest of at least 10 people in the family and then we made visit arrangements for all 10 family members. After enrolling at least 10 people, we would continue trying within each family to get 5 to 10 more people to enroll until either we had 10 to 15 family members or 12 months had elapsed from the time of initial proband contact. If we had reached 12 months of active recruitment without enrolling 10 to 15 family members, we would stop recruitment activities toward that family and focus on enrolling other families with larger numbers of interested relatives. In addition, this family would be eliminated from this study.

3.4 STUDY PROCEDURES

Knowing the importance and potential difficulty of recruiting at least ten (10) family members from any one family regarding the completion of this study, procedures, including blood work and questionnaires' administration, would be performed at one of three locations: (1) the Bellefield clinic in Oakland, (2) a prearranged meeting place close to the participant's home, or (3) the participant's home. Options 2 or 3 would be carried out in the event that any person could not or would not come to the clinic, did wish to participate, no other arrangements could be made and the meeting place was within 60 miles from the Pittsburgh/Oakland area. Arrangements would be made for blood draws to ensure participant safety and comfort and stability of glucose, lipid and insulin for later analysis. This method would involve us (the researchers) going to a subject's home to enroll them and any interested family members that might be present and to complete the clinic visit for as many participants as possible. Since we were collecting fasting blood samples, all visits for this study would be done first thing in the morning.

If none of these arrangements could be made, then this participant was excluded from participating in this study. Children aged 14 to 17 were asked to complete the same procedures as adults as all questionnaires and study procedures were appropriate for ages 14 and older.

3.4.1 Consenting

A written informed consent was obtained from each participant after proband screening procedures and after at least 10 family members had verbally agreed to participate. No study procedures were performed on any subject prior to obtaining a written informed consent except for screening procedures on the proband, at which time no identifying information was collected about specific family members from the proband or about the proband themselves. We had requested and obtained a waiver to document written informed consent of the screening process, which took place over the phone. For children 14 to 17 years old, we obtained written informed consent from them and from their parents/legal guardians before their enrollment. If future studies desired to use stored genetic specimens of these children, who turn 18 years of age by the time of planned use of these specimens, re-consenting these children using the consent form for adults aged 18 years or older will be performed.

Obtaining participant written consent was arranged in two ways: 1) at the Bellefield clinic, 2) at a home-based location convenient to the participant or at their home that we must travel to but was within 60 miles from the Pittsburgh/Oakland area, or 3) long-distance (greater than 60 miles from the Pittsburgh/Oakland area) by mail. Consent procedures changed according to the arrangement made with each individual or group. *For clinic visits*, we gave the participant duplicate copies of the consent form and asked them to sign and initial both, one being their copy and one being ours. We informed them that we would answer any questions they might have about the study before they signed the consent forms. We would let them read the form, initial at the bottom of each page except for the signature page, and sign the last page. We would ask them if they had any questions at that time and invited them to ask any questions of us throughout the visit should they arise. We would review the study protocol with them as they read the consent form, ask them open-ended questions about the study to ensure they have read and understood the consent form and then take our copy of the consent form and leave them with their copy to keep for their records. We would sign the investigator signature of our copy and put it in the participant's file. We would then begin study procedures in a private interview area in an inner room of the clinic. *For home-based and home visits*, we would meet the participant or group of participants at a prearranged meeting area. The same procedures for

obtaining consent were followed as those used in the clinic visit. We would mail them the consent form for adults(aged 18 years and older) with Consent Form Letter and instructions (to initial bottom of each page, sign, and keep one copy for their records) and with a pre-paid, self-addressed return envelope. We instructed them in this mailing to call our office with any questions they might have about this study at any time throughout the consent process, but preferably before they sign the consent form and to call our office regarding any questions, to get verbal instruction if they would like it, or for any review they would like before signing the consent. Any questions they ask would be addressed over the phone. They would mail back our copy of the completed consent form for investigator signature and we would put this in their participant file. If there were any problems (blanks in initials or signature sections) on the consent form when we received the copy they have mailed back, we would re-mail the consent forms to the participant until it was completed accurately before enrolling them in this study. For all participants, we would go over the consent form with them by discussing procedures, risks, and benefits of study participation. We would also ask them open-ended questions about the study to determine if they truly understood what study participation entailed (i.e., How long will the visit take?, What will we do during this visit today?, How many questionnaires will you be filling out in this study?, How many tablespoons of blood will we be collecting from you today?). Consent forms, questionnaires and clinic notes would be dated and the time put on them to verify that consent was given prior to implementation of any study procedures.

3.4.2 Clinic Procedures

For out-of-town participants, blood samples were not obtained for glucose, lipid, insulin, and testosterone analyses. This was due to the technical unfeasibility of appropriately processing those samples prior to shipment to the Heinz laboratory in Pittsburgh for later analysis. For those participants, DNA was collected by using buccal swabs instead. Instructions for gathering buccal swabs together with two buccals were mailed to each interested out-of-town participant. We also asked out-of-town participants to mail back their used buccals, within one week of use, in self-addressed postage-paid envelopes, which we provided. Furthermore, questionnaire data for such participants were collected over the phone by the study coordinator. **For in-town participants,** the below procedures were performed.

3.4.2.1 Genotype Analyses

Subjects completed their participation in this study in one 1-hour visit only. Each participant's visit included a fasting blood draw or buccal swabs to ascertain their DNA with a focus on the presence or absence of specific metabolic pathway related alleles (peroxisome proliferator activated receptor gamma (γ){PPAR-λ}, insulin receptor substrate-1{IRS-1}) or inflammatory pathway (Interleukin-6{IL-6}). Buccal swabs, a noninvasive way to collect DNA by stroking the inner facial cheek with cotton tipped applicator tips, were used for participants for whom we encountered difficulty in obtaining a blood sample.

Genomic DNA was assessed for blood samples drawn from 101 participants total and genomic DNA from problematic samples, low-yield or OD 260/280-ratio, was re-extracted. DNA was extracted from a 1-1.5 ml aliquot of red blood cell contaminated buffy coat using a simple salting-out procedure (666). This procedure provides for a red cell lysis step followed by DNA extraction from the white blood cell fraction. Purified DNA was spectrophotometrically quantified using A260/A280 absorbance values. All DNA preparations were divided into two aliquots; one aliquot served as the working aliquot and the second aliquot was held in reserve in case of loss of the working aliquot. The working aliquot was equivalent to a working DNA dilution of 100 ng/µl, which was prepared in a total volume of 50ul of sterile water. Both of these aliquots were stored at -20 º C in the Epidemiology Genetics Laboratory at the University of Pittsburgh, Graduate School of Public Health. In addition, a 1-1.5 ml aliquot of residual blood was stored frozen at -80º C from all patients in case there was ever a need for additional DNA or if there was a question about sample mix-up. This extra 1-1.5 ml aliquot of residual blood was stored at Heinz Nutrition Laboratory at the University of Pittsburgh, Graduate School of Public Health. Our original plan was to tagSNP PPARλ, IRS-1, IL-6 and CRP genes. This plan changed due to limited resources and the replacement plan was to genotype only the non- synonymous SNPs in these genes. No non-synonymous SNPs were found in the CRP gene and therefore, no SNPs were genotyped for in this gene. Therefore, we attempted to genotype for the PPARλ Pro12Ala (rs1801282), IRS-1 G972R (rs1801278), IL-6 G-174C (rs1800795) and V162D (rs2069860) variants. The working solution was serially distributed to 1/10 using Tris-EDTA (TE) buffer for Taq SNP analysis. The SNPs were genotyped for using the Taqman genotyping

platform, by the ABI (Applied Biosystems, Foster City CA), which utilizes a 5' nuclease protocol (667). The assays, which were used to genotype for these four SNPs were: For the Pro12Ala SNP, the assay ID # was C-1129864-10; for IRS-1 G972R,C- 2384392-20; for IL-6 V162D,C-15860129-10; for the IL-6 G-174C SNP, we used an assay custom designed and manufactured by the ABI; the forward/reverse primers which were used to define the amplified region were:

Forward primer, GACGACCTAAGCTGCACTTTTC;

Reverse primer, GGGCTGATTGGAAACCTTATTAAGATTG.

The **reporter sequences** which were used to actually type the polymorphism were **Reporter1** (VIC) (CCTTTAGCATCGCAAGAC); **Reporter2** (FAM) (CTTTAGCATGGCAAGAC).

Furthermore, to check on the validity of the genotyping results, we genotyped 167 duplicate samples and found no genotyping errors.

3.4.2.2 Laboratory Analyses

In all in-town participants, a fasting blood draw also included the following: (1) test for fasting insulin(I0) levels, (2) test for fasting glucose(G0) levels, (3) test for blood lipid levels, including total cholesterol (TC), HDLc, LDLc, and triglycerides, (4) test for inflammatory markers' serum levels, specifically Interleukin 6 (IL-6) and C-Reactive Protein(CRP) levels. In addition, total testosterone levels were measured in female pariticipants. All these measurements were performed at the Heinz Nutrion Laboratory under the direction of the Dr. Rhobert Evans. The laboratory is carefully monitored and participates in the Centers of Disease Control standardization programs.

3.4.2.2.1 Fasting Insulin and Glucose Measurements

Insulin was measured using an RIA procedure developed by Linco Research, Inc. Cross-reactivity of the antibody with human proinsulin was under 0.2%. Briefly, samples were mixed with ^{125}I-insulin and insulin antibody and then incubated at room temperature for 18 to 24 hours. The insulin-antibody complex was precipitated during a 20 min incubation at 4EC and subsequently sedimented by centrifugation for 15 min at 3,000g at 4EC. Finally, the supernatant was decanted and the pellets counted. Under these conditions the limit of sensitivity was 2μU/ml and the response was linear up to 200μU/ml. Standards, blanks, quality controls and a control

pool were run simultaneously with all samples. The coefficient of variation between runs was 2.6 ∀ 0.7 (10) %.

Serum glucose was quantitatively determined by an enzymic determination read at 340/380 nm with a procedure similar to that described by Bondar and Mead (668), utilizing the coupled enzyme reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase. Using the Sigma Diagnostics glucose [HK] 20 reagent, serum was diluted 1:101. The mixture was then incubated for four minutes at 37EC and read at 340/380 nm using the Abbott VP Supersystem spectrophotometer. The increase in absorbance at 340/380 was directly proportional to the glucose concentration of the sample. The coefficient of variation between runs was1.8%.

3.4.2.2.2 Blood Lipids

Total cholesterol was determined using the enzymatic method of Allain et al (669). This procedure involves the hydrolysis of cholesterol esters by cholesterol esterase, oxidation of cholesterol by cholesterol oxidase with formation of hydrogen peroxide and finally, a peroxidase catalyzed reaction of hydrogen peroxide with 4-aminoantipyrine and phenol to form Quinoneimine dye. The intensity of the color produced was directly proportional to the total cholesterol concentration in the sample. Duplicate samples with standards, control sera and serum calibrators were included in each run. The coefficient of variation between runs was 1.3%.

LDL was calculated indirectly using Friedewald equation (670):

LDL_c = Total Cholesterol - HDL_c - 0.2 (total TG). For samples whose TG > 400 mg/dL, the below LDL Direct method was used.

LDL Direct was measured directly using an automated spectrophotometric assay, LDL Direct Liquid Select, from Equal Diagnostics. The principle of the assay involved solubilizing non LDL particles and removing their cholesterol and then solubilizing the LDL and measuring their cholesterol content. In brief, 3µl of sample was incubated with 300µl of buffer containing detergent, cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine and ascorbic acid oxidase for 5 min. at room temperature. Then, 100 µl of buffer containing detergent and N, N-bis (4-sulphobutyl) -m- toluidine-disodium was added and the mixture incubated at room temperature for 10 min. The absorbance was then read at 550/650 nm. Standards (100-200

mg/dL), blanks and control pools were run with each assay. The coefficient of variation was 2.0 $\pm$ 0.3%(22).

HDL cholesterol was determined after selective precipitation by heparin/manganese chloride and removal by centrifugation of very low density (VLDL) and low density lipoprotein (LDL) (671). The cholesterol was measured as described below for total cholesterol. Duplicate samples, standards and control sera were included in each run. The coefficient of variation between runs was 2.1%.

Triglycerides were determined enzymatically using the procedure of Bucolo et al (672). Briefly, the triglycerides were hydrolyzed by lipase; the glycerol phosphorylated by ATP and glycerol kinase to yield glycerol-1-phosphate and ADP. The ADP was then rephosphorylated by phosphoenolpyruvate in the presence of pyruvate kinase. Finally, lactate dehydrogenase was used to catalyze the reduction of pyruvate by NADH to give lactate and NAD. The reaction was monitored spectrophotometrically and the decrease in absorbance at 340 nm was directly proportional to the concentration of pyruvate and, hence, to the original triglyceride content. Duplicate samples, standards and control sera were included in each run. Coefficient of variation between runs was 1.7%.

3.4.2.2.3 Total Testosterone

Total testosterone was measured in duplicate using a commercial EIA kit (DSL-10-4000) purchased from Diagnostic Systems Laboratories (Webster, Texas). In brief, samples (50 µl serum or plasma) were mixed with enzyme conjugate solution and testosterone antiserum and incubated for one hour at room temperature with rapid shaking. The plate was then washed x5, TMB chromogen solution added and the plate incubated at room temperature for 30 minutes with rapid shaking. Stop solution (0.2 M H_2SO_4) was added and the absorbance read at 450 nm with background correction at 600 or 620 nm. Standards (0.1 to 25 ng/ml), blanks, controls and a pooled laboratory control were run with each assay.

3.4.2.2.4 Interleukin-6 (IL6) and C - reactive protein (CRP)

IL-6 was measured, in duplicate, using a commercial high sensitive ELISA kit purchased from R&D Systems (HS600). In brief, samples 200 µl were incubated at room temperature (RT) for 14-20 hours in microplate wells coated with murine monoclonal antibody against IL-6. The

plates were washed (x4), 200 μl of conjugate (alkaline phosphatase/polyclonal antibody against IL-6) added and the samples further incubated for 6 hrs at RT. The plates were again washed (x4) and 50 μl of substrate (NADPH) then added. The samples were incubated for 60 minutes at RT, 50 μl of amplifier (alcohol dehydrogenase/diaphorase) added and the plates incubated for 30 minutes at RT. The reaction was stopped with sulfuric acid (50 μl of 2N) and the absorbance read at 490 nm with correction at 650 nm. Standards (0.15 to 10 pg/ml), controls and a pooled laboratory control were run with each assay.

CRP was measured using reagents obtained from Olympus America, Inc. (Melville, NY). In this procedure the CRP in the sample reacts with goat anti-CRP-antibodies coated on latex particles. The increase in absorbance was measured turbidimetrically. Blanks, controls and standards (0.5 to 20 mg/L) were simultaneously run with all samples. The intra-assay and the inter-assay coefficient of variations were 5.5% and 3.0% respectively.

3.4.2.3 Anthropometry Analyses

In all in-town participants, each clinic visit included blood pressure, heart rate assessments as well as anthropometric measurements. Anthropometric measurements included weight, height, waist and hip circumferences. Blood pressure was obtained from each subject using a standard mercury sphygmomanometer. Systolic and diastolic blood pressures were determined as the mean of two measures at 60-s intervals with the subject sitting quietly and the cuff deflated between measures. Weight was measured at a standing position on balance beam scales to the nearest pound, while subjects wore no shoes. Standing height was measured on a wall-mounted stadiometer to the nearest inch while subjects wore no shoes as well. Body mass index was calculated as weight (kg) divided by height (m^2). Waist and hip circumferences were determined as the mean of two tape measurements, taken to the nearest 0.1 cm, at the umbilicus and at the greatest diameter respectively. All anthropometry measurements were made using the same equipment for each patient.

3.4.2.4 Questionnaire Data Collection

Each participant was interviewed to complete three questionnaires as well (the clinic questionnaire, the SF-36 version 2 Quality of Life questionnaire and the Paffenbarger physical activity questionnaire) **(SEE APPENDIX A)**. The clinic questionnaire includes information on

basic demographics (name, address, phone number, date of birth, social security number, ethnicity, marital status, educational history, and occupational status), medical history, current medications, family history, lifestyle description and habits (lifetime smoking and current drinkinging patterns), anthropometrics (weight, height, and weight gain or loss in the last year), and reproductive, menstrual and endogenous hormone history (for women only). The SF-36 Quality of life questionnaire includes questions about individual's perception of his/her own health (type of activity which an individual thinks is limited due to personal health problems and extent of limitation, frequency and extent of interference of individual's physical or emotional health problem with regular daily activities, including social activities during the past month, and assessment of individual's physical and psychological feelings in the past month). The Paffenbarger physical activity questionnaire addresses physical activity questions (number of blocks/flights an individual walks/climbs up each day, usual pace of walking, type, frequency and average time per episode of any physical activity practiced in the past year and length of practicing a specific sport, level of exertion during exercise, and assessment of time allocation to different types of activities on a typical day).

3.4.3 Participant's Research data Confidentiality

All records related to subject involvement in this research study were stored in a locked file cabinet. Participant identity on these records was indicated by a case number rather than by name, and the information linking these case numbers with their identity was kept separate from the research records. Genetic information and biological samples were marked with case numbers rather than participant's name to protect confidentiality. Questionnaires were administered in a private area away from other people to ensure an individual's privacy and research data collected was kept confidential in locked file cabinets in the inner office of the CHARM study office, access of which was limited to CHARM and GeneIRP study staff only. Research records will be maintained for at least five years after study completion per University policy. Blood specimens/buccals will be kept for up to five years after this study end. Should the subject chose to withdraw from this study after blood or buccals had been collected their sample or buccals would be rendered anonymous and destroyed. Meanwhile, the blood samples/buccals

were stored at the Heinz Nutrition Laboratory/ Epidemiology Genetics Laboratory at the University of Pittsburgh, Graduate School of Public Health respectively.

3.4.4 Potential Benefits and Costs Associated with this Study

Participants in this study received a free cardiovascular health risk assessment based upon body measure assessments, blood lipid levels, blood insulin and glucose levels, and an honorarium for travel, time and inconvenience compensation. Participants did not receive results of genetic testing, as these are not clinically significant at this time. Overall benefits of this study include progression of scientific knowledge regarding the genetic basis of insulin resistance in PCOS. All procedures in this study were free of charge to research subjects and their insurance providers.

3.5 MEASUREMENT OF EXPOSURE AND OUTCOME VARIABLES

3.5.1 Exposure variables

3.5.1.1 Main Exposure Variable (Genotypes of Selected Variants)

Genomic DNA was assessed for blood samples drawn from 101 participants total and problematic samples, low-yield or OD260/280-ratio samples, were analyzed a second time. DNA was extracted from a 1-1.5 ml aliquot of red blood cell contaminated buffy coat using a simple salting-out procedure(666). This procedure provides for a red cell lysis step followed by DNA extraction from the white blood cell fraction. Purified DNA was spectrophotometrically quantified using A260/A280 absorbance values. All DNA preparations were divided into two aliquots; one aliquot served as the working aliquot and the second aliquot was held in reserve in case of loss of the working aliquot. The working aliquot was equivalent to a working DNA dilution of 100 ng/μl, which was prepared in a total volume of 50ul of sterile water. Both of these aliquots were stored at -20ºC in the Epidemiology Genetics Laboratory at the University of Pittsburgh, Graduate School of Public Health. In addition, a 1-1.5 ml aliquot of residual blood

was stored frozen at -80º C from all patients in case there was ever a need for additional DNA or if there was a question about sample mix-up. This extra 1-1.5 ml aliquot of residual blood was stored at Heinz Nutrition Laboratory at the University of Pittsburgh, Graduate School of Public Health. Our original plan was to tagSNP PPARλ, IRS-1, IL-6 and CRP genes. This plan changed due to limited resources and the replacement plan was to genotype only the non-synonymous SNPS in these genes. Therefore, we attempted to genotype, in our 101 individuals, the PPARλ Pro12Ala (rs1801282), IRS-1 G972R (rs1801278), IL-6 G-174C (rs1800795) and V162D (rs2069860) variants. No non-synonymous SNPs were found in the CRP gene and therefore, no SNPs were genotyped for in this gene. The working solution was serially distributed to 1/10 using Tris- EDTA (TE) buffer for Taq SNP analysis. The SNPs were genotyped using the Taqman genotyping platform, by the ABI (Applied Biosystems, Foster City CA), which utilizes a 5' nuclease protocol (667). The assays, which were used to genotype for these four SNPs were: For the Pro12Ala SNP, the assay ID # was C-1129864-10; for IRS-1 G972R,C- 2384392-20; for IL-6 V162D,C-15860129-10; for the IL-6 G-174C SNP, we used an assay custom designed and manufactured by the ABI; the primers which were used to define the amplified region were:
Forward primer, GACGACCTAAGCTGCACTTTTC;
Reverse primer, GGGCTGATTGGAAACCTTATTAAGATTG.
The **reporter sequences** which were used to actually type the polymorphism were **Reporter1** (VIC) (CCTTTAGCATCGCAAGAC); **Reporter2** (FAM) (CTTTAGCATGGCAAGAC).

Furthermore, to check on the validity of our genotyping results, we genotyped 167 duplicate samples and found no genotyping errors.

3.5.1.2 Other Exposure Variables

These included laboratory measurements, anthropometric and other physical measurements, and other covariates. Laboratory measurements (fasting insulin, fasting glucose, lipids, serum IL-6, CRP and testosterone levels) were obtained from analyses of the individual blood samples at the Heinz Laboratory at University of Pittsburgh, Graduate School of Public Health under the direction of Dr. Rhobert Evans. All other variables, which were also used in the linear and logistic regression models, variance components association test and variance components related to quantitative traits (age, race, gender, menopause status, smoking, alcohol consumption,

blood pressure and anthropometric measurements)were obtained from the questionnaires completed for each participant.

3.5.2 Outcome Variables

In our study, we evaluated for linkage and association two dichotomous outcome variables; the Insulin Resistance (Main Outcome Variable) and Metabolic Syndrome(secondary outcome variable) phenotypes. Both these outcome variables are defined below.

Criteria used to determine significant results of linkage analyses were the presence or absence of specific genetic variants (P12A, G972R and G-174C and V162D) given the phenotypic absence or presence of insulin resistance(IR)(as defined below),PCOS/insulin resistance (defined by clinical diagnosis of PCOS or Insulin resistance phenotype as defined below) and metabolic syndrome (MS).

3.5.2.1 Main Outcome Variable (Insulin Resistance)

Although the concept of insulin resistance is relatively easy to understand, quantitative assessment of insulin sensitivity and the ability to determine exactly who is insulin-resistant present a more challenging task. The hyperinsulinemic-euglycelmic clamp technique is the "gold standard" technique for measuring insulin sensitivity (673). However, this and other similar clamp techniques are expensive, time-consuming and labor-intensive. Alternative tests include the frequently sampled intravenous glucose tolerance test (FSlVGTT), insulin tolerance test (ITT), insulin sensitivity test (IST), and continuous infusion of glucose with model assessment (CIGMA).A major limitation for use of these tests , however, is that all of these methods require IV access and multiple venipunctures. The oral glucose tolerance test (OGTT) does not require IV access but does involve several venipunctures and several hours of patient and technician time. Several fasting or "homeostatic" models have been proposed as noninvasive measurements of insulin sensitivity, and each has correlated reasonably well with clamp techniques (674-675). The fasting insulin level (I0), fasting glucose/insulin ratio (G0/I0 ratio), homeostasis model assessment (HOMA), and quantitative insulin sensitivity check index (QUICKI) have been the most frequently used techniques in clinical investigations. These tests are based on fasting glucose and fasting insulin and use straightforward mathematical calculations to assess insulin

sensitivity. The former two tests are applicable only to patients without glucose intolerance and the latter two had better reflect underlying physiologic features of IR in both diabetic and non-diabetic subjects (676,677). In other words, hyperglycemia (fasting glucose >=110 mg/dL) essentially negates the value of I0 and most probably negate the value of G0/I0, but less likely to negate the values of HOMA and QUICKI depending on the severity of the β-cell dysfunction. One common weakness of all these models, however, is that they assume the relationship between glucose and insulin is linear when, in fact, it is parabolic (678-679,676).

Fasting serum insulin (I0) is a direct inexpensive measure of insulin sensitivity; I0 increases as degree of insulin resistance increases. However, the fasting insulin level must be interpreted in the context of the amount of peripheral insulin sensitivity, the degree of pancreatic beta cell function, and the contribution of the liver to glucose production (680). With these limitations in mind, one study found that a fasting insulin >=20 μU/mL in white women and >=23 μU/mL in Mexican-American women probably indicates insulin resistance in women with PCOS (681). The ratio of fasting serum glucose to fasting serum insulin (G0/I0 ratio) is easily calculated, with lower values depicting higher degrees of insulin resistance. In a study involving obese, non-Hispanic, white individuals with PCOS living in southern Pennsylvania, "insulin resistance" was determined by performance of FSIVGTT on 15 normal and 40 PCOS women. The authors, using an SI below the 10th percentile of age, ethnicity, and weight-matched control women as evidence of insulin resistance, found that a G0/I0 ratio of less than 4.5 is 95 % sensitive and 84% specific for insulin resistance in a group of women with PCOS when compared with the control group (674). The main limitation of this parameter is that it was derived from the data of a group of obese women (BMI>26 kg/m^2) and, therefore, is unlikely to be a good measure of insulin resistance in non-obese PCOS patients. Another limitation of this parameter is that the G0/I0 ratio of less than 4.5 was based on the distribution of SI of a very small sample size of controls. In another group of women living in west Texas, a G0/I0 ratio of <=7.2 was found to suggest insulin resistance in white PCOS women, whereas a G0/I0 ratio <=4.0 was more appropriate for Mexican-American PCOS women (681). These findings suggest that these screening values should be population-specific. The homeostasis model assessment (HOMA) has been widely used in clinical research to assess insulin sensitivity (682) and several recent studies have demonstrated that the HOMA approach to estimating insulin sensitivity is useful in epidemiological studies (683-684). HOMA is defined as the product of the fasting

values of glucose (expressed as mmol/L) and insulin (expressed as μU/mL) divided by a constant (I0× G0)/22.5, with higher values showing higher degrees of insulin resistance. As mentioned earlier, unlike I0 and the G0/I0 ratio, the HOMA calculation better compensates for fasting hyperglycemia (676) and better correlates with clamp techniques than either I0 or G0/I0 (682). HOMA has also been used to study insulin resistance among PCOS patients of differing ethnic origins. In a group of women living in west Texas(681), a HOMA value of > 3.8 was found to suggest insulin resistance in white women suspected to have PCOS, whereas a HOMA value of >4.5 was more appropriate for Mexican-American women suspected to have PCOS. Like HOMA, the quantitative insulin sensitivity check index (QUICKI) can be applied to normoglycemic and hyperglycemic patients (685), with lower values showing higher degrees of insulin resistance. QUICKI is derived by calculating the inverse of the sum of logarithmically expressed values of fasting glucose and insulin (686): $[1]/ [\log (I_0) + \log (G_0)]$. Many investigators believe that QUICKI is superior to HOMA as a way of determining insulin sensitivity (686), although the two values correlate well(r=0.77(685); in our study r=0.89) and several studies showed similar correlations of each of QUICKI and HOMA with insulin sensitivity obtained by minimal models (687). It is noteworthy to mention that three mathematical models predicting insulin sensitivity as measured by euglycemic clamp have been constructed recently (688). However, this study involved 72 women with PCOS diagnosed on the basis ultrasonographic evidence of polycystic ovaries, in association with a history of menstrual irregularities, indicating chronic anovulation. Eighty-one non-hirsute, normally menstruating women with normal ovaries according to ultrasound, and age and BMI matched as the PCOS women formed the reference group for the calculation of the normal percentiles of insulin sensitivity. The three models were based on waist-circumference and fasting insulin, serum triglycerides, or sub scapular skin fold. The authors suggested that these mathematical models were derived from an unselected population of PCOS patients with BMIs of 17.6-37.4 kg/m^2, making this model applicable to both lean and obese patients. However, a major limitation of this study is that it was conducted in Europe and the criterion the authors used for PCOS diagnosis relied mostly on ovarian morphology. This diagnostic criterion is inconsistent with the NICHD criteria for PCOS diagnosis, which most researchers and clinicians use in the U.S. Thus, these mathematical models may not appropriate for use in our PCOS families.

Establishing limits for normal degrees of insulin sensitivity is arbitrary. A World Health Organization consensus group recently concluded that the insulin sensitivity index (SI) of the lowest 25% of a general population can be considered insulin-resistant (689). The European Group for the Study of Insulin Resistance took a more restricted view, defining insulin resistance as the SI of the lowest 10% of a nonobese, nondiabetic, normotensive white population (690). Legro and his associates also used the SI of the lowest 10% of an obese, non-PCOS population to define insulin resistance (674).

Our data collection procedures restricted us to the use of the fasting "homeostasis" models to measure insulin sensitivity. The ideal cutoff point for determining insulin resistance status in our PCOS families would be extracted from percentiles obtained from population-based studies on healthy subjects; the cutoff point from such kind of studies should be "ideally" age, gender, ethnic and BMI specific, give that all these variables affect the insulin resistance phenotype. Given that HOMA is a better measure of insulin sensitivity than either I0 or G0/I0, provides a useful model to assess insulin resistance in epidemiological studies in which only fasting samples are available(691) and has been selectively used- and not QUICKI, which is the only suggested slightly better fasting index of insulin sensitivity than HOMA- in population-based studies which evaluated the patterns of insulin resistance in the general population, we chose to use HOMA-IR to identify insulin resistant subjects in our PCOS families. Three cross-sectional population-based studies applied the HOMA-IR model to evaluate patterns of insulin resistance in the general population. One study was the San Antonio Heart study, a population-based study of diabetes and cardiovascular disease in 1,686 Mexican Americans and 779 non-Hispanic whites aged 25-64 years (691). The second cross-sectional study was another population-based study involving 1,226 Spanish individuals aged 18-65 years (692). Both studies reported mean HOMA-IR by glucose tolerance status and neither study showed a distribution of the HOMA-IR by glucose tolerance status. The latter study reported an unadjusted mean HOMA-IR± S.D. of 1.7± 1.5 for the 626 Normal glucose tolerant (NGT) Spanish subjects. The San Antonio heart study reported an age and sex adjusted mean of HOMA-IR±S.E. of 2.1± 0.2(or equivalently, a mean HOMA-IR± S.D of 2.1±5.2) for the 683 NGT non-Hispanic whites. The third cross sectional population-based study described the age- and sex-specific distributions of HOMA-IR in a representative sample of 2,244 Quebec children and adolescents aged 9,13 and 16 years. The 95th percentile values for HOMA-IR for boys and girls aged 13 and 16 years were

reported to be 3.28 and 3.31, 3.86 and 3.10 respectively(693). Another two case control studies should be mentioned in this regard as well. The first case-control study performed an OGTT on white and Mexican American women living in Texas to determine whether a single cutoff point for HOMA-IR could differentiate insulin resistant subjects from those with normal insulin sensitivity. 83 PCOS cases were identified according to NICHD diagnostic criteria and 19 non-obese ovulatory women volunteered as controls. The diagnosis of IR was made if one or more of the insulin levels obtained at 1,2, and 3 hours exceeded the upper limit of normal value for insulin at the appropriate stage of OGTT. In their white population (n=65), HOMA values >3.8 were 81% sensitive and 77.3% specific with the area under the curve 0.857± 0.056(681). However, major limitations of this screening value are: 1) it is based on a relatively small sample size (n=65). 2) It is derived from both PCOS women, some of whom are probably glucose intolerant and T2 Diabetics, and control women.3) it is derived from an obese white population with a mean BMI=32 kg/m^2. Moreover, this study did not involve African Americans, an ethnic group which we have in our PCOS families. The other most recent case-control study (694) comprised of 271 African American and white PCOS patients and 260 African American and white healthy eumenorrheic, premenopausal nonhirsute, control women who responded to posted advertisement. The presence of PCOS was defined by NICHD criteria. The HOMA-%β-cell function was calculated as [20 x I0 in μU/ml]/ [G0 in mmol/L -3.5] and the HOMA-IR was calculated as defined earlier. The values for HOMA-IR and HOMA-%β-cell values among controls were adjusted for age, race and BMI, and these adjusted values were then used to establish the normal limit for HOMA-IR(upper 95th percentile) and HOMA-%β-cell (Lower 5th percentile) as follows: adjusted 95th percentile HOMA-IR among controls =3.9mol x μU/L ; adjusted 5th percentile HOMA-%β-cell among controls=59.2%. Major strengths of the normal limits for HOMA-IR and HOMA-%β-cell in this study are that these limits: **1)** were based on a large population of healthy controls (n=271). **2)** were based on a **distribution of adjusted values** (for age, race and BMI) of HOMA-IR and HOMA-%β-cell among healthy controls, and thus these adjusted 95th percentiles HOMA-IR and the adjusted 5th percentile HOMA-%β-cell could be reasonably applied to other populations**. 3)** provide both a cutoff point, defined by adjusted 5th percentile HOMA-%β-cell< 59.2%, which helps in identifying subjects with abnormal β cell function and another cutoff point for identifying insulin resistant subjects, defined by adjusted 95th percentile HOMA-IR >3.9 mmol x μU/ml . We believe that these adjusted HOMA-IR and

adjusted HOMA-%β-cell cutoff points are the best available in the literature and we used them in differentiating IR adults (≥18years) from those adults with normal insulin sensitivity in our PCOS families. For children aged 14 -17, we used age sex specific 95th percentile HOMA-IR cutoff points as were reported for Caucasian boys or girls ages 13 and 16 in the population-based study which was mentioned earlier(693). For boys age 13 and 16, 95th percentile HOMA-IR cutoff points were reported as 3.28 and 3.31 respectively. We assumed equal increment of 0.01 per one-year increase in age and the corresponding 95th percentile HOMA-IR cutoff points for boys ages 14, 15, 16 and 17 were calculated to be 3.29, 3.30, 3.31 and 3.32 respectively. For girls aged 13 and 16, 95th percentile HOMA-IR cutoff points were reported as 3.86 and 3.10 respectively. We assumed equal increment of 0.25 per one-year decrease in age and the corresponding 95th percentile HOMA-IR cutoff points for girls ages 14, 15, 16 and 17 were calculated to be 3.61, 3.35 ,3.10 and 2.85 respectively. The ideal cutoff point for identifying IR subjects in our PCOS families would be age gender ethnic BMI specific and derived from a population-based study, since each of these variables affect the development of the insulin resistance phenotype. However, no such data is available in the literature. Therefore, our chosen cutoff points- age, BMI and race adjusted for adults and age-sex specific for children- were the best available and not the ideal reference points.

For our study, HOMA-IR and HOMA-%β-cell function were calculated as defined previously (695): HOMA-IR= [I0 in in μU/ml x G0 in mmol/l]/ [22.5]; HOMA-%β-cell function =[20 x I0 in μU/ml]/[G0 in mmol/l -3.5] . We calculated individual HOMA-IR's and HOMA-%β-cell function and compared them with normal limits for HOMA-IR and HOMA-%β-cell function as defined above: an adult was identified as insulin resistant if he/she has a HOMA-IR>3.9 mmol x μU/ml ; an adult was identified as having an abnormal β-cell function will if he/she has a HOMA-%β-cell <59.2%. An adolescent was identified as insulin resistant if he/she has a HOMA-IR > the above defined age-sex specific limits. Since HOMA-IR is known to be less predictive in subjects with glucose intolerance or type two (T2) diabetics, we considered all T2 diabetics in our study, including those who are on anti-diabetic medications, insulin resistant and with abnormal B-cell function regardless of what his/her HOMA-IR result indicated. We also examined closely the fasting insulin and HOMA-%β-cell function of subjects with impaired fasting glucose (IFG). Any such subjects with abnormal β-cell function were considered insulin resistant regardless of what his/her HOMA-IR result indicated.

Though the HOMA-IR measurement has been reported to have a high degree of correlation with IR assessed by the euglycemic hyperglycemic clamp (696-697), a potential limitation is the possible insensitivity of the HOMA-IR to estimate IR. The HOMA-IR seems to be less predictive in subjects with glucose intolerance (698-699), a potential uncertainty in our study, because 20%-30% of patients with PCOS might have glucose intolerance (11, 30,700).

3.5.2.2 Secondary Outcome Variables (Metabolic Syndrome)

3.5.2.2.1 Adults

For this study, we used the National Cholesterol Education Program (NCEP) Adult treatment Panel III guidelines for definition of MS in our adult population (701). The NCEP-ATP III has created an operational definition of the metabolic syndrome: the co-occurrence of three or more of the abnormalities mentioned in the **below table.**

Table 7: NCEP Adult Treatment Panel III diagnostic Criteria for Metabolic Syndrome in ADULTS

ATP III diagnostic criteria (≥ three of the following)	
Abdominal/central obesity	**Waist circumference: >102 cm** (40 in) in **men**, **>88 cm** (35 in) in **women**
Hypertriglyceridemia	**≥150 mg per dL**
Low HDL cholesterol	**<40 mg per dL** (<1.036 mmol per L) for **men,** **<50 mg per dL** (<1.295 mmol per L) for **women**
High blood pressure	**≥130/85 mm Hg** or documented use of antihypertensive therapy
High fasting glucose	**≥110 mg per dL** (>=6.1 mmol per L)† or diagnosed or treated for type 2 diabetes

3.5.2.2.2 Adolescents

Although no universally accepted definition for the metabolic syndrome in adolescents has been formulated, previously developed criteria (702) have been widely used in the literature that are based on values from national references for cholesterol, blood pressure, and glucose for children and adolescents. Using these criteria, the metabolic syndrome in our adolescent population was defined as having ≥3 components from the National Cholesterol Education Panel definition,

modified for age. Adolescents who had a waist circumference ≥90th percentile for age and gender from the NHANES III (1988-1994) population were classified as having abdominal obesity. Systolic and diastolic hypertension was defined by having blood pressure values ≥90th percentile for age, sex, and height or currently using antihypertensive medication. Participants were classified with hypertriglyceridemia if they had TG values ≥110 mg/dL, low HDL if they had values ≤40 mg/dL, and elevated fasting glucose if they had serum glucose values ≥110 mg/dL, were T2diabetics or on anti-diabetic medications **(see Table below).**

Table 8: NCEP Adult Treatment Panel III diagnostic Criteria for MS, modified for age in ADOLESCENTS

(≥ three of the following)	
Abdominal/central obesity	**Waist circumference:** ≥ 90th percentile for age and gender
Hypertriglyceridemia	≥ **110 mg per dL**
Low HDL cholesterol	≤ **40 mg per dL** (≤1.036 mmol per L)
High blood pressure	**SBP and/or DBP:** ≥ 90th percentile for age, gender, and height **or** documented use of antihypertensive therapy
High fasting glucose	≥ **110 mg per dL** (>=6.1 mmol per L) or diagnosed or treated for type 2 diabetes

3.5.2.3 Other Outcome Variables (Quantitative)

These included components of Metabolic Syndrome (Waist, TG, HDL, SBP, DBP and fasting glucose), serum inflammatory markers' (IL6/CRP) and total testosterone levels. The measurement methods of these outcomes were discussed earlier.

4.0 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

4.1 DATA ANALYSIS

4.1.1 Specific Aims 1 and 2: Not applicable.

4.1.2 Specific Aims 3 and 4

The two main steps in the analysis of these two specific aims were as follows: (1) the analysis of the SNP data to locate putative insulin resistance susceptibility genes in PCOS families (2) the analysis of the candidate genes to determine whether specific alleles in these putative IR susceptibility genes associate with severity/status of insulin resistance, metabolic syndrome (MS) or any of its components, serum inflammation (CRP, IL-6) and total testosterone levels in PCOS families. The three candidate genes represent genes that have either received some interest in the literature as potentially associated with insulin resistance or PCOS. As such they were each an a priori hypothesis for testing and did not require adjustment of the type I error (α=0.05). Non-parametric methods of linkage and association analyses were used to analyze data for the candidate genes in the multigenerational, multiplex PCOS families. We applied a number of different analyses methods to the family data where each highlighted either an evidence for linkage or association. For linkage analyses, we applied a non-parametric linkage method (Variance Components). For association analyses, we used variance components association test for quantitative traits and a family-based association test (FBAT) for dichotomous traits, a variation of the transmission disequilibrium test (TDT), and linear regression and logistic regression models. TDT examines the transmission of alleles from parents to affected offspring (PCOS/insulin resistant, metabolic syndrome) looking for a deviation from the expected ratio where deviation suggests linkage disequilibrium/association between the allele and a putative disease locus. This method requires selection of a single nuclear family from any extended

pedigrees when testing for linkage disequilibrium. In our study, we did not have enough number of families to select independent nuclear families, each from one extended pedigree. On the other hand, selecting multiple trios (both parents + an affected (PCOS/IR) offspring) from the same extended family would invalidate the association results obtained by TDT. Therefore, it would be desirable to have a valid test of linkage disequilibrium/association that can use all potentially informative data. The FBAT and variance components association test are two such tests. One study (703) found that: 1) when extended pedigree data are available, power simulations demonstrated that substantial gains in power can be attained by using such kind of tests rather than available methods that use only a subset of the data (like TDT) 2) FBAT and variance components association test remain more powerful even when there is misclassification of unaffected individuals. Therefore, FBAT and variance components association test were used to examine associations between specific alleles (genotypes) of the selected variants, at each of IRS-1, PPARλ and IL-6 with insulin resistance severity/status, metabolic syndrome status and its components, serum levels of inflammatory markers (CRP or IL-6) and testosterone. Linear and logistic regression models were used for this same purpose as well. The effect of variation at the candidate genes on variation in insulin resistance, metabolic syndrome and its components, serum levels of inflammatory marker (CRP/IL-6) and total testosterone levels was analyzed using linear regression model for continuous variables [insulin resistance severity, components of MS, serum levels of inflammatory markers (CRP or IL-6) and total testosterone levels) and logistic regression model for categorical variables (Insulin resistance status and Metabolic Syndrome). The main difference between FBAT/variance components association tests and linear and logistic regression models is that the former tests takes into account dependency between observations as opposed to the latter models. Linear and Logistic regression analyses were carried out at three levels: total sample, females and males, using SPSS version 13.0(704). On the other hand, variance components association test was performed in total sample and females, using SOLAR software (705) and each of FBAT and variance components linkage analyses were carried out in total sample only, using FBAT software (706) and MERLIN software (707) respectively. For association analyses, we used the wild genotype for each SNP as the reference category (For each of the Pro12Ala PPARλ and G-174C IL6 SNPs, the CC genotype was the reference category, according to PERLEGEN data; Pro12Pro genotype for Pro12Ala SNP and C-174C for G-174C IL6 SNP). Furthermore, for purpose of total analyses,

menopause status, ever/ current oral contraceptive(OC) use, ever/ current hormone replacement therapy(HRT) use, men were included in the premenopausal, OC and HRT non-users' categories respectively. By doing this and adjusting for gender in all our analyses, we are assuming that our men fall into the premenopausal female category and into the female OC or HRT non-users' categories. We found this important to do so that we make use of the total sample, given its small size, in our analyses in general and linkage analyses in particular while adjusting for important covariates which were found to be significantly associated/are known to be associated with the studied outcome. For subgroup analyses in females, men were treated as missing for each of the aforementioned variables. Physical activity was measured according to a standard formula which is used to calculate Kilo-calories/week (Kcal/WK) from responses to Paffenbarger physical activity questionnaire (708).

We started the linear regression analyses by checking for outliers, defined as data points that were more than 3 standard deviations from the mean. We examined the plots of the distribution of the continuous variables and remove outliers from analyses, when necessary; normality of each of the continuous variables (HOMA-IR, CRR, IL6 or testosterone serum levels, and various components of the MS- Waist, TG, HDL, SBP, DBP and fasting glucose) was assessed and transformations of the not-normally distributed variables were carried out prior to carrying out the linear regression model analyses. If no transformation helped normalizing the distribution of the variable, the variable would be used in its original distribution after excluding the outliers. Univariate and multivariate regression analyses were both performed. We initially carried out univariate regression analyses to identify significant associations of potential covariates with the outcome of interest; a p-value of ≤ 0.1 was used as a criterion to include a covariate in the multivariate regression analyses of the studied outcome. The effects of several environmental covariates on attenuation in insulin resistance severity/status, serum inflammation(IL6 or CRP) levels, serum testosterone levels, metabolic syndrome(MS) or various components of MS- Waist, TG, HDL, SBP, DBP and fasting glucose- were considered: age, race, gender, BMI, physical activity, smoking, and alcohol consumption. Other covariates, which were reported to have an association with serum inflammation level, were considered as well: menopause status, hormone use, fasting insulin, fasting glucose, triglycerides and total cholesterol. Serum Inflammatory markers' levels were considered as covariates in the regression models where insulin resistance was the outcome and vice versa. The logistics of the multivariate

regression analyses was as follows. We forced in order, irrespective of significance status, the studied SNP, age, gender and race into each model in total analyses. In subgroup analysis, we forced, regardless of significance status, the studied SNP, age and race into each model in females; however, the studied SNP and age but not race were forced into each model in males. This was because of the small sample size (n=29), few African American males (n=6) and the resulting instability of the model when too many parameters entered the regression model. We also forced 1) covariates (IR/fasting insulin, PCOS and BMI), which were found to be significantly univariately (or after adjusting for potential confounders) associated with the outcome 2) covariates, which are well established in the literature to associate with the outcome under study (current anti-diabetic medication use when HOMA-IR was the outcome, current OC use and current HRT use when testosterone was the outcome). Furthermore, we determined the sequence of the variables to be forced into each model a priori, which was the following. To evaluate the unadjusted effect of the SNP on the trait of interest, the SNP was first entered into the model followed by age, gender (applicable in total analysis only), race (in males, depending on significance status), IR/fasting insulin (if significant univariately or after adjusting for age, BMI or/and PCOS ($P \leq 0.1$)), PCOS status (if significant univariately or after adjusting for age and BMI ($P \leq 0.1$)-Total and Female analyses only) and BMI in a hierarchical fashion. Current antidiabetic medication use was forced after BMI into the model, where ln-HOMA-IR was the outcome; current OC and current HRT use were forced into the model after BMI, where ln-Testosterone was the outcome. All other covariates which were significantly univariately associated with a dependent variable were entered in a stepwise fashion. Variables were selected in a stepwise (forward and backward) procedure by determining which was the next variable which when added to the subset offered the largest increase in the improvement of the fit, as measured by the deviance difference, and by examining those variables already in the model whose exclusion would not now lead to a significant loss in the fit of the model. We adjusted for the effect of one SNP in the model of the other SNP if the p-value of the former SNP was < 0.05 in its final regression model; if adjustment for one SNP is needed in the model of the other SNP, adjustment was performed before the stepwise procedure so that we evaluate which other variables would still enter the model after adjusting for the effect of the two SNPs together. We tested for gene-gene interactions if the p-value for each of the two SNPs was < 0.1 in its separate final regression model. We evaluated potential gene-environment interactions as well; such

interactions were identified through any change in significance status of the SNP whenever an environmental covariate enters the multivariate regression model. In interpreting the results of the association analyses, p-values of < 0.05 and < 0.1 were considered significant and borderline significant respectively. Distributional assumptions were rechecked by analysis of the distribution of the residuals (the errors) from the final linear model analysis.

For purposes of variance components association test and linkage analyses on quantitative traits, we used the results of multivariate regression analyses in constructing the models; In addition to the studied SNP, only covariates which were found to significantly associate with the outcome in the multivariate regression model (p-value<0.1) were included in the variance components association test and linkage models. An exception to this is that we adjusted, irrespective of significance status, for anti-T2DM medication use / current OC and HRT use when ln HOMA-IR and ln-Testosterone serum levels were the outcomes respectively.

4.1.3 Specific Aim 5:

We assessed the frequencies of the alleles of each of Pro12Ala variant of PPARλ gene, Gly97Arg variant of IRS-1 gene and G-174C variant of IL-6 gene and compared these allele frequencies with population-based frequencies which were obtained from National Cancer Institute NCI data base or The NCBI Single Nucleotide Polymorphism Database (dbSNP).

4.2 POWER CALCULATIONS

4.2.1 Specific Aims 1, 2 and 5

Since **specific aim 1** only addresses the ability to recruit 10-15 family members from ten different families with prevalent PCOS with no other outcome variables, there were no power calculations for this aim. Our goal was to show that we could recruit multiplex families with PCOS from the Pittsburgh community, with the longer-term aim (as part of a subsequent proposal) to recruit additional families and perform a linkage analysis to identify genes affecting risk of developing insulin resistance in PCOS families. **Specific aim 2** only addressed genotyping the ten probands and their multiplex, multigenerational family members to study insulin resistance and inflammation markers in families with PCOS. **Specific Aim 5** only addressed comparing allele frequencies of the insulin resistance and inflammatory markers in the studied PCOS families with population frequencies using NCBI dbSNP or NCI databases and therefore no power calculations were needed for these aims as well.

4.2.2 Specific Aim 3

We calculated the power for linkage analyses using SOLAR software (705). Assuming an Ala12 allele frequency similar to what we found in our population of 0.1, no recombination between the Ala12 allele and the phenotype marker locus and assuming that the Pro12Ala variant accounted for 24% variation in quantitative trait of interest (for e.g., HOMA-IR), we had only 5% power to detect significant linkage (LOD score >3) and 13% power to detect suggestive linkage (LOD score>2).

4.2.3 Specific Aim 4

Three sets of power calculations have been performed for this specific aim. The first was for the variance components association test, the second for the family based association test (FBAT) and this was assuming dependency between observations. The third set of power calculations was for linear and logistic regression analyses, and this was assuming independence between observations.

4.2.3.1 Variance Components Association Test

To determine whether we would have sufficient power to detect the effect of a candidate gene on a quantitative trait of interest (for e.g., HOMA-IR), we performed simulation studies. We assumed that we would have information on 5 families with 20-25 members each. We also assumed that the variant at a candidate locus would account for 5% of the variation in HOMA-IR. Based on 200 simulations of these five families, we tested for an association between the simulated quantitative insulin resistance trait and the simulated candidate gene using the program QTDT (709). This program is used to test for an association using data on families. We determined that 75% of the time we would obtain a p-value < 0.05. Thus, we will have 75% power to evaluate the effect of a candidate gene on a quantitative trait with a total recruitment of 100 to 125 participants from five families.

4.2.3.2 FBAT

To determine whether we would have sufficient power to detect the effect of a candidate gene on a dichotomous trait of interest (for e.g., insulin resistance status), we used the FBAT software (706), assumed an Ala12 allele frequency of 0.1 that was similar to what we found in our population and an overall prevalence of insulin resistance of 20% in the general population (710) and found that we had 23% power and 52% power to detect an OR of 2 and 3 respectively. Moreover, assuming an Ala12 allele frequency of 0.1 that was similar to what we found in our population, an overall prevalence of insulin resistance of 50% in PCOS families (16), we had 11% power and 21% power to detect an OR of 2 and 3 respectively.

4.2.3.3 Logistic/Linear Regression Analyses

For **logistic regression** model analyses, we used unmatched case-control 1:1, a main gene effect only, an allele frequency of 6%, a population risk of 60%, and an odds ratio of 1.5-2.5 by 0.5 increments. These selections were based on the following. The Arg972 allele of the G972R variant in IRS-1 has been reported to have an overall frequency of approximately 6% in the general population (325). Furthermore, according to HAPMAP release 16, a similar frequency has been found for this Arg972 allele in Caucasians (5.8%); in African Americans, it was found to be 10%(711); since the majority of our population will be Caucasians, in our power calculations we assumed the allele frequency of Arg972 to be 6%. About 50%-70% of all women with PCOS have some degree of Insulin resistance (16) and one family study found that 69% of siblings and parents of PCOS individuals in five families are hyperinsulinaemic (167); in our power calculations, we assumed that the risk of insulin resistance in PCOS families is 60%. We assumed that the odds of developing a dichotomous trait of interest(for e.g., insulin resistance status) among carriers for R972 allele(R972R+G972R) of the G972R variant in IRS-1 compared to subjects homozygous for the G972 allele is 1.5, 2 or 2.5(345). This is also supported by findings on G-174C variant in IL-6 (711-712). These studies used a dominant model (345; 712-713). We also assumed a dominant model and a significance level of 0.05, 2-sided. We performed the power calculations for total and subgroup analysis (Total sample, N=101; Females, N=72; Males, N=29). For **linear regression** model analyses, we assumed that the variant at a candidate locus would account for 2%, 5%, or 10 % of the variation ($R^2{}_G$) in the quantitative measure of the trait of interest (for e.g., severity of insulin resistance (HOMA-IR)). We used a mean HOMA_IR±SD of 5.8±0.5 in PCOS population (714).This mean HOMA value was based on 408 premeopausal US women with PCOS, the majority of which were Caucasians and African Americans(714). We assumed an allele frequency of 6% for Arg972 of the Gly972Arg in IRS-1 (325,609), log-additive, recessive, or dominant models, a significance level of 0.05, 2-sided. We performed the power calculations for total and subgroup analysis (Total sample, N=101; Females, N=72; Males, N=29). These power calculations were carried out using Quanto software (715). A summary of the power calculations are tabulated below (**Tables below**):

Table 9: Results of Power Calculation for Logistic Regression Analyses: Outcome= MS status, IR status

Odds Ratio	**Sample Size**		
	Total analysis	**Females' Analyses**	**Males' Analysis**
	101	**72**	**29**
1.5	15%	12%	7%
2	33%	25%	13%
2.5	50%	38%	18%

$\alpha = 0.05$, 2-sided, Dominant Model

Table 10: Results of Power Calculation for Linear Regression Analyses: Outcome= IR severity, serum CRP/IL-6 & total Testosterone levels, Components of MS

	Dominant or Recessive or Log-Additive		
R^2_G *	**Sample Size**		
	Total analysis	**Females' Analyses**	**Males' Analysis**
	101	**72**	**29**
2%	30%	23%	12%
5%	62%	49%	23%
10%	90%	79%	42%

$\alpha = 0.05$, 2-sided

* Estimated variation in the quantitative measure of phenotype under study, which could be accounted for by a variant at a candidate locus.

5.0 RESULTS

5.1 RECRUITMENT OF PCOS FAMILIES

We achieved our recruitment goal of 100-125 individuals by identifying probands either through the ongoing CHARM study population or through responses received through an advertisement in the Pittsburgh Post Gazette, other newspapers or magazines and, therefore, did not have to initiate recruitment through PCOSA or endocrinologists' offices, as was originally proposed in our protocol. Furthermore, we were able to reach our recruitment goal by enrolling families who lived within 60 miles of the Pittsburgh area. In total, 101 individuals were recruited from 9 multigenerational extended families who had at least 2 clinically diagnosed PCOS cases; eight of the recruited families were Caucasian and the remaining was African American **(SEE APPENDIX B)**. The African American family was composed of 19 family members and the distribution of the number of individuals in the 8 Caucasian families was as follows:(1(16 individuals), 2 (15 individuals), 1(12 individuals), 1(9 individuals), 1(7individuals), 1(5 individuals) and 1(3 individuals). The logistics of the recruitment process was as follows. An attempt was made to recruit the families from a total of 61 probands; 47 CHARM PCOS cases and 14 other women who responded to the newspaper or University of Pittsburgh ODIX service ads about this study. The results for our efforts in contacting the 47 CHARM PCOS women were as follows: 3(6%) were eligible and participated in our study together with their families, 23(49%) were not eligible, 5(11%) indicated no interest to participate in the study, 15(32%) did not return the postcard to indicate interest in participation and 1(2%) was eligible but had all family members outside Pittsburgh. We postponed recruiting this latter family until the end of the recruitment process to determine if there is a need to do that; our preference was to recruit the families from Pittsburgh area for the feasibility of obtaining and properly processing the collected blood samples. The results for our efforts in contacting women who responded to our

ads were as follows: 6 (43%) were eligible and participated in our study together with their families, 5(36%) were not eligible and 3(21%) did not return the postcard, which we sent them for a few times, confirming interest in participation.

In summary out of the 61 PCOS probands whom we attempted to contact, 10(16%) were eligible; 9(15%) were recruited together with their families and 1(1%) was not recruited for the reason mentioned earlier. A total of 28(46%) indicated interest but were not eligible, 5 (8%) indicated no interest to participate, 18(30%) did not return the postcard indicating interest in participation. As mentioned earlier, one out of the recruited nine families was African American with 19 individuals total. This represents 20% of total recruited individuals (19/101), as was originally proposed in our recruitment plan. Thus, our attempt to recruit subjects in respective proportion to the demographics of Pittsburgh and the surrounding area and/or the patient population of the University of Pittsburgh Medical Center was successful. Although we were enrolling entire families and not selecting on basis of gender, we were able to recruit 29 men vs. 72 women and therefore not be able to meet the estimated national ratio of men: women of approximately 1:1. This is expected however, in any epidemiologic study.

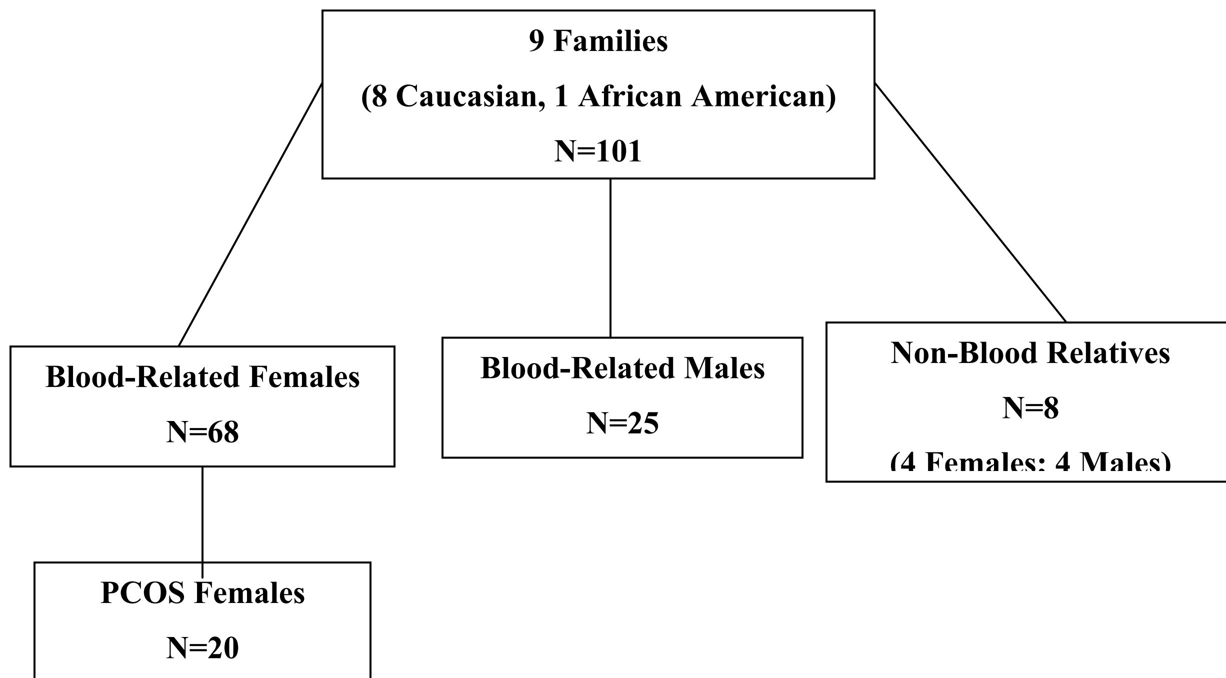


Figure 4: Chart of Study Participants

5.2 GENOTYPING RESULTS

We genotyped the 101 individuals for PPARλ P12A (rs1801282), IRS-1 G972R (rs1801278), IL-6 G-174C (rs1800795) and V162D (rs2069860) variants. The IRS-1 G972R (rs1801278) and IL-6 V162D (rs2069860) variants were present in a low frequency in our study population of PCOS families (MAF<5%; IRS-1 G972R SNP, MAF=0.5%; IL6 V162D SNP, MAF=3.8%) and therefore the SNP- phenotype analyses were restricted to PPARλ P12A and IL-6 G-174C SNPs. We were not able to test for Hardy-Weinberg equilibrium; this is because we had few unrelated individuals in our pedigrees (parents and spouses of offspring).

5.3 DESCRIPTIVE RESULTS

5.3.1 Demographic and Biologic Descriptors

5.3.1.1 PCOS cases

Table 11 shows the demographic, anthropometric and cardiovascular characteristics, at time of interview, of the 20 PCOS cases in our 9 families. A total of 17 or 85% of the women were 18 years or older and, therefore the majority of the PCOS cases were in the adult range. However, this should not undermine the significance of having three (15%), 17 years or younger girls with a clinical diagnosis of PCOS. The mean age for all cases was 37.40 years (± 15.39); 16.67 years (± 0.58) for 17 years or younger (17-) girls and 41.06 years (±13.65) for 18 years or older (18+) women. The mean Body Mass Index (BMI) was 34.39 kg/m2 (± 8.97) for the total group of PCOS women [after excluding one outlier for BMI for an 18+ years PCOS woman ,33.11 kg/m2 (± 7.07)]; 29.86 kg/m2 (±5.56) for 17- years girls and 35.19 kg/m2 (± 9.34) for 18+ years women [after excluding one outlier, 33.71 kg/m2 (± 7.30)]. According to the standard CDC chart of girls' Body Mass index, a new recommended method to judge if a girl child is overweight, obese, normal or underweight (717), our PCOS adolescent girls fall into the overweight category. Moreover, a mean BMI of 35.19 kg/m2 for our adult women complies with the "obese" definition (≥ 30 kg/m2) of the CDC (718). The mean waist-to-hip ratio (WHR) was calculated to

be 0.84 (± 0.07) for total PCOS cases; 0.83 (±0.06) for 17- years girls and 0.84 (± 0.07) for 18+ years women. According to the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK)(719), women with waist-to-hip ratios greater than 0.8 are generally considered to have "excess fat" and be at higher risk for obesity-linked diseases, including arthritis, heart disease, hypertension and diabetes. This WHR cutoff point indicates that our adult PCOS cases are at increased risk for obesity-linked diseases. The mean waist was 99.94 cm (± 17.88) for total group of PCOS cases; 92.75 cm (±6.38) for 17- years girls and 101.2 (± 19.05) for 18+ years women. According to the Adult Treatment Panel III (ATP III) diagnostic criteria for metabolic syndrome (701), women with waists of >88 cm are considered to be centrally obese. Our findings strongly support the heightened risk of our adult PCOS cases for metabolic syndrome and cardiovascular disease. The mean systolic blood pressure (SBP)/ mean diastolic blood pressure (DBP) was 121.95mm (± 12.73) / 78.05 mm (± 10.84) for all PCOS cases[after excluding one outlier for SBP and DBP for an adult PCOS woman ,119.68mm (± 7.92)/ 76.42 mm (± 8.24)]; 113.33 mm (± 5.03) / 67.67 mm (± 12.50) for 17- years girls and 123.47 mm (± 13.15) / 79.88 mm (± 9.80)[after excluding one outlier, 120.88mm (± 7.89)/78.06mm (± 6.52)]. According to the American Heart Association (AHA) recommended blood pressure levels (720), individuals with a SBP range of 120-139 mm or DBP range of 80-89 mm are considered pre-hypertensive. These BP cutoff levels suggest that our PCOS women 18+ years were, on average, pre-hypertensive. The lipid profile for our PCOS cases was as follows. The mean total serum cholesterol was 227.80 mg/dl (± 33.74) for total group of PCOS females; 188.33 mg/dl (± 40.77) for 17- years girls and 234.76 mg/dl (± 28.29) for 18+ years women. The mean LDL cholesterol was 145.55 mg/dl (±35.91) for total PCOS cases; 118.33 mg/dl (± 44.11) for 17- years girls and 150.35 mg/dl (± 33.54) for 18+ years women. The mean total HDL cholesterol for all PCOS cases was 52.00 mg/dl (±13.16)[after excluding one outlier for HDL for an adult PCOS woman, 49.65 mg/dl (± 8.12)]; 50.90 mg/dl (± 7.81) for 17- years girls and 52.19 mg/dl (± 14.06)[after excluding one outlier ,49.41mg/dl (± 8.40)] for 18+ years women. The mean triglycerides for total group of PCOS females were 151.40 mg/dl (±64.05); 95.00mg/dl (± 66.01) for 17- years girls and 161.35 mg/dl (± 60.21) for 18+ years women. According to the ATP III high blood cholesterol criteria (721), total cholesterol (TC) and LDL cholesterol values of 200-239 mg/dl and 130-159 mg/dl respectively are considered borderline high. Furthermore, total HDL cholesterol values of < 50 mg/dl in women are considered low and triglyceride values (TG) of ≥

150 mg/dl are considered to be high. The total and LDL cholesterol values for our 18 years or older PCOS cases appear to fall close to the top bound of "borderline high" category and ,therefore, close to the lower bound of the "high" category. Moreover, according to these criteria these women, on average, are said to have hypertriglyceridemia. According to the 1991 NCEP recommendations on Blood Cholesterol in Children and Adolescents (722), total cholesterol and LDL cholesterol values of 170-199 mg/dl and 110-129 mg/dl respectively are considered borderline high. Furthermore, total HDL cholesterol values of 35-45 mg/dl are considered borderline low and triglyceride values of ≥ 130 mg/dl are considered to be high. On average, our adolescent PCOS cases had "borderline high" TC, LDL cholesterol and TG levels, reflecting their increased BMI. Furthermore, it is worth noting that our PCOS 17- years girls had lower HDL mean value than that of 18+ years PCOS women; this is indeed noting the limitation that such observation is based on a much smaller sample size for the adolescent subgroup. The mean HOMA-IR was 6.67 (± 7.28) for all PCOS cases; 4.38 (± 2.91) for 17- years girls and 7.08 (± 7.8) for 18+ years women. According to our criteria for identifying insulin resistant adults (HOMA-IR >3.9) or adolescents (age-sex specific; 16 years girls HOMA-IR >3.10, 17 years girls HOMA-IR >2.85), our PCOS cases were, on average, insulin resistant. Excluding the four T2 diabetics (One 17- year girl and three18+ years women), the mean HOMA-IR for total PCOS women became 4.98 (± 2.98); 2.82 (± 1.49) for 17- years girls and 5.29 (± 3.04) for 18+ years women. The majority of our PCOS women were white, 18 (90%) and the remaining 2 (10%) were African Americans.

In summary our PCOS cases were, on average, overweight to obese, dyslipidemic and insulin resistant.

Table 11: Distribution of Salient Demographic, Anthropometric and Cardiovascular Characteristics among PCOS Cases by Adult Status (N=20)

Characteristic	**PCOS Cases**					
	≤17 (N=3)*		**≥18(N=17)***		**TOTAL(N=20)***	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Age	16.67	0.58	41.06	13.65	37.40	15.39
BMI(Kg/m^2)	29.86	5.56	35.19	9.34	34.39	8.97
*BMI(Kg/m^2)***	_____	_____	*33.71(n=16)*	*7.30*	*33.11(n=19)*	*7.07*
WHR	0.83	0.06	0.84	0.07	0.84	0.07
Waist(cm)	92.75	6.38	101.2	19.05	99.94	17.88
SBP(mm Hg)	113.33	5.03	123.47	13.15	121.95	12.73
*SBP(mm Hg)***	_____	_____	*120.88(n=16)*	*7.89*	*119.68(n=19)*	*7.92*
DBP(mm Hg)	67.67	12.50	79.88	9.8	78.05	10.84
*DBP(mm Hg)***	_____	_____	*78.06(n=16)*	*6.52*	*76.42(n=19)*	*8.24*
Total Cholesterol(mg/dl)	188.33	40.77	234.76	28.29	227.80	33.74
HDLT(mg/dl)	50.90	7.81	52.19	14.06	52.00	13.16
*HDLT(mg/dl)***	_____	_____	*49.41(n=16)*	*8.40*	*49.65(n=19)*	*8.12*
LDL(mg/dl)	118.33	44.11	150.35	33.54	145.55	35.91
Triglycerides(mg/dl)	95.00	66.01	161.35	60.21	151.40	64.05
HOMA-IR***	4.38	2.91	7.08	7.8	6.67	7.28
16 years(N=1)	1.76	_____	_____	_____	_____	_____
17years(N=2)	5.69	2.58	_____	_____	_____	_____
HOMA-IR******	**2.82(n=2)**	**1.49**	**5.29(n=14)**	**3.04**	**4.98(n=16)**	**2.98**
16 years(N=1)	1.76	_____	_____	_____	_____	_____
17 years(N=1)	3.87	_____	_____	_____	_____	_____
Race	**No.**	**%**	**No.**	**%**	**No.**	**%**
Caucasian	2	67	16	94.1	18	90
African American	1	33	1	5.9	2	10
TOTAL	**3**	**100**	**17**	**100**	**20.00**	**100.00**

*Unless otherwise specified in table, for ≤ 17 years N =3; for ≥ 18 years N=17; Total N= 20.

***Italicized* Characteristics imply means calculated after excluding outliers.

*** IR defined by HOMA_IR (>3.9 for 18yrs+;>2.85 for 17yrs;>3.10 for 16 yrs).

****Bolded characteristics imply means calculated after excluding T2 diabetics.

_____ means N.A.

Table 12 shows that 8 (40%) of our PCOS cases were currently married, 7(35%) never married, 4(20%) separated or divorced and 1(5%) was widowed. **Table 13** describes the distribution of our PCOS cases by years of schooling and job classification. A total of 2 (10%) adolescent PCOS girls did not complete high school, 6(30%) had a high school degree(1 was an adolescent PCOS case), 4(20%) had some post high school, 3(15%) had a college degree, while the remainder 5(25%) were educated past college and therefore, 18 (90%) of our PCOS cases had at least a high school degree. Five (25%) of the PCOS cases classified themselves as professional; 3(15%) as managerial, 1(5%) as clerical, 4(20%) as homemakers; 1(5%) as unemployed; and 6(30%) categorized themselves as other.

Table 12: Marital Status among PCOS Cases (N=20)

Marital Status	**PCOS Cases**	
	No.	**%**
Married	8	40
Divorced	3	15
Widowed	1	5
Separated	1	5
Never Married *	7	35
TOTAL	**20**	**100**

* This includes all 3 PCOS cases who are ≤ 17 years.

Table 13: Distribution of Years of Education Completed and Job Classification among PCOS Cases (N=20)

Year of Education Completed	PCOS Probands	
	No.	**%**
≤12*	8	40
13-15	4	20
16	3	15
≥17	5	25
TOTAL	**20**	**100**
Mean Years of Education ±S.D.	**14.05±2.31**	
Job Classification Distribution	**No.**	**%**
Professional	5	25
Managerial	3	15
Clerical	1	5
Homemaker	4	20
Unemployed-Not Disabled	1	5
Other**	6	30
TOTAL	**20**	**100**

* 2 adolescent PCOS girls did not complete high school and the remaining PCOS adolescent girl had a high school degree.

**Student, Federal Security Officer, home business.

Table 14 presents the distribution of PCOS cases by their doctor-diagnosed conditions. Four (20%) of our PCOS cases were diagnosed with T2DM, 6(30%) with hypertension, 1(5%) with angina, 1(5%) with stroke, 2(10%) with cancer, 4(20%) with nervous or emotional problem and none was diagnosed with heart attack or myocardial infarction, bypass surgery or angioplasty or circulation problems.

Table 14: Doctor Diagnosed Conditions among PCOS Cases (N=20)

Doctor Diagnosed Condition	PCOS Cases	
	No.	**%**
T2DM		
Yes	4	20
No	16	80
TOTAL	**20**	**100**
Hypertension		
Yes	6	30
No	14	70
TOTAL	**20**	**100**
Circulation Problems		
Yes	0	0
No	20	100
TOTAL	**20**	**100**
Bypass Surgery or Angioplasty		
Yes	0	0
No	20	100
TOTAL	**20**	**100**
Angina		
Yes	1	5
No	19	95
TOTAL	**20**	**100**
Heart Attack or MI		
Yes	0	0
No	20	100
TOTAL	**20**	**100**
Stroke		
Yes	1	5
No	19	95
TOTAL	**20**	**100**
Cancer		
Yes	2	10
No	18	90
TOTAL	**20**	**100**
Emotional/Nervous Problem		
Yes	4	20
No	16	80
TOTAL	**20**	**100**

Table 15 presents the distribution of our adolescent and adult PCOS cases by the results of the fasting blood specimen and clinic visit measurements. In this table, impaired fasting glucose (IFG) was defined as 110 mg/dl ≤ fasting glucose (G0) ≤125 mg/dl, not T2 diabetic (clinically or lab diagnosed) and not on anti-diabetic medication. Insulin Resistance (IR) was defined as either IR-No T2DM, IR-T2DM or IR-Self reported. IR-No T2DM are subjects who were detected as insulin resistant by HOMA-IR criteria (HOMA-IR >3.9 for 18yrs+;Girls: >2.85 for 17yrs;>3.10 for 16 yrs;>3.35 for 15 yrs and >3.61 for 14 yrs) and were not clinical or lab-diagnosed T2 diabetics; IR-T2DM are clinical or/and lab-diagnosed T2 diabetic subjects; IR-Self reported are subjects who reported themselves as having a clinical diagnosis of IR, were not clinical or lab-diagnosed T2 diabetics and were not detected by HOMA as IR. T2DM are T2diabetics who are either clinically diagnosed or/and diagnosed according to our fasting glucose lab results (G0≥126 mg/dl). IFG/IR/T2DM are subjects who had any of the glucose abnormalities (IFG or/and IR-not T2DM, or T2DM). **Table 16** shows the distribution of the number of components of metabolic syndrome among the 20 PCOS cases by adult status.

In total, 2 of our 20 PCOS cases (10%) had IFG, 15(75%) had IR, 4(20%) had T2DM and 15(75%) had IFG/IR/T2DM. A total of eleven(55%) satisfied the metabolic syndrome (MS) ATP III diagnostic criteria; 5 (25%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS and the remaining 6 (30%) had 4 components or more of this criteria. Of the remaining 9 PCOS cases, 7 (35%) had 1-2 components and 2(10%) had 0 components **(Tables 15 & 16).** Among the group of the 3 adolescent PCOS cases, 0% had IFG, 3 (100%) had IR, 1(33.3%) had T2DM, 3 (100%) had IFG/IR/T2DM and 0% had MS **(Table 15).** However, all 3 adolescent PCOS cases (100%) had 1-2 components of the MS diagnostic criteria **(Table 16)**. Among the group of our 17 adult PCOS women, 2(~12%) had IFG, 12(~71%) had IR, 3 (~18%) had T2DM and 12 (~71%) adult PCOS cases had IFG/IR/T2DM. Moreover, 11(~ 65%) had MS; 5 (~29%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS and 6(~35%) have 4 components or more of this criteria. Other 4(~24%) had 1-2 components and 2 (~12%) had 0 components **(Tables15 & 16).** Therefore, in total 75% of our PCOS cases had some kind of glucose abnormality, 55% had MS with 90 % carrying at least 1 component of the MS ATPIII diagnostic criteria. It is worth noting that all 3 adolescent PCOS cases presented with some kind of metabolic abnormality known to be

associated with PCOS (IR or T2DM). This is indeed noting the limitation of the small sample size upon which this observation is based.

Table 15: Distribution of Impaired Fasting Glucose (IFG), Insulin Resistance (IR), T2DM and Metabolic Syndrome (MS) among PCOS cases By Adult Status (N=20)

Characteristic	PCOS Cases					
	≤17 years(N=3)		≥18 years(N=17)		TOTAL(N=20)	
	No.	%	No.	%	No.	%
IFG*						
Yes	0	0	2	11.8	2	10
No	3	100	15	88.2	18	90
TOTAL	3	**100**	**17**	**100**	**20**	**100**
IR						
IR_No T2DM**	1	33.3	8	47.1	9	45
IR_T2DM**	1	33.3	3	17.6	4	20
IR-Self Reported**	1	33.3	1	5.9	2	10
Yes	3	100	12	70.6	15	75
No	0	0	5	29.4	5	25
TOTAL	**3**	**100**	**17**	**100**	**20**	**100**
T2DM**						
Yes	1	33.3	3	17.6	4	20
No	2	66.7	14	82.4	16	80
TOTAL	**3**	**100**	**17**	**100**	**200**	**100**
IFG/IR/T2DM**						
Yes	3	100	12	70.6	15	75
No	0	0	5	29.4	5	25
TOTAL	**3**	**100**	**17**	**100**	**20**	**100**
MS***						
Yes	0	0	11	64.7	11	55
No	3	100	6	35.3	9	45
TOTAL	**3**	**100**	**17**	**100**	**20**	**100**

*__IFG__ is defined as 110≤G0≤125 mg/dl, were not T2 diabetics and were not on anti-diabetic medicate

** **IR-No T2DM** are subjects who were detected as IR by HOMA-IR criteria (>3.9 for 18yrs+;Girls: >2.85 for 17yrs; >3.10 for 16 yrs; >3.35 for 15 yrs; >3.61 for 14 yrs) AND were not T2 diabetics. **IR-T2DM** are T2 diabetic subjects. **IR-self reported** are subjects who self-reported themselves as doctor-diagnosed with IR and were on Metformin for this purpose , were not detected by HOMA-IR criteria as IR AND were not T2 diabetics.****T2DM** is defined as clinically diagnosed T2DM cases as well as cases identified according to our G0 lab results; **T2DM** was defined as G0 ≥ 126 mg/dl.**IFG/IR/T2DM** are subjects who had any of the glucose abnormalities (IFG only, IR+IFG, IR only or T2DM).

*****MS** was defined according to ATPIII Diagnostic Criteria in Adults and modified for age in Adolescents.

Table 16: Number of Metabolic Syndrome (MS) Components among PCOS cases By Adult Status (N=20)

Number of MS Components	PCOS Cases					
	≤17 years		≥ 18 years		TOTAL	
	No.	%	No.	%	No.	%
0	0	0	2	11.8	2	10
1-2	3	100	4	23.5	7	35
3	0	0	5	29.4	5	25
4+	0	0	6	35.3	6	30
TOTAL	**3**	**100**	**17**	**100**	**20**	**100**

***MS** was defined according to ATPIII Diagnostic Criteria in Adults and modified for age in Adolescents.

Table 17 presents the distribution of PCOS cases by menopause status together with the distribution of post-menopausal women by cause of post-menopause. 14(70%) were pre-menopausal, including the 3 adolescent PCOS cases. 6(30%) were post-menopausal; 3(50%) had both hysterectomy and ovariectomy, 1(17%) had ovariectomy only and 2 (33%) had natural menopause. Therefore, the majority of our PCOS cases were in the pre-menopausal range and the majority of our post-menopausal women (4, 67%) went into post-menopause through surgical procedures.

Table 17: Menopause Status Together with History of Hysterectomy and Ovariectomy among PCOS Cases(N=20)

Menopause Status	PCOS Probands	
	No.	%
Pre-menopause*	14	70
Post-menopause**	6	30
TOTAL	**20**	**100**
Distribution of Postmenopausal Women by hysterectomy and ovariectomy Status	**No.**	**%**
Hysterectomy with Ovariectomy	3	50
Ovariectomy Only	1	17
Hysterectomy Only	0	0
Natural Menopause	2	30
TOTAL	**6**	**100**

*All 3 Adolescent PCOS cases were premenopausal

**Post-menopause status was defined by absence of period for ≥ 6 months before clinic visit

Table 18 describes the conception status, number of pregnancies and live births among our 17 adult PCOS cases. A total of 13 (76.5%) were conceived; 4 (23.5%) were conceived once, 6(35.5%) 2-4 times and 3(17.6%) 5-7 times. The distribution of the number of live births among the adult PCOS women was as follows: 10(58.8%) had 1-2 live births and 3 (17.6%) 3-4 live births. The mean age of our adult PCOS women was 41.06 years ± 13.65 and the mean number of conceptions was 2.24 (± 2.28) with a mean number of live births of 1.53 (± 1.18). A comparison was made between the mean number of live births of our adult PCOS cases with that of the 244 CHARM I PCOS women; the CHARM I PCOS cases were ≥ 18 years, had a mean age of 35.32 years (±7.5) and a mean number of live births of 0.930 (± 1.43). The comparisons suggest that our group of adult PCOS cases had a slightly higher mean number of live births. This could be partly attributed to the fact that our PCOS women were, on average, older (mean age 41.06 years ± 13.65) than the CHARM PCOS cases (35.32 years ±7.5). Another reason could be the significant improvement in the effectiveness of the fertility drugs, which are available in today's market compared to those available 10 years ago.

Table 18: Conception Status, Number of Pregnancies and live Births among PCOS Cases who are 18 years+(N=17)

Reproductive Characteristic	PCOS Cases ≥ 18 years(N=17)	
	No.	%
Conception Status		
Yes	13	76.5
No	4	23.5
TOTAL	**17**	**100**
No. Of pregnancies		
0	4	23.5
1	4	23.5
2-4	6	35.3
5+	3	17.6
TOTAL	**17**	**100**
No. Of Live Births		
0	4	23.5
1-2	10	58.8
3+	3	17.6
TOTAL	**17**	**100**
Mean Age±S.D.	**41.06±13.65**	
Mean No. of pregnancies±S.D.	**2.24±2.28**	
Mean No. Of Live Births±S.D.	**1.53±1.18**	

In total, we had 101 males and females; 72 females and 29 males. Fifty-six females (~78%) and all participating 29 males (100%) were 18 years or older, resulting in the majority of our population being in the adult range (85 total, 84%). A total of 82 participants (81%) were white and the remaining 19(19%) were African Americans. Among the 101 participants, we had 8 not-blood related subjects; 4 men and 4 women; this group of subjects includes spouses of probands or spouses of a proband's blood relative and was excluded from the analyses described in Tables 19-32. The analyses in Tables 19-32, therefore, was restricted to probands and blood- relatives (Probands and Female blood-relatives N=68 and Male Blood-Relatives N=25).

5.3.1.2 Probands and Blood-Related Females

Table 19 presents the average number of menstrual periods probands and blood-related women had in their 20's by PCOS status. For the purpose of this analysis, 21 blood-related women were < 20 years old and 4 other women were on birth control pills throughout their 20's; therefore, 25 women were not eligible for this analysis. As expected, only 3(~21%) of the PCOS women had regular periods in their 20's, 7 (50%) had ≤ 8 periods a year, 3 (21%) had 9-11 periods a year and the remaining 1(~7%) had 13 or more periods a year. In contrast, 25(~86%) of the non-PCOS women had regular periods in their 20's, only 2(~7%) had ≤ 8 periods a year, 1(~3%) had 9-11 periods a year and the remaining 1(~3%) had 13 or more periods a year.

Table 19: Average Periods per Year Probands and Female Relatives had in their 20's by PCOS Status (N=47) *

Average Periods Per Year	PCOS (N=17)*		Non-PCOS (N=30)*	
	≥20 yrs			
	No.	%	No.	%
0-5	5	35.7	0	0
6-8	2	14.3	2	6.9
9-11	3	21.4	1	3.4
12	3	21.4	25	86.2
13+	1	7.1	1	3.4
TOTAL	**14**	**100**	**29**	**100**
Not Applicable**	3		1	
TOTAL	**17**	**100**	**30**	**100**

*21 Blood-related Females in our study population were < 20 years old and therefore not eligible for this analysis.

** 3 PCOS and 1 non-PCOS women were on OC throughout their 20's.

Tables 20, 21 and 22 show the demographic, anthropometric and cardiovascular characteristics, at time of interview, of the 9 PCOS probands and their female relatives, male relatives and probands and their male and female relatives respectively. Female relatives include mothers, sisters, daughters, 1st, 2nd or 3rd cousins; aunts; grandmothers; granddaughters or nieces; male relatives include fathers, brothers, sons, 1st, 2nd or 3rd cousins, uncles or nephews.

The mean age of the 9 PCOS **probands** (all > 18 years) was 44.00 years (± 8.63) with a mean BMI of 35.67 kg/m^2 (± 11.60)[after excluding one outlier 32.77 kg/m^2 (± 8.20)], which complies with the “obese” definition (≥30 kg/m^2) of the CDC(718). The mean waist-to-hip ratio (WHR) was calculated to be 0.85(± 0.09). According to the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK)(719), women with waist-to-hip ratios (WHR) greater than 0.8 are generally considered to have "excess fat" and be at higher risk for obesity-linked diseases, including arthritis, heart disease, hypertension and diabetes. This WHR cutoff point indicates that our Probands are at increased risk for such diseases. The mean waist was 102.53 cm (± 23.31). According to the Adult Treatment Panel III (ATP III) diagnostic criteria for metabolic syndrome(701), women with waists of >88 cm are considered to be centrally obese. Our findings strongly support the heightened risk of our probands for metabolic syndrome and cardiovascular disease. The mean systolic blood pressure (SBP)/ mean diastolic blood pressure (DBP) was 120.23 (± 7.40) / 77.78 mm (± 5.04). According to the AHA recommended blood pressure levels (720), individuals with a SBP range of 120-139 mm or DBP range of 80-89 mm are considered pre-hypertensive. These BP cutoff levels suggest that our probands were, on average, pre-hypertensive. The lipid profile for our probands was as follows. The mean total serum cholesterol (TC) was 232.44 mg/dl (± 31.77), mean total HDL cholesterol (HDL) was 51.19 mg/dl (±17.94); excluding one outlier for HDL, the mean HDL became 45.5 mg/dl (± 5.9), mean LDL cholesterol (LDL) was 155.11 mg/dl (±39.70) and mean triglycerides (TG) were 130.44 mg/dl (±48.12). According to the ATP III high blood cholesterol criteria (721), total cholesterol and LDL cholesterol values of 200-239 mg/dl and 130-159 mg/dl respectively are considered borderline high. Furthermore, total HDL cholesterol values of < 50 mg/dl in women are considered low and triglycerides values of ≥ 150 mg/dl are considered to be high. The total and LDL cholesterol values for our probands appear to fall close to the top bound of “borderline high” category and, therefore, close to the lower bound of the “high” category. The mean HOMA-IR was 8.12 (± 10.44) (after excluding the two T2 diabetics, 4.54 ± 2.36). According to

our criteria for identifying insulin resistant adults (HOMA-IR >3.9), our probands were, on average, insulin resistant. The majority of our probands were white, 8 (89%) and the remaining 1 (11%) was African Americans **(Table 20).** In summary, our PCOS probands were on average obese, at heightened risk of obesity-linked diseases (MS and CVD, T2DM, hypertension-HTN), pre-hypertensive, had abnormal lipid profile with borderline high TC and LDL levels and insulin resistant.

Among the 5 **mothers** of the PCOS probands, 1(20%) had a clinical diagnosis of PCOS. The values for the different demographic, anthropometric and cardiovascular characteristics for PCOS vs. non-PCOS mothers were as follows. The age was 73.00 years (± 0.00) vs.66.75 years (± 10.97), BMI was 31.23 kg/m^2 (± 0.00) vs. 34.37 kg/m^2 (± 9.73), WHR was 0.80(± 0.00) vs. 0.86(± 0.02), waist was 92.75 cm (± 0.00) vs. 96.08 cm (± 14.60), SBP/ mean DBP was 130.00 (± 0.00) / 80.00 mm (± 0.00) vs. 130.00 (± 10.07) / 76.25 mm (± 7.50); TC was 242.00 mg/dl (± 0.00) vs. 186.00 mg/dl (± 42.41), total HDL cholesterol was 56.40 mg/dl (±0.00) vs. 48.58 mg/dl (±4.81), LDL cholesterol was 130.00 mg/dl (±0.00) vs. 116.50 mg/dl (±41.06), TG were 280.00 mg/dl (±0.00) vs. 105.75 mg/dl (±25.16) and HOMA-IR of the PCOS vs. non-PCOS mothers was 4.97 (± 0.00) vs. 5.46 (± 3.61) (after excluding the two non-PCOS T2 diabetic mothers, 3.83±0.02) respectively. The results indicate that the mothers of our PCOS probands were obese, at increased risk for obesity-linked diseases (MS, CVD, T2DM, HTN), pre-hypertensive and insulin resistant. This is according to the CDC definition(718),the ATP III diagnostic criteria for metabolic syndrome(701), the AHA recommended blood pressure levels(720) and our criteria for identifying insulin resistant adults (HOMA-IR >3.9). Moreover, the PCOS mother had an abnormal lipid profile with high TC and TG levels and borderline high LDL levels and the non-PCOS mothers had low HDL levels, according to the ATP III high blood cholesterol criteria(721).The majority of the mothers of our PCOS probands were white, 4 (80%)**(Table 20).**

Among the twelve **sisters** of the PCOS probands, 2 (~17%) had PCOS. The means for the different demographic, anthropometric and cardiovascular characteristics for PCOS vs. non-PCOS sisters were as follows. The mean age was 47.50 years (± 9.19) vs. 41.40 years (± 10.48), mean BMI was 25.40 kg/m^2 (± 4.39) vs. 27.80 kg/m^2 (± 5.00), mean WHR was calculated to be 0.84(± 0.07) vs. 0.83(± 0.09), mean waist was 86.00 cm (± 21.21) vs. 84.31 cm (± 13.38), mean SBP/ DBP of 141.50mm (± 33.23) / 89.50 mm (± 27.58) (after excluding 1 outlier ,118.00mm ± 0.00/ 70mm ± 0.00) vs. 115.50mm (± 6.59) / 75.50 mm (± 6.92), mean TC was 233.00 mg/dl

(±50.91) vs. 234.20 mg/dl (±43.24), mean HDL cholesterol was 66.05 mg/dl (±5.30) vs. 53.90 mg/dl (±14.71), mean LDL cholesterol was 138.00 mg/dl (±55.15) vs. 148.10 mg/dl (±41.55) and the mean TG were 145.00 mg/dl (±2.83) vs. 163.20 mg/dl (±155.97)(excluding an outlier in the non-PCOS sisters group,123.78 mg/dl ± 99.41) and the mean HOMA-IR of the PCOS vs. non-PCOS sisters was 2.20(± 0.45) vs. 4.06 (± 2.73) (after excluding the two non-PCOS T2 diabetic sisters, 2.94±0.98) respectively. The results indicate that the sisters of our PCOS probands were overweight, at increased risk for obesity-linked diseases (including (MS, CVD, T2DM, and HTN)), had borderline high total and LDL cholesterol levels. This is according to the CDC definition (718), the NIDDK criteria(719) and the ATP III high blood cholesterol criteria (721) respectively. Moreover, on average the PCOS sisters of our probands were hypertensive, according to the AHA recommended blood pressure levels(720) and the non-PCOS sisters were hypertriglyceridemic and insulin resistant, according to the ATP III high blood cholesterol criteria(721) and our criteria for identifying insulin resistant adults (HOMA-IR >3.9) respectively. It is noteworthy to mention that the finding of IR in non-PCOS sisters may also apply to PCOS sisters; however, we did not have enough PCOS sisters to validate this phenotype. The majority of the sisters of our probands were white, 11 (92%) **(Table 20).**

Among the 9 **daughters** of our probands, 5 (~56%) had PCOS; 4(44.4%) ≤ 17 years and 8(89%) were white. The values for the different demographic, anthropometric and cardiovascular characteristics for PCOS (n=3; 2=17yrs, 1=16yrs) vs. non-PCOS (n=1; 14yrs) adolescent daughters were as follows. The mean age was 16.67 years (± 0.58) vs. 14.00 years (± 0.00, mean BMI was 29.86 kg/m^2 (± 5.56) vs. 21.20 kg/m^2 (± 0.00), mean WHR of 0.8 (± 0.1) vs. 0.8 (±0.0), mean waist of 92.75 cm (± 6.38) vs. 77.50cm (± 0.00), mean SBP/ DBP of 113.33 mm (± 5.03) / 67.67 mm (± 12.50) vs. 107.00 mm (± 0.00) / 71.00 mm (± 0.00), mean total serum cholesterol was 188.33 mg/dl (±40.77) vs. 163.00 mg/dl (±0.00), mean total HDL cholesterol was 50.90 mg/dl (±7.81) vs. 58.60 mg/dl (±0.00), mean LDL cholesterol was 118.33 mg/dl (±44.11) vs. 90 mg/dl (±0.00) and the mean triglycerides were 95.00 mg/dl (±66.01) vs. 71.00 mg/dl (±0.00), and the mean HOMA-IR of the PCOS vs. non-PCOS adolescent daughters was 4.38(± 2.91 for total PCOS adolescent daughters) (1.76,16yrs; 5.69,17yrs) [after excluding the T2 diabetic PCOS adolescent daughters, 2.82±1.49 for total PCOS adolescents; 1.76,16yrs;3.87,17 yrs] vs. 4.11 (± 0.00) respectively. The results demonstrate that the PCOS adolescent daughters were overweight, had borderline high TC, LDL cholesterol and TG levels,

according to the standard CDC chart of girls' BMI (717), and the 1991 NCEP recommendations on Blood Cholesterol in Children and Adolescents respectively (722). Moreover, the adolescent daughters of the PCOS probands were, on average, insulin resistant, according to our criteria for identifying insulin resistant adolescents (age-sex dependent; 14 years girls HOMA-IR >3.61; 16years >3.10; 17 years,>2.85) **(Table 20)**. It is worth noting that one (33.3%) PCOS daughter had a T2DM diagnosis.

Among the 9 daughters of the probands, 5(56.0%) ≥18 years (2 had PCOS). The means for the various demographic, anthropometric and cardiovascular characteristics for PCOS (n=2) vs. non-PCOS (n=3) adult daughters. The mean age was 28.00 years (± 8.49) vs. 24.67 years (± 9.87), mean BMI was 40.07 kg/m^2 (± 0.41) vs. 22.29 kg/m^2 (± 5.11), mean WHR was 0.83(± 0.03) vs. 0.77(± 0.07), mean waist was 110.75 cm (± 1.77) vs. 72.67 cm (± 12.50), mean systolic blood pressure (SBP)/ mean diastolic blood pressure (DBP) was 120.50 mm (± 9.19) / 81.00 mm (± 12.73) vs. 107.00 mm (± 6.56) / 71.67 mm (± 10.26), mean total serum cholesterol was 223.00 mg/dl (±22.63) vs. 209.33 mg/dl (±14.05), mean total HDL cholesterol was 48.65 mg/dl (±4.03) vs. 70.17 mg/dl (±14.21), mean LDL cholesterol was 136.50 mg/dl (±21.92) vs. 120.0 mg/dl (±14.1) and the mean triglycerides were 191.00 mg/dl (±22.63) vs. 95.67 mg/dl (±41.55), and the mean HOMA-IR of the PCOS vs. non-PCOS adult daughters was 10.00±3.34 (after excluding an outlier for fasting insulin level ,7.63±0.00) vs. 3.04±0.36, respectively. The results indicate that the adult PCOS daughters were obese, at increased risk for obesity-linked diseases (such as MS, CVD, T2DM and HTN), pre-hypertensive, had an abnormal lipid profile(borderline high total and LDL cholesterol, low HDL and high TG levels) and were insulin resistant, according to the CDC definition(718), the NIDDK criteria(719) and the Adult the ATP III diagnostic criteria for metabolic syndrome(701), the AHA recommended blood pressure levels(720), the ATP III high blood cholesterol criteria(721) and according to our criteria for identifying insulin resistant adults (HOMA-IR >3.9) respectively. Moreover, the non-PCOS adult daughters had borderline high TC levels, according to the ATP III high blood cholesterol criteria (721) **(Table 20).**

Among the 33 **other female blood-relatives** of probands, defined as 1st, 2nd or 3rd cousins; aunts; grandmothers; granddaughters or nieces, 3 (~9%) had PCOS and were ≥ 18 years old; 12(36.4%) ≤ 17 years and 24(73%) were white.The 12 adolescent non-PCOS female relatives had a mean age of 15.40 years (± 1.34), BMI of 24.41 kg/m^2 (± 4.77), WHR of 0.81(±

0.09, waist of 78.67 cm (± 14.25), SBP/ DBP of 109.33 mm (± 7.04) / 69.42 mm (± 5.55), mean total serum cholesterol was 152.08 mg/dl (±22.96), mean total HDL cholesterol was 42.25 mg/dl (±8.01), mean LDL cholesterol was 94.25 mg/dl (±15.73) and the mean triglycerides were 77.25 mg/dl (±27.49) and mean HOMA-IR for these adolescent non-PCOS female relatives was 3.83±1.01: 14yrs, 3.32±1.09 (n=4); 15yrs, 3.25±0.72 (n=3); 16yrs, 5.09±0.00 (n=1); 17yrs, 4.15± 1.08(n=4). The results indicate that the adolescent non-PCOS other female relatives were on average "at risk of overweight", had borderline low mean HDL cholesterol levels and were insulin resistant, according to the standard CDC chart of girls' BMI(717), the 1991 NCEP recommendations on Blood Cholesterol in Children and Adolescents(722), and our age-sex specific criteria for identifying insulin resistant adolescent girls (HOMA-IR >3.61 for 14 yrs; >3.35 for 15 yrs; >3.10 for 16 yrs and >2.85 for 17yrs) respectively. It is worth noting, however, that 1 out of the 12 non-PCOS adolescents (~8%) falls into the pre-hypertensive category (90th-95th percentile for age, gender and height) and another non-PCOS adolescent female (8%) falls into the hypertensive category (≥120/80 mm Hg) , according to the NHLBI fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents(723) **(Table 20).**

Among the remaining 21 (64.0%) adult female relatives of probands, 3 had PCOS. The means for the various demographic, anthropometric and cardiovascular characteristics for PCOS (n=3) vs. non-PCOS (n=18) adult female relatives of our probands were as follows. The mean age was 26.00 years (± 3.61) vs. 42.11 years (± 19.12), mean BMI was 38.36 kg/m^2 (± 2.46) vs. 29.75 kg/m^2 (± 6.98), mean WHR was 0.85(± 0.06) vs. 0.86(± 0.07), mean waist was 103.85 cm (± 11.27) vs. 92.14 cm (± 15.43), mean SBP/DBP was 120.67 mm (± 12.58) / 79.00 mm (± 9.54) vs. 122.50 mm (± 17.68) [after excluding an outlier,119.8± 13.97) / 75.06 mm (± 9.69)], mean total serum cholesterol was 248.33 mg/dl (±18.58) vs. 195.17 mg/dl (±36.76), mean total HDL cholesterol was 46.93 mg/dl (±3.63) vs. 51.58 mg/dl (±11.35), mean LDL cholesterol was 160.33 mg/dl (±12.06) vs. 117.94 mg/dl (±37.76) and the mean triglycerides were 205.67 mg/dl (±63.61) vs. 128.39 mg/dl (±75.12), and the mean HOMA-IR of the PCOS vs. non-PCOS adult female relatives was 5.98±1.83 vs. 4.23±2.30 (after excluding the 2 non-PCOS T2Diabetics, 3.77±1.87), respectively. The results indicate that the adult other female blood relatives were obese in the PCOS subgroup and overweight in the non-PCOS subgroup, at increased risk for obesity-linked diseases (MS, CVD, T2DM and HTN), pre-hypertensive and insulin resistant,

according to the CDC definition(718), the NIDDK criteria(719) and the ATP III diagnostic criteria for metabolic syndrome(701), the AHA recommended blood pressure levels(720) and our criteria for identifying insulin resistant adults (HOMA-IR >3.9) respectively. Moreover, the adult PCOS other female blood-relatives had and abnormal lipid profile characterized by high total, LDL cholesterol, TG and low HDL levels, according to the ATP III high blood cholesterol criteria (721) **(Table 20).**

Of the **68 women in the study**, 9 were probands and 59 were blood-related females. Out of these 68 females, 16 (~24%) were 17 years or younger, 20 (~29%), including the 9 probands (all ≥ 18yrs) and 11 blood-related females (3 daughters ≤ 17- yrs and 8 female relatives ≥ 18yrs), had PCOS. The means for the various demographic, anthropometric and cardiovascular characteristics for PCOS (n=3; 2=17yrs, 1=16yrs) vs. non-PCOS (n=13; 5=14yrs, 3=15yrs; 1=16yrs and 4=17yrs) adolescent female relatives were as follows. The mean age was 16.67 years (± 0.58) vs. 15.29years (± 1.34), mean BMI was 29.86 kg/m^2 (± 5.56) vs. 24.16 kg/m^2 (± 4.65), mean WHR was 0.83(± 0.06) vs. 0.81(± 0.09), mean waist was 92.75 cm (± 6.38) vs. 78.58cm (± 13.65), mean SBP/ DBP was 113.33 mm (± 5.03) / 67.67 mm (± 12.50) vs. 109.15 mm (± 6.77) / 69.54 mm (± 5.33), mean total serum cholesterol was 188.33 mg/dl (±40.77) vs. 152.92 mg/dl (±22.19), mean total HDL cholesterol was 50.90 mg/dl (±7.81) vs. 43.51 mg/dl (±8.91), mean LDL cholesterol was 118.33 mg/dl (±44.11) vs. 93.92 mg/dl (±15.10) and the mean triglycerides were 95.00 mg/dl (±66.01) vs. 76.77 mg/dl (±26.37), respectively. The mean HOMA-IR of the PCOS vs. non-PCOS adolescent female relatives was 4.38(± 2.91) (after excluding the T2 diabetic PCOS adolescent, 2.82±1.49) vs. 3.85 (± 0.97). The mean HOMA-IR of the adolescent PCOS female relatives was distributed by age as follows: 16yrs, 1.76±0.00 (n=1); 17yrs, 5.69± 2.58(n=2) (after excluding the T2 diabetic 17-yr old PCOS girl, 3.87±0.00). The mean HOMA-IR of the adolescent non-PCOS female relatives was distributed by age as follows: 14yrs, 3.74±0.97 (n=4); 15yrs, 3.25±0.72 (n=3); 16yrs, 5.09±0.00 (n=1); 17yrs, 4.15± 1.08(n=4). The results show that the adolescent female relatives to our PCOS probands were overweight in the PCOS subgroup and “at risk of overweight” in the non-PCOS subgroup and were insulin resistant, according to the standard CDC chart of girls’ BMI (717) and our criteria for identifying insulin resistant girl adolescent (HOMA-IR >3.61 for 14 yrs ; >3.35 for 15 yrs; ;>3.10 for 16 yrs and >2.85 for 17yrs) respectively. It is worth noting, however, that 1 out of the 13 non-PCOS adolescents (~8%) was pre-hypertensive (90th-95th percentile for age, gender and

height) and another non-PCOS adolescent female(~8%) falls into the hypertensive category (≥120/80 mm Hg), according to the NHLBI fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (723). Moreover,on average, our adolescent PCOS blood-related females had "borderline high" TC, LDL cholesterol and TG levels whereas the non-PCOS blood-related females had low HDL levels, according to the 1991 NCEP recommendations on Blood Cholesterol in Children and Adolescents(722) **(Table20).**

Among the remaining 52 (~ 76.0%) adult blood-related females, including the 9 PCOS probands, 17 had PCOS. The means for the various demographic, anthropometric and cardiovascular characteristics for PCOS (n=17, 9 of whom were the probands) vs. non-PCOS (n=35) adult blood-related females were as follows. The mean age was 41.06 years (± 13.65) vs. 43.23 years (± 18.04), mean BMI was 35.19 kg/m^2 (± 9.34)(after excluding an outlier, 33.71 kg/m^2 ± 7.30) vs. 29.08 kg/m^2 (± 7.00), mean WHR was calculated to be 0.84(± 0.07) vs. 0.84(± 0.07), mean waist was 101.2 cm (± 19.05) vs. 88.68 cm (± 15.34), mean SBP/DBP were 123.47 mm (± 13.15) [after excluding an outlier, 120.88 mm ± 7.89] / 79.88 mm (± 9.8)[after excluding an outlier,78.06 mm ± 6.52] vs. 120.03 mm (± 14.67)[after excluding an outlier, 118.62 ± 12.25] / 75.03 mm (± 8.48), mean total serum cholesterol was 234.76 mg/dl (±28.29) vs. 206.49 mg/dl (±41.04), mean total HDL cholesterol was 52.19 mg/dl (±14.06)[after excluding an outlier, 49.41 mg/dl ±8.40] vs. 53.49 mg/dl (±12.84), mean LDL cholesterol was 150.35 mg/dl (±33.52) vs. 126.57 mg/dl (±39.01) and the mean triglycerides were 161.35 mg/dl (±60.21) vs. 132.94 mg/dl (±99.54)[after excluding an outlier,121.62±74.22], respectively. The mean HOMA-IR of the adult PCOS probands and and their PCOS blood-related females vs. the non-PCOS blood-related females was 7.08±7.8[after excluding 3 PCOS T2diabetics, 5.29±3.04] [4.75±2.35, after excluding 3 PCOS T2diabetics and 1 outlier for fasting insulin for a not-T2 diabetic PCOS woman] vs. 4.22±2.47 (after excluding 6 non-PCOS T2diabetics, 3.45±1.51).The results demonstrate that the adult female relatives were obese in the PCOS subgroup and overweight in the non-PCOS subgroup, at increased risk for obesity-linked diseases (including MS, T2DM, CVD and HTN), pre-hypertensive and insulin resistant, according to the CDC definition (718), the NIDDK criteria (719) and the ATP III diagnostic criteria for metabolic syndrome(701), the AHA recommended blood pressure levels(720) and our criteria for identifying insulin resistant adults (HOMA-IR >3.9) respectively. Moreover, the adult PCOS probands and their PCOS adult blood-related females had borderline high TC and LDL and high TG levels and the adult non-

PCOS blood-related females of our PCOS probands had borderline high mean TC levels, according to the ATP III high blood cholesterol criteria (721). It is also worth mentioning that the mean HOMA-IR for the PCOS probands and their PCOS adult blood-related females was much higher than that of the adult non-PCOS blood-related females **(Table 20).**

Table 20: Distribution of Salient Demographic and Cardiovascular Characteristics by Proband Family Relation Status (Females Only)(N=68)

Characteristic	Proband Family Female Relation Status																	
	Probands(N=9)*		Mothers(N=5)*				Sisters(N=12)*				Daughters(N=9)*							
	All PCOS		PCOS(n=1)		Non PCOS(n=4)		PCOS(n=2)		Non PCOS (n=10)		PCOS(n=5)				Non PCOS(n=4)			
	≥ 18 yrs		≥ 18 yrs				≥ 18 yrs				≤ 17 yrs (n=3)		≥ 18 yrs(n=2)		≤17yrs(n=1)		≥ 18 yrs(n=3)	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Age	44.0	8.6	73.0	_	66.8	11.0	47.5	9.2	41.4	10.5	16.7	0.6	28.0	8.5	14.0	_	24.7	9.87
BMI(Kg/m^2)	35.7	11.6	31.2	_	34.4	9.7	25.4	4.4	27.8	5.0	29.9	5.6	40.1	0.4	21.2	_	22.3	5.11
*BMI(Kg/m^2)***	*32.77(n=8)*	*8.2*		_												_		
WHR	0.9	0.1	0.8	_	0.9	0.0	0.8	0.1	0.8	0.1	0.8	0.1	0.8	0.0	0.8	_	0.8	0.07
Waist(cm)	102.5	23.3	92.8	_	96.1	14.6	86.0	21.2	84.3	13.4	92.8	6.4	110.8	1.8	77.5	_	72.7	12.50
SBP(mm Hg)	120.2	7.4	130.0	_	130.0	10.1	141.5	33.2	115.5	6.6	113.3	5.0	120.5	9.2	107.0	_	107.0	6.56
*SBP(mm Hg)***				_			*118(n=1)*									_		
DBP(mm Hg)	77.8	5.0	80.0	_	76.3	7.5	89.5	27.6	75.5	6.9	67.7	12.5	81.0	12.7	71.0	_	71.7	10.26
*DBP(mm Hg)***				_			*70(n=1)*	*0.0*	*75.5*	*6.9*						_		
TC(mg/dl)	232.4	31.8	242.0	_	186.0	42.4	233.0	50.9	234.2	43.2	188.3	40.8	223.0	22.6	163.0	_	209.3	14.05
HDLT(mg/dl)	51.2	17.9	56.4	_	48.6	4.8	66.1	5.3	53.9	14.7	50.9	7.8	48.7	4.0	58.6	_	70.2	14.21
*HDLT(mg/dl)***	*45.5(n=8)*	*5.9*		_												_		
LDL(mg/dl)	155.1	39.7	130.0	_	116.5	41.1	138.0	55.2	148.1	41.6	118.3	44.1	136.5	21.9	90.0	_	120.0	14.1
TG (mg/dl)	130.4	48.1	280.0	_	105.8	25.2	145.0	2.8	163.2	156.0	95.0	66.0	191.0	22.6	71.0	_	95.7	41.55
*TG(mg/dl)***				_					*123.78(n=9)*	*99.4*						_		
HOMA-IR***	8.1	10.4	5.0	_	5.5	3.6	2.2	0.5	4.1	2.7	4.4	2.9	10.0	3.3	4.1	_	3.0	0.36
14 yrs				_											4.1	_		
15 yrs				_												_		
16 yrs				_							1.76(n=1)	0.0				_		
17yrs				_							5.69(n=2)	2.6				_		
≥ 18 yrs	8.1	10.4	5.0	_	5.5	3.6	2.2	0.5	4.1	2.7			10.0	3.3		_	3.04(n=3)	0.36

Table 20 **(Cont'd)**

Characteristic	Proband Family Female Relation Status																	
	Probands(N=9)*		Mothers(N=5)*				Sisters(N=12)*				Daughters(N=9)*							
	All PCOS		PCOS(n=1)		Non PCOS(n=4)		PCOS(n=2)		Non PCOS(n=10)		PCOS(n=5)				Non PCOS(n=4)			
	≥ 18 yrs		≥ 18 yrs				≥ 18 yrs				≤ 17 yrs (n=3)		≥ 18 yrs(n=2)		≤17yrs(n=1)		≥ 18 yrs(n=3)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.								
HOMA-IR****	**4.54(n=7)**	**2.4**	**N.A.**	_	**3.83(n=2)**	**0.0**	____	____	**2.94(n=8)**	**1.0**	**2.82(n=2)**	**1.5**	____	____	____	_	____	____
14 yrs	____	____	____	_	____	____	____	____	____	____	____	____	____	____	____	_	____	____
16 yrs	____	____	____	_	____	____	____	____	____	____	1.76(n=1)	0.0	____	____	____	_	____	____
17yrs	____	____	____	_	____	____	____	____	____	____	3.87(n=1)	0.0	____	____	____	_	____	____
≥ 18 yrs	**4.54(n=7)**	**2.4**	**N.A.**	_	**3.83(n=2)**	**0.0**	____	____	**2.94(n=8)**	**1.0**	____	____	____	____	____	_	____	____
Race	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Caucasian	8	89	1	100	3	75	2	100	9	90	2	67	2	100	1	100	3	100
African American	1	11	0	0	1	25	0	0	1	10	1	33	0	0	0	0	0	0
TOTAL	**9**	**100**	**1**	**100**	**4**	**100**	**2**	**100**	**10**	**100**	**3**	**100**	**2**	**100**	**1**	**100**	**3**	**100**

Table 20 **(Cont'd)**

Characteristic	Proband Family Female Relation Status													
	Other Female Blood-Relatives(N=33)*						Probands and TOTAL Female Relatives (N=68)*							
	PCOS(n=3)		Non PCOS(n=30)				PCOS(n=20)				Non PCOS(n=48)			
	≥ 18 yrs		≤ 17 yrs (n=12)		≥ 18 yrs(n=18)		≤ 17 yrs (n=3)		≥ 18 yrs(n=17)		≤ 17 yrs (n=13)		≥ 18 yrs(n=35)	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Age	26.0	3.6	15.4	1.3	42.1	19.1	16.7	0.6	41.1	13.7	15.3	1.3	43.2	18.0
BMI(Kg/m^2)	38.4	2.5	24.4	4.8	29.7	7.0	29.9	5.6	35.2	9.3	24.2	4.6	29.1	7.0
*BMI(Kg/m^2)***									*33.71(N=16)*	*7.3*				
WHR	0.8	0.1	0.8	0.1	0.9	0.1	0.8	0.1	0.8	0.1	0.8	0.1	0.8	0.1
Waist(cm)	103.9	11.3	78.7	14.2	92.1	15.4	92.8	6.4	101.2	19.1	78.6	13.6	88.7	15.3
SBP(mm Hg)	120.7	12.6	109.3	7.0	122.5	17.7	113.3	5.0	123.5	13.2	109.2	6.8	120.0	14.7
*SBP(mm Hg)***					*119.8(n=17)*	*14.0*			*120.88(N=16)*	*7.9*			*118.62(n=34)*	*12.2*
DBP(mm Hg)	79.0	9.5	69.4	5.6	75.1	9.7	67.7	12.5	79.9	9.8	69.5	5.3	75.0	8.5
*DBP(mm Hg)***									*78.06(N=16)*	*6.5*				
TC(mg/dl)	248.3	18.6	152.1	23.0	195.2	36.8	188.3	40.8	234.8	28.3	152.9	22.2	206.5	41.0
HDLT(mg/dl)	46.9	3.6	42.3	8.0	51.6	11.3	50.9	7.8	52.2	14.1	43.5	8.9	53.5	12.8
*HDLT(mg/dl)***									*49.41(N=16)*	*8.4*				
LDL(mg/dl)	160.3	12.1	94.3	15.7	117.9	37.8	118.3	44.1	150.4	33.5	93.9	15.1	126.6	39.0
TG (mg/dl)	205.7	63.6	77.3	27.5	128.4	75.1	95.0	66.0	161.4	60.2	76.8	26.4	132.9	99.5
*TG(mg/dl)***													*121.62(n=34)*	*74.7*
HOMA-IR***	6.0	1.8	3.8	1.0	4.2	2.3	4.38(n=3)	2.9	7.1	7.8	3.9	1.0	4.2	2.5
14 yrs			3.32(n=4)	1.1							3.74(n=5)	1.0		
15 yrs			3.25(n=3)	0.7							3.25(n=3)	0.7		
16 yrs			5.09(n=1)	0.0			1.76(n=1)				5.09(n=1)	0.0		
17yrs			4.15(n=4)	1.1			5.69(n=2)	2.6			4.15(n=4)	1.1		
≥ 18 yrs	6.0	1.8			4.2	2.3			7.1	7.8			4.2	2.5

Table 20**(Cont'd)**

Characteristic	**Proband Family Female Relation Status**															
	Other Female Blood-Relatives(N=33)*						**Probands and TOTAL Female Relatives (N=68)***									
	PCOS(n=3)		**Non PCOS(n=30)**				**PCOS(n=20)**				**Non PCOS(n=48)**					
	≥ 18 yrs		**≤ 17 yrs (n=12)**		**≥ 18 yrs(n=18)**		**≤ 17 yrs (n=3)**		**≥ 18 yrs(n=17)**		**≤ 17 yrs (n=13)**		**≥ 18 yrs(n=35)**			
HOMA-IR******	_____	_____	_____	_____	**3.73(n=16)**	**1.9**	**2.82(n=2)**	**1.5**	**5.29(n=14)**	**3.0**	_____	_____	**3.45(n=29)**	**1.5**		
14 yrs	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____				
16 yrs	_____	_____	_____	_____	_____	_____	1.8	_____	_____	_____	_____	_____				
17yrs	_____	_____	_____	_____	_____	_____	3.87(n=1)	_____	_____	_____	_____	_____				
≥ 18 yrs	_____	_____	_____	_____	**3.73(n=16)**	**1.9**	_____	_____	**5.29(n=14)**	**3.0**	_____	_____	**3.45(n=29)**	**1.5**		
Race	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Caucasian	3	100	9	75	12	67	2	66.7	16	94.1	10	77	27	77.1		
African American	0	0	3	25	6	33	1	33.3	1	5.9	3	23	8	22.9		
TOTAL	**3**	**100**	**12**	**100**	**18**	**100**	**3**	**100**	**17**	**100**	**13**	**100**	**35**	**100**		

*Unless otherwise specified in table, For Probands N =9; Mothers N=5; Sisters N= 12; Daughters N=9; other female-Blood Relatives N=33; **other female Blood-Relatives** include 1st, 2nd

or 3rd cousin, niece, aunt, grandma and granddaughter. **Probands and Total Female Blood-Relatives** N=68 (excluding the 4 Not-Blood related females).

***Italicized* Charcateristics imply means calculated after excluding outliers.

*** **IR** defined by HOMA_IR (>3.9 for 18yrs+;>2.85 for 17yrs;>3.10 for 16 yrs; >3.35 for 15 yrs; >3.61 for 14 yrs

******Bolded** characteristics imply means calculated after excluding T2 diabetics.

_____ means N.A brcause no outliers or T2 Diabetics found.

N.A. means cannot be calculated because there were so subjects left.

5.3.1.3 Blood-Related Males

Table 21 shows that we had a total of 25 male blood-relatives to probands. Out of the total 25 participating males, 2(80%) were white and all 29(100%) were adults.

The 6 **fathers** of the PCOS probands had a mean age of 76.83 years (± 11.99), BMI of 29.15 kg/m^2 (± 5.73), WHR of 0.97(± 0.08), waist of 104.75 cm (± 12.2) and a mean SBP/ DBP of 127.5 (± 20.87) / 71.33 mm (± 18.52) [after excluding one outlier,78.20±8.67]. The lipid profile for the fathers of our PCOS probands was as follows: the mean total serum cholesterol was 201.33 mg/dl (± 64.04), mean total HDL cholesterol was 39.55mg/dl (±11.84), mean LDL cholesterol was 125.67 mg/dl (±56.02) and the mean triglycerides were 188.33 mg/dl (±139.24)[after excluding one outlier,135mg/dl ±53.87]. Their mean HOMA-IR was 6.84± 7.45[after excluding the 3 T2 diabetics, 3.74± 1.93]. The results indicate that the fathers of our PCOS probands were overweight, at heightened risk for metabolic syndrome and cardiovascular disease, pre-hypertensive, dyslipidemic, characterized by borderline high TC, high TG and low HDL levels and insulin resistant. This is according to the CDC definition (25.0-29.9kg/m^2)(718), the ATP III diagnostic criteria for metabolic syndrome (waist >102cm) (701) and the AHA recommended blood pressure levels720, the ATP III high blood cholesterol criteria(721), and our criteria for identifying insulin resistant adults (HOMA-IR >3.9), respectively**(Table 21)**.

The 8 **brothers** of our PCOS probands had a mean age of 40.88 years (± 11.51), BMI of 28.04 kg/m^2 (± 4.90), WHR of 0.93(± 0.04), waist of 97.65 cm (± 14.46) and a mean SBP/ DBP of 123.25 (± 16.42) / 80.56 mm (± 9.83). Their lipid profile was as follows: mean total serum cholesterol was 198.63 mg/dl (± 42.10), HDL cholesterol was 49.61mg/dl (±14.55), LDL cholesterol was125.50 mg/dl (±32.17) and triglycerides were 118.13 mg/dl (±94.04). The mean HOMA-IR for the brothers of our PCOS probands was 3.64± 2.08 [after excluding the T2 diabetic,3.06± 1.38]. The results show that that the brothers of our PCOS probands were overweight, pre-hypertensive and had borderline high TC levels. This is according to the CDC definition (25.0-29.9kg/m^2)(718), the AHA recommended blood pressure levels(720); and the ATP III high blood cholesterol criteria(721) respectively **(Table 21).**

The only participating **son** of our PCOS probands was 18 years old, had a BMI of 19.15 kg/m^2 , a WHR of 0.84, a waist of 81.50 cm and a mean SBP/ DBP of 119mm / 75 mm. His lipid profile was as follows: TC, 166 mg/dl; HDL cholesterol, 50.3 mg/dl; LDL cholesterol, 99 mg/dl and TG were 85 mg/dl. The HOMA-IR value of this son was 3.82 **(Table 21).**

The 10 **other blood-related males** to our PCOS probands, defined as 1st, 2nd or 3rd cousin, or uncle, had a mean age of 35.40 years (± 14.79), BMI of 31.28 kg/m^2 (± 8.92)[after excluding an outlier, 28.80 kg/m^2 ±4.48], WHR of 0.94(± 0.05), waist of 103.37 cm (± 20.44)[after excluding an outlier, 98.24 cm± 13.20] and a mean SBP/ DBP of 125.30 (± 13.66) / 78.40 mm (± 7.99). The lipid profile of the blood-related males was as follows: The mean total serum cholesterol was 195.60 mg/dl (± 56.06), mean total HDL cholesterol was 48.18 mg/dl (±13.01), mean LDL cholesterol was 121.90 mg/dl (±51.54) and the mean triglycerides were 127.90 mg/dl (±93.37). Their mean HOMA-IR was 3.84± 2.12 [after excluding the T2 diabetic, 3.97± 2.21]. The results demonstrate that the other blood-related males to our PCOS probands were overweight, at heightened risk for metabolic syndrome and cardiovascular disease and pre-hypertensive. This is according to the CDC definition(718), the ATP III diagnostic criteria for metabolic syndrome(701), and the AHA recommended blood pressure levels(720) **(Table 21).**

Table 21: Distribution of Salient Demographic and Cardiovascular Characteristics by Proband Family Relation Status (Males Only) (N=25)

Characteristic	Proband Family Male Relation Status									
	Fathers(N=6)*		Brothers(N=8)*		Sons(N=1)*		Other Male Blood-Relatives (N=10)*		TOTAL Male Blood-Relatives (N=25)*	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Age	76.83	11.99	40.88	11.51	18.00	0.00	35.4	14.79	46.40	21.83
BMI(Kg/m^2)	29.15	5.73	28.04	4.90	19.15	0.00	31.28	8.92	29.25	7.08
*BMI(Kg/m^2)***	____	____	____	____	____	____	*28.80(n=9)*	*4.48*	*28.23(n=24)*	*5.04*
WHR	0.97(n=4)	0.078	0.93	0.04	0.84	0.00	0.94	0.05	0.94(n=23)	0.05
Waist(cm)	104.75(n=4)	12.2	97.65	14.46	81.50	0.00	103.4	20.44	100.67(n=23)	16.85
*Waist(cm)***	____	____	____	____	____	____	*98.24(n=9)*	*13.20*	*98.45(n=22)*	*13.38*
SBP(mm Hg)	127.5	20.87	123.25	16.42	119.00	0.00	125.3	13.66	124.92	15.60
DBP(mm Hg)	71.33	18.52	80.56	9.83	75.00	0.00	78.4	7.99	77.26	11.69
*DBP(mm Hg)***	*78.20*	*8.67*	____	____	____	____	____	____	*78.93(n=24)*	*8.32*
TC(mg/dl)	201.33	64.04	198.63	42.10	166.00	0.00	195.6	56.06	196.76	50.95
HDLT(mg/dl)	39.55	11.84	49.61	14.55	50.30	0.00	48.18	13.01	46.65	13.09
LDL(mg/dl)	125.67	56.02	125.5	32.17	99.00	0.00	121.9	51.54	123.04	44.50
TG(mg/dl)	188.33	139.24	118.13	94.09	85.00	0.00	127.9	93.37	137.56	103.99
*TG(mg/dl)***	*135(n=5)*	*53.87*	____	____	____	____	____	____	*124.33(n=24)*	*81.98*
HOMA-IR***	6.84	7.45	3.64	2.08	3.82	0.00	3.84	2.12	4.50	4.04
HOMA-IR******	**3.74(n=3)**	**1.93**	**3.06(n=7)**	**1.38**	____	____	**3.97(n=9)**	**2.21**	**3.61(n=20)**	**1.79**
Race	**No.**	**%**	**No.**	**%**	**No.**	%	**No.**	**%**	**No.**	**%**
Caucasian	**5**	**83**	**8**	**100**	**1**	100	**6**	**60**	**20**	**80**
African American	**1**	**17**	**0**	**0**	**0**	0	**4**	**40**	**5**	**20**
TOTAL	**6**	**100**	**8**	**100**	**1**	100	**10**	**100**	**25**	**100**

**Unless otherwise specified in table, For Fathers N=6; Brothers N= 8; Sons N=1; other Male Relatives N=10 and Total N=25 (excluding the 4 Not-Blood related males).

***Italicized* Charcateristics imply means calculated after excluding outliers.

*** **IR** defined by HOMA_IR(>3.9 for 18yrs+;>2.85 for 17yrs;>3.10 for 16 yrs; >3.35 for 15 yrs; >3.61 for 14 yrs).

******Bolded** characteristics imply means calculated after excluding T2 diabetics.

_____ means N.A brcause no outliers or T2 Diabetics found.

5.3.1.4 Conclusions

The results for the different demographic, anthropometric and cardiovascular characteristics for PCOS probands and their adult blood-related females, blood related males, total adult blood relatives as well as blood-related adolescents are summarized below.

The 52 **adult blood-related females**, including the 9 probands had a mean age of 42.52 years (± 16.63), BMI of 31.08 kg/m^2 (± 8.27) [after excluding an outlier 30.53 kg/m^2 ±7.35], WHR of 0.84(± 0.07), waist of 92.78 cm (± 17.49), SBP/ DBP of 121.15 (± 14.61)[after excluding an outlier,119.34±11.01] / 76.62 mm (± 9.13)[after excluding an outlier,75.98±7.98] and a mean HOMA-IR of 5.15± 5.00[after excluding the 9 T2 diabetics, 4.05± 2.27]. Moreover, the lipid profile for the probands and their adult blood-related females was as follows: the mean TC was 215.73 mg/dl (± 39.42), total HDL cholesterol was 53.07 mg/dl (±13.13)[after excluding one outlier, 52.21mg/dl±11.70), LDL cholesterol was 134.35 mg/dl (±38.66) and TG were 142.33 mg/dl (±89.01)[after excluding one outlier,134.86 mg/dl ±72.13]. The results demonstrate that the probands and their adult blood-related female were obese, at increased risk of obesity-linked diseases (including MS, CVD, T2DM and HTN), prehypertensive, insulin resistant, and had borderline high levels of TC and LDL cholesterol. This is according to the CDC definition of overweight and obesity in adults (718), the ATP III diagnostic criteria for metabolic syndrome (701), the AHA recommended blood pressure levels(720), our criteria for identifying insulin resistant adults (HOMA-IR >3.9) and the ATP III high blood cholesterol criteria(721), respectively. Moreover, 43 (83%) of the 52 adult blood-related females were white **(Table 22).**

The total 25 **blood-related males** within our PCOS families had a mean age of 46.40 years (± 21.83). They had a mean BMI of 29.25 kg/m^2 (± 7.08) [after excluding an outlier 28.23 kg/m^2 ±5.04], WHR of 0.94(± 0.05) , waist of 100.67 cm (± 16.85)[after excluding an outlier, 98.45 ±13.38], SBP/ DBP of 124.92 (± 15.60) / 77.26 mm (± 11.69)[after excluding an outlier 78.93±8.32] and a mean HOMA-IR of 4.50± 4.04[after excluding the 5 T2 diabetics, 3.61± 1.79]. The lipid profile for the blood-related males of our PCOS probands was as follows: the mean total serum cholesterol was 196.76 mg/dl (± 50.95), HDL cholesterol was 46.65 mg/dl (±13.09), LDL cholesterol was 123.04 mg/dl (±44.50) and TG were 137.56 mg/dl (±103.99)[after excluding one outlier, 124.33mg/dl ±81.98]. The results suggest that the blood-

related males of our PCOS probands were overweight, pre-hypertensive and insulin resistant. This is according to the CDC definition(718), the AHA recommended blood pressure levels(720) and our criteria for identifying insulin resistant adults (HOMA-IR >3.9) respectively. It is noteworthy to mention that the mean total cholesterol levels for the blood-related males of our probands appears to approach the definition of "borderline high" category, according to the ATP III high blood cholesterol criteria(721). Moreover, 20 (80%) of the 25 blood related males were white **(Table 22).**

The mean age for all **77 adult male and female blood relatives** was 43.78 years (± 18.42). They had a mean BMI of 30.48 kg/m^2 (± 7.90) [after excluding 2 outliers, 29.80 kg/m^2 ±6.75], WHR of 0.87(± 0.08), waist of 95.20 cm (± 17.57)[after excluding one outlier, 94.46 cm ± 16.50], SBP/ DBP of 122.38 mm (± 14.65)[after excluding 2 outliers, 121.20 mm±12.89] / 76.82 mm (± 9.96)[after excluding 2 outliers 76.93±8.15] and a mean HOMA-IR of 4.94± 4.69[after excluding the 14 adult T2 diabetics, 3.91± 2.12]. The mean total serum cholesterol was 209.57 mg/dl (±44.07), mean total HDL cholesterol was 50.99 mg/dl (±13.38)[after excluding one outlier, 50.38±12.37], mean LDL cholesterol was 130.68 mg/dl (±40.70) and the mean triglycerides were 140.71 mg/dl (±93.47)[after excluding two outliers 131.49 mg/dl ±75.02]. The results indicate that the participating adult blood-relatives were obese, pre-hypertensive, insulin resistant, and had borderline-high TC and LDL levels. This is according to the CDC definition of overweight and obesity in adults(718), the AHA recommended blood pressure levels(720), our criteria for identifying insulin resistant adults (HOMA-IR >3.9) and the ATP III high blood cholesterol criteria(721), respectively. Moreover, 63 (82%) of the 77 adult blood relatives to our PCOS probands were white **(Table 22).**

The mean age for all **16 adolescent blood relatives** to our PCOS probands (all were females) was 15.55 years (± 1.34). They had a mean BMI of 25.23 kg/m^2 (± 5.17), WHR of 0.81(± 0.08), waist of 81.24cm (± 13.68), SBP/ DBP of 109.94mm(± 6.55) / 69.19mm (± 6.65) and a mean HOMA-IR of 3.95± 1.39. The distribution of mean HOMA-IR by age was as follows: 14yrs, 3.74±0.97 (n=5); 15yrs, 3.25±0.72 (n=3); 16yrs, 3.42±2.35 (n=2); 17yrs, 4.66± 1.64(n=6). After excluding the T2 diabetic17-year old female, the mean HOMA-IR became 3.72±1.05and the mean HOMA-IR for the 17 year old adolescents became: 17yrs, 4.09± 0.95(n=5). Moreover, the lipid profile for the blood related adolescents was as follows. The mean

total serum cholesterol was 159.56 mg/dl (± 28.26), mean total HDL cholesterol was 44.89 mg/dl (±8.98), mean LDL cholesterol was 98.50 mg/dl (±23.21) and the mean triglycerides were 80.19 mg/dl (±34.52). The results show that the participating adolescent blood-relatives were on average at risk of overweight, insulin resistant and had an HDL level within the borderline low category. This is according to the standard CDC chart of girls' and boys' BMI(717), our criteria for identifying insulin resistant girl adolescent and the 1991 NCEP recommendations on Blood Cholesterol in Children and Adolescents(722) respectively. Moreover, 12 (75%) of the 16 adolescent blood relatives were white **(Table 22).**

Table 22: Distribution of Salient Demographic and Cardiovascular Characteristics among Probands & their Participating Blood-Relatives

Characteristic	Probands and Female Blood-Relatives (N=68)*				Male Blood-Relatives (N=25)*		Probands and Adult Blood-Relatives (N=77)*	
	≤ 17 yrs (n=16)		≥ 18 yrs(n=52)		≥ 18 yrs		≥ 18 yrs	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Age	15.55	1.34	42.52	16.63	46.40	21.83	43.78	18.42
BMI(Kg/m^2)	25.23	5.17	31.08	8.27	29.25	7.08	30.48	7.90
*BMI(Kg/m^2)***	____	____	*30.53(n=51)*	*7.35*	*28.23(n=24)*	*5.04*	*29.80(n=75)*	*6.75*
WHR	0.81	0.08	0.84	0.07	0.94(n=23)	0.05	0.87	0.08
Waist(cm)	81.24	13.68	92.78	17.49	100.67(n=23)	16.85	95.20(n=75)	17.57
*Waist(cm)***	____	____	____	____	*98.45(n=22)*	*13.38*	94.46(n=74)	16.50
SBP(mm Hg)	109.94	6.55	121.15	14.16	124.92	15.60	122.38	14.65
*SBP(mm Hg)***	____	____	*119.34(n=50)*	*11.01*	____	____	*121.20(n=75)*	*12.89*
DBP(mm Hg)	69.19	6.65	76.62	9.13	77.26	11.69	76.82	9.96
*DBP(mm Hg)***	____	____	*75.98(n=51)*	*7.98*	*78.93(n=24)*	*8.32*	*76.93(n=75)*	*8.15*
TC(mg/dl)	159.56	28.62	215.73	39.42	196.76	50.95	209.57	44.07
HDLT(mg/dl)	44.89	8.98	53.07	13.13	46.65	13.09	50.99	13.38
*HDLT(mg/dl)***	____	____	*52.21(n=51)*	*11.70*	____	____	*50.38(n=76)*	*12.37*
LDL(mg/dl)	98.5	23.21	134.35	38.66	123.04	44.50	130.68	40.70
TG (mg/dl)	80.19	34.52	142.23	89.01	137.56	103.99	140.71	93.47
*TG(mg/dl)***	____	____	*134.86(n=51)*	*72.13*	*124.33(n=24)*	*81.98*	*131.49(n=75)*	*75.02*
HOMA-IR***	3.95	1.39	5.15	5.00	4.50	4.04	4.94	4.69
14 yrs	3.74(n=5)	0.97	____	____	____	____	____	____
15 yrs	3.25(n=3)	0.72	____	____	____	____	____	____
16 yrs	3.42(n=2)	2.35	____	____	____	____	____	____
17yrs	4.66(n=6)	1.64	____	____	____	____	____	____
≥ 18 yrs	____	____	5.15	5.00	4.50	4.04	4.94	4.69

Table 22 **(Cont'd)**

Characteristic	**Probands and Female Blood-Relatives (N=68)***				**Male Blood-Relatives (N=25)***		**Probands and Adult Blood-Relatives (N=77)***	
	≤ 17 yrs (n=16)		**≥ 18 yrs(n=52)**		**≥ 18 yrs**		**≥ 18 yrs**	
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
HOMA-IR******	**3.72(n=15)**	**1.05**	**4.05(n=43)**	**2.27**	**3.61(n=20)**	**1.79**	**3.91(n=63)**	**2.12**
14 yrs	3.74(n=5)	0.97	_____	_____	_____	_____	_____	_____
15 yrs	3.25(n=3)	0.72	_____	_____	_____	_____	_____	_____
16 yrs	3.42(n=2)	2.35	_____	_____	_____	_____	_____	_____
17yrs	**4.09(n=5)**	**0.95**	_____	_____	_____	_____	_____	_____
≥ 18 yrs	_____	_____	**4.05(n=43)**	**2.27**	**3.61(n=20)**	**1.79**	**3.91(n=63)**	**2.12**
Race	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Caucasian	12	75	43	83	20	80	63	82
African American	4	25	9	17	5	20	14	18
TOTAL	**16**	**100**	**52**	**100**	**25**	**100**	**77**	**100**

*Unless otherwise specified in table, probands and female-Blood Relatives N=68 and total male Blood-Relatives N=25. **Probands and Total Female Blood-Relatives** N=68 (excluding the 4 Not-Blood related females); **Total Male Blood-Relatives** N=25 (excluding the 4 Not-Blood related males). **Female Blood-Relatives** include mothers, sisters, daughters, 1st, 2nd or 3rd cousin, niece, aunt, grandma and granddaughter. **Male Blood Relatives** include fathers, brothers, sons 1st, 2nd or 3rd cousin, nephew or uncle.

***Italicized* Charcateristics imply means calculated after excluding outliers.

*** **IR** defined by HOMA_IR (>3.9 for 18yrs+;>2.85 for 17yrs;>3.10 for 16 yrs; >3.35 for 15 yrs; >3.61 for 14 yrs).

******Bolded** characteristics imply means calculated after excluding T2 diabetics.

_____ means N.A brcause no outliers or T2 Diabetics found.

5.3.2 Hormonal and Inflammatory Profile

5.3.2.1 Probands and Blood-Related Females

Table 23 presents the distribution of hormonal and inflammatory markers' levels for pre-menopausal blood-related females (N=52), including 6 probands, by PCOS and adult status. Among these females, there were 14 (27%) PCOS cases; 3 were ≤ 17 yrs, and 38 (73%) non-PCOS females; 13 of whom were ≤ 17 yrs. We will present these findings for adolescents and adults by PCOS status separately. We will also present the findings in the total group of pre-menopausal females by PCOS status.

Among the 16 pre-menopausal adolescents, the mean age, BMI and WHR for PCOS females (n=3) vs. non-PCOS females (n=13) were 16.67 years (±0.58) vs. 15.29 years (±1.34), 29.86 Kg/m^2 (±5.56) vs. 24.16 Kg/m^2 (±4.65) and 0.83(±0.06) vs. 0.81(±0.09) respectively. The mean serum testosterone, C-reactive protein (CRP) and Interleukin-6 (IL6) levels were respectively as follows: 1.26 ng/ml(±0.44), 1.96 mg/L(±2.66) and 2.62 pg/ml(±2.71) for PCOS adolescents vs.1.39 ng/ml(±0.94), 0.32 mg/L(±0.44) and 1.73 pg/ml(±1.40) for non-PCOS adolescents. After excluding the PCOS adolescent who was currently using OC, the mean testosterone, CRP and IL6 levels in the PCOS vs. non-PCOS premenopausal adolescents became: 1.50 ng/ml (±0.23) vs. 1.39 ng/ml(±0.94) , 2.55 mg/L (±3.48)vs. 0.32 mg/L(±0.44) and 3.51 pg/ml (±3.15)vs.1.73 pg/ml(±1.40). The distribution of PCOS adolescents vs. non-PCOS adolescents by testosterone tertiles was as follows:1(33.3%) vs. 5(38.5%) in the lowest tertile(≤ 0.84 ng/ml), 1(33.3%)vs.4(30.8%) in the middle tertile(0.85-1.45 ng/ml) and 1(33.3%)vs.4(30.8%) in the highest tertile (1.46+ ng/ml). After excluding the PCOS adolescent who was on OC, this distribution in the PCOS adolescent group became: (0%) vs. 5(38.5%) in the lowest tertile (≤ 0.84 ng/ml), 1(50%) vs. 4(30.8%) in the middle tertile (0.85-1.45 ng/ml) and 1(50%) vs. 4 (30.8%) in the highest tertile (1.46+ ng/ml). The distribution of PCOS adolescents vs. non-PCOS adolescents by reported clinical cutoff points of testosterone(724) was as follows:1(33.3%) vs. 6(46.2%) in the normal cutoff point category (≤ 0.99 ng/ml), 2(66.7%)vs. 4(30.8%) in the middle category (1-1.67 ng/ml) and 0(0%)vs.3(23.1%) in the highest category

(1.68+ ng/ml). After excluding the PCOS adolescent who was on OC, this distribution in the PCOS vs. non-PCOS adolescents became: (0%) vs. 6 (46.2%) (≤ 0.99 ng/ml) category, 2(100%) vs. 4(30.8%)(1-1.67 ng/ml) category and 0(0%) vs. 3(23.1%) (1.68+ ng/ml)category. The distribution of PCOS vs. non-PCOS adolescents by CRP tertiles was as follows:1(33.3%)vs. 11(84.6%) in the lowest tertile(≤ 0.57 mg/L), 1(33.3%)vs. 2(15.4%) in the middle tertile(0.58-2.45 mg/L) and 1(33.3%)vs. 0(0%) in the highest tertile(2.46+ mg/L). After excluding the PCOS adolescent who was on OC, this distribution became: 1(50%) vs. 11(84.6%) (≤ 0.57 mg/L) tertile, 0(0%) vs. 2(15.4%) (0.58-2.45 mg/L) tertile and 1(50%) vs. 0(0%) (2.46+ mg/L) tertile. The distribution of PCOS adolescents vs. non-PCOS adolescents by reported clinical cutoff points of CRP (725) was as follows: 2(66.7%)vs.11(84.6%) in the normal cutoff point category (≤ 0.99 mg/L), 0(0%)vs. 2(15.4%) in the middle category (1-3 mg/L) and 1(33.3%)vs.0(0%) in the highest category (3.01+ mg/L). After excluding the PCOS adolescent who was on OC, this distribution became: 1(50%) vs.11(84.6%)(≤ 0.99 mg/L) category, 0(0%) vs. 2(15.4%)(1-3 mg/L) category and 1(50%) vs.0(0%)(3.01 mg/L) category. The distribution of PCOS adolescents vs. non-PCOS adolescents by IL6 tertiles was as follows:1(33.3%)vs.8(61.5%) in the lowest tertile(≤ 0.84 pg/ml), 1(33.3%)vs.1(7.7%) in the middle tertile(0.85-1.45 pg/ml) and 1(33.3%)vs.4(30.8%) in the highest tertile(1.46+ pg/ml). After excluding the PCOS adolescent who was on OC, this distribution became: (0%)vs.8(61.5%) (≤ 0.84 ng/ml) tertile, 1(50%)vs.1(7.7%)(0.85-1.45 ng/ml)tertile and 1(50%)vs.4(30.8%) in the highest tertile(1.46+ ng/ml).

In summary, our 15 pre-menopausal adolescents (2 PCOS and 13 non-PCOS), who were not current OC and HRT users, had mean testosterone levels that are higher than the recommended normal levels (>1ng/ml) (724). Our PCOS adolescents had mean CRP levels much higher than the recommended levels (>1mg/L) as well (~2.5 times higher) (725). A total of 2 (100%) PCOS vs. 8(62%) non-PCOS and 2(100%) PCOS vs. 7 (~54%) non-PCOS had testosterone levels in the top 2 tertiles and higher than the recommended normal levels(>1ng/ml) respectively (724). As for CRP levels, a total of 1(50%) PCOS vs. 2(15%) non-PCOS had CRP levels higher than the recommended normal levels respectively (>1mg/L) (725). It is noteworthy that the PCOS adolescent had CRP levels > 3 mg/L whereas the non-PCOS girls had their CRP levels within the range of 1-3 mg/L. CRP concentrations slightly above 1mg/L (typical CRP

concentration for healthy subjects) have been associated with increased risk of coronary heart disease (726); CRP levels > 3 mg/L have been associated with precarious outcomes after a myocardial infarct (727). In addition, 2(100%) PCOS vs. 5(~39%) non-PCOS had serum IL6 levels in the top 2 tertiles. The results, although based on small number of observations, suggest that high hormonal and inflammation levels are prevalent among our pre-menopausal PCOS and non-PCOS blood-related adolescents to PCOS women.

Among the 36 pre-menopausal adult women, the mean age, BMI and WHR for PCOS females (n=11) vs. non-PCOS females (n=25) were 36.45 years (±9.20) vs. 34.56 years (±12.00), 33.56 Kg/m^2 (±8.47) vs. 26.88 Kg/m^2 (±5.81) and 0.83(±0.07) vs. 0.84(±0.08) respectively. There was 1 missing value for testosterone levels in PCOS women. The mean testosterone, CRP and IL6 levels were respectively as follows: 1.35 ng/ml (±0.72), 6.07 mg/L(±5.62) and 2.43 pg/ml(±1.14) for PCOS women vs.1.24 ng/ml(±0.66), 1.70 mg/L(±2.37) and 1.92 pg/ml(±1.29) for non-PCOS women. There were 7 PCOS and 3 non-PCOS pre-menopausal women who were currently using OC or HRT. After excluding these women, the mean testosterone, CRP and IL6 levels for PCOS vs. non-PCOS women became: 1.68 ng/ml(±1.00) vs. 1.29 ng/ml(±0.66), 3.27 mg/L(±2.10)vs.1.64 mg/L(±2.33) and 2.76 pg/ml(±0.77) vs.1.97 pg/ml(±1.27). The distribution of the pre-menopausal PCOS vs. non-PCOS women by testosterone tertiles was as follows:3(30%)vs. 8(32%) in the lowest tertile(≤ 0.84 ng/ml), 3(30%)vs. 10(40%) in the middle tertile(0.85-1.45 ng/ml) and 4(40%)vs. 7(28%) in the highest tertile(1.46+ ng/ml). After excluding the 10 women who were on OC or HRT, this distribution became: 1(33.3%)vs. 6(27.3%) in the lowest tertile(≤ 0.84 ng/ml), 0(0%)vs. 10(45.5%) in the middle tertile(0.85-1.45 ng/ml) and 2(66.7%)vs. 6(27.3%) in the highest tertile(1.46+ ng/ml). Their distribution by reported clinical cutoff points of testosterone (724) (PCOS vs. non-PCOS) was as follows: 3(30%)vs. 11(44%) in the normal cutoff point category (≤ 0.99 ng/ml), 4(40%)vs. 9(36%) in the middle category (1-1.67 ng/ml) and 3(30%)vs. 5(20%) in the highest category (1.68+ ng/ml). After excluding the 10 women who were on OC or HRT, this distribution became: 1(33.3%) vs. 9(40.9%) (≤ 0.99 ng/ml) category, 1(33.3%)vs.8(36.4%)(1-1.67 ng/ml) category and 1(33.3%)vs. 5(22.7%)(1.68+ ng/ml) category. The distribution of the pre-menopausal PCOS vs. non-PCOS women by CRP tertiles was as follows:0(0%)vs. 10(40%) in the lowest tertile(≤ 0.57 mg/L), 3(27.3%)vs. 11(44%) in the middle tertile(0.58-2.45 mg/L) and 8(72.7%)vs. 4(16%) in the

highest tertile(2.46+ mg/L). After excluding the 10 women who were on OC or HRT, this distribution became: (0%)vs.8(36.4%) (≤ 0.57 mg/L) tertile, 2(50%)vs.11(50%)(0.58-2.45 mg/L) tertile and 2(50%)vs.3(13.6%)(2.46+ mg/L) tertile. Their distribution by reported clinical cutoff points of CRP(725)(PCOS vs. non-PCOS) was as follows:0(0%)vs. 13(52%) in the normal cutoff point category (≤ 0.99 mg/L), 3(27.3%)vs. 8(32%) in the middle category (1-3 mg/L) and 8(72.7%)vs. 4(16%) in the highest category (3.01+ mg/L). After excluding the 10 women who were on OC or HRT, this distribution became 0 (0%) vs.11 (50%) (≤ 0.99 mg/L) category, 2(50%)vs. 8(36.4%)(1-3 mg/L) category and 2(50%)vs. 3(13.6%) (3.01 mg/L) category. The distribution of premenopausal PCOS vs. non-PCOS women by IL6 tertiles was as follows:2(18.2%)vs. 8(32%) in the lowest tertile(≤ 0.84 pg/ml), 4(36.4%)vs. 10(40%) in the middle tertile(0.85-1.45 pg/ml) and 5(45.5%)vs. 7(28%) in the highest tertile(1.46+ pg/ml). After excluding the 10 women who were on OC or HRT, this distribution became: 0(0%)vs. 6(27.3%) (≤ 0.84 ng/ml) tertile, 1(25%)vs.10(45.5%)(0.85-1.45 ng/ml) tertile and 3(75%)vs. 6(27.3%) (1.46+ ng/ml) tertile.

In summary, our 26 adult pre-menopausal women (4 PCOS and 22 non-PCOS), who were not current OC and HRT users, had higher testosterone (>1ng/ml) and CRP(>1mg/L) levels than those recommended in healthy subjects (724,725 respectively). In addition, 2(66%) of the PCOS vs. 13(59%) of the non-PCOS pre-menopausal women had testosterone levels above the recommended clinical cutoff point(>1ng/ml)(724), 4(100%) vs. 14(64%) and 4(100%) vs. 11(50%) had CRP levels in the top two tertiles and above those recommended in healthy subjects(>1mg/L)(725) respectively. CRP concentrations slightly above 1mg/L (typical CRP concentration for healthy subjects) have been associated with with increased risk of coronary heart disease (726). All 4 PCOS vs. 16(73%) of the non-PCOS pre-menopausal women had serum IL6 levels in the top 2 tertiles. The results suggest that high hormonal and inflammation levels are prevalent among our adult pre-menopausal PCOS and non-PCOS blood-related.

Examining the distribution of hormonal and inflammatory markers' levels among the total 52 pre-menopausal women by PCOS status (14 PCOS and 38 non-PCOS), we obtained the following results

Among the 14 pre-menopausal PCOS females, the mean age, BMI and WHR were 32.21 years (±11.67), 32.77 Kg/m^2 (±7.90) and 0.83(±0.07) respectively. After excluding the 8 pre-menopausal PCOS females who were current OC or HRT users, the mean testosterone, CRP and IL6 levels and the distribution of the remaining 6 pre-menopausal PCOS females by testosterone/CRP tertiles and clinical cutoff points and IL6 tertiles was as follows. The mean testosterone, CRP and IL6 levels were respectively as follows: 1.61 ng/ml (±0.72), 3.03 mg/L(±2.28) and 3.01 pg/ml(±1.58). The distribution of these females by testosterone tertiles was as follows: 1(20%) in the lowest tertile (≤ 0.84 ng/ml), 1(20%) in the middle tertile (0.85-1.45 ng/ml) and 3(60%) in the highest tertile(1.46+ ng/ml). Their distribution by reported clinical cutoff points of testosterone (724) was as follows: 1(20%) in the normal cutoff point category (≤ 0.99 ng/ml), 3(60%) in the middle category (1-1.67 ng/ml) and 1(20%) in the highest category (1.68+ ng/ml). Our pre-menopausal PCOS females had the following distributions by CRP and IL6 levels. Their distribution by CRP tertiles was as follows: 1(17%) in the lowest tertile(≤ 0.57 mg/L), 2(33%) in the middle tertile(0.58-2.45 mg/L) and 3(50%) in the highest tertile(2.46+ mg/L). By reported clinical cutoff points of CRP (725), their distribution was as follows: 1(17%) in the normal cutoff point category (≤ 0.99 mg/L), 2(33%) in the middle category (1-3 mg/L) and 3(50%) in the highest category (3.01+ mg/L). As for the IL6 levels, the distribution of these pre-menopausal PCOS females was as follows: 0(0%) in the lowest tertile (≤ 1.14 pg/ml), 2(33%) in the middle tertile (1.15-2.42 pg/ml) and 3 (67%) in the highest tertile (2.43+ pg/ml).

Among the 38 pre-menopausal non-PCOS females, the mean age, BMI and WHR were 27.97 years (±13.41), 25.95 Kg/m^2 (±5.53) and 0.83(±0.08) respectively. After excluding the 3 pre-menopausal non-PCOS females who were current OC or HRT users, the mean testosterone, CRP and IL6 levels and the distribution of the 35 pre-menopausal non-PCOS females by testosterone/CRP tertiles and clinical cutoff points and IL6 tertiles was as follows. The mean testosterone, CRP and IL6 levels were respectively as follows: 1.33 ng/ml (±0.76), 1.15 mg/L(±1.96) and 1.88 pg/ml(±1.30). The distribution of these females by testosterone tertiles was as follows: 11(31%) in the lowest tertile (≤ 0.84 ng/ml), 14(40%) in the middle tertile (0.85-1.45 ng/ml) and 10(29%) in the highest tertile(1.46+ ng/ml). Their distribution by meaningful reported cutoff points of testosterone (724) was as follows: 15(43%) in the normal cutoff point category (≤ 0.99 ng/ml), 12(34%) in the middle category (1-1.67 ng/ml) and 8(23%)

in the highest category (1.68+ ng/ml). Our pre-menopausal non-PCOS females had the following distributions by CRP and IL6 levels. Their distribution by CRP tertiles was as follows: 19(54%) in the lowest tertile (≤ 0.57 mg/L), 13(37%) in the middle tertile(0.58-2.45 mg/L) and 3(9%) in the highest tertile(2.46+ mg/L). By meaningful clinical cutoff points of CRP (725), their distribution was as follows: 22(63%) in the normal cutoff point category (≤ 0.99 mg/L), 10(29%) in the middle category (1-3 mg/L) and 3(9%) in the highest category (3.01+ mg/L). As for the IL6 levels, the distribution of the non-PCOS pre-menopausal women was as follows: 14(40%) in the lowest tertile (≤ 1.14 pg/ml), 11(31%) in the middle tertile (1.15-2.42 pg/ml) and 10 (29%) in the highest tertile (2.43+ pg/ml).

In conclusion, our 6 PCOS and 35 non-PCOS pre-menopausal women, who were not current OC and HRT users, had mean testosterone and CRP levels, which are higher than the recommended normal levels (>1ng/ml ; >1mg/L, respectively)(724,725). A total of 4 (80%) PCOS vs. 24(69%) non-PCOS and 4(80%) PCOS vs.20 (57%) non-PCOS had testosterone levels in the top 2 tertiles and higher than the recommended normal levels(>1ng/ml) (724) respectively. As for CRP levels, a total of 5 (83%) PCOS vs. 16(46%) non-PCOS and 5(83%) PCOS vs. 13 (38%) non-PCOS had CRP levels in the top 2 tertiles and higher than the recommended normal levels (>1mg/L)(725) respectively. Moreover, 3(50%) PCOS vs. 3(9%) non-PCOS had CRP levels > 3 mg/L. CRP concentrations slightly above 1mg/L (typical CRP concentration for healthy adults) and > 3 mg/L have been associated with with increased risk of coronary heart disease and precarious outcomes after a myocardial infarct respectively (726,727 respectively). In addition, 6(100%) PCOS vs. 21(60%) non-PCOS had serum IL6 levels in the top 2 tertiles. The results show that high hormonal and inflammation levels are prevalent among our pre-menopausal PCOS and non-PCOS blood-related females to PCOS women **(Table 23).**

Table 23: Clinical Characteristics and Distribution of Hormonal and Inflammatory Markers' levels among 6 Pre-menopausal Probands and their pre-menopausal Female Blood-Relatives (N=52)

Hormonal or Inflammatory Marker	**Adult Status**								**PCOS status**			
	PCOS (N=3)		**Non-PCOS (N=13)**		**PCOS(N=11)**		**Non-PCOS(N=25)**		**PCOS(N=14)**		**Non-PCOS(N=38)**	
	≤17 yrs				**≥18 yrs**							
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
Age	16.67	0.58	15.29	1.34	36.45	9.20	34.56	12.00	32.21	11.67	27.97	13.41
BMI(Kg/m^2)	29.86	5.56	24.16	4.65	33.56	8.47	26.88	5.81	32.77	7.90	25.95	5.53
WHR	0.83	0.06	0.81	0.09	0.83	0.07	0.84	0.08	0.83	0.07	0.83	0.08
Testosterone(ng/ml)*	1.26(**1.50**)	0.44(**0.23**)	1.39	0.94	1.35(**1.68**)	0.72(**1.00**)	1.24(**1.29**)	0.66(**0.66**)	1.33(**1.61**)	0.65(**0.72**)	1.29(**1.33**)	0.76(**0.76**)
C_Reactine Protein (CRP,mg/L)	1.96(**2.55**)	2.66(**3.48**)	0.32	0.44	6.07(**3.27**)	5.62(**2.10**)	1.70(**1.64**)	2.37(**2.33**)	5.19(**3.03**)	5.34(**2.28**)	1.23(**1.15**)	2.03(**1.96**)
Interleukin_6(IL-6, pg/ml)	2.62(**3.51**)	2.71(**3.15**)	1.73	1.40	2.43(**2.76**)	1.14(**0.77**)	1.92(**1.97**)	1.29(**1.27**)	2.47(**3.01**)	1.46(**1.58**)	1.86(**1.88**)	1.31(**1.30**)
Testosterone Tertiles (ng/ml)*	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
<= .84	1(**0**)	33.3(**0**)	5	38.5	3(**1**)	30(**33.3**)	8(**6**)	32(**27.3**)	4(**1**)	31(**20**)	13(**11**)	34(**31**)
.85 - 1.45	1(**1**)	33.3(**50**)	4	30.8	3(**0**)	30(**0**)	10(**10**)	40(**45.5**)	4(**1**)	31(**20**)	14(**14**)	37(**40**)
1.46+	1(**1**)	33.3(**50**)	4	30.8	4(**2**)	40(**66.7**)	7(**6**)	28(**27.3**)	5(**3**)	38(**60**)	11(**10**)	29(**29**)
TOTAL	3(**2**)	100(**100**)	13	100	10(**3**)	100(**100**)	25(**22**)	100(**100**)	13(**5**)	100(**100**)	38(**35**)	100(**100**)
Testosterone (ng/ml)*	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
<= 0.99	1(**0**)	33.3(**0**)	6	46.2	3(**1**)	30(**33.3**)	11(**9**)	44(**40.9**)	4(**1**)	31(**20**)	17(**15**)	45(**43**)
1.00-1.67	2(**2**)	66.7(**100**)	4	30.8	4(**1**)	40(**33.3**)	9(**8**)	36(**36.4**)	6(**3**)	46(**60**)	13(**12**)	34(**34**)
1.68+	0(**0**)	0(**0**)	3	23.1	3(**1**)	30(**33.3**)	5(**5**)	20(**22.7**)	3(**1**)	23(**20**)	8(**8**)	21(**23**)
TOTAL	3(**2**)	100(**100**)	13	100	10(**3**)	100(**100**)	25(**22**)	100(**100**)	13(**5**)	100(**100**)	38(**35**)	100(**100**)
CRP Tertiles (mg/L)	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
<= .57	1(**1**)	33.3(**50**)	11	84.6	0(**0**)	0	10(**8**)	40(**36.4**)	1(**1**)	7(**17**)	21(**19**)	55(**54**)
.58 - 2.45	1(**0**)	33.3(**0**)	2	15.4	3(**2**)	27.3(**50**)	11(**11**)	44(**50**)	4(**2**)	29(**33**)	13(**13**)	34(**37**)
2.46+	1(**1**)	33.3(**50**)	0	0	8(**2**)	72.7(**50**)	4(**3**)	16(**13.6**)	9(**3**)	64(**50**)	4(**3**)	11(**9**)
TOTAL	3(**2**)	100(**100**)	13	100	11(**4**)	100(**100**)	25(**22**)	100(**100**)	14(**6**)	100(**100**)	38(**35**)	100(**100**)

Table 23 **(Cont'd)**

Hormonal or Inflammatory Marker	**Adult Status**								**PCOS status**			
	PCOS (N=3)		**Non-PCOS (N=13)**		**PCOS(N=11)**		**Non-PCOS(N=25)**		**PCOS(N=14)**		**Non-PCOS(N=38)**	
	≤17 yrs				**≥18 yrs**							
CRP (mg/L)	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
<= 0.99	2(**1**)	66.7(**50**)	11	84.6	0(**0**)	0(**0**)	13(**11**)	52(**50**)	2(**1**)	14(**17**)	24(**22**)	63(**63**)
1-3.00	0(**0**)	0(**0**)	2	15.4	3(**2**)	27.3(**50**)	8(**8**)	32(**36.4**)	3(**2**)	21(**33**)	10(**10**)	26(**29**)
3.01+	1(**1**)	33.3(**50**)	0	0	8(**2**)	72.7(**50**)	4(**3**)	16(**13.6**)	9(**3**)	64(**50**)	4(**3**)	11(**9**)
TOTAL	3(**2**)	**100**	13	100	11(**4**)	100(**100**)	25(**22**)	100(**100**)	14(**6**)	100(**100**)	38(**35**)	100(**100**)
IL-6 Tertiles (pg/ml)	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
<= 1.14	1(**0**)	33.3(**0**)	8	61.5	2(**0**)	18.2(**0**)	8(**6**)	32(**27.3**)	3(**0**)	21(**0**)	16(**14**)	42(**40**)
1.15 - 2.42	1(**1**)	33.3(**50**)	1	7.7	4(**1**)	36.4(**25**)	10(**10**)	40(**45.5**)	5(**2**)	36(**33**)	11(**11**)	29(**31**)
2.43+	1(**1**)	33.3(**50**)	4	30.8	5(**3**)	45.5(**75**)	7(**6**)	28(**27.3**)	6(**4**)	43(**67**)	11(**10**)	29(**29**)
TOTAL	3(**2**)	100(**100**)	13	100	11(**4**)	100(**100**)	25(**22**)	100(**100**)	14(**6**)	100(**100**)	38(**35**)	100(**100**)

Bolded values in parentheses represent values for females not currently using OC or HRT**.** A total of 11 pre-menopausal women are current OC or HRT users.

*****1 value for testosterone was missing for a PCOS case ≥ 18 yrs, not currently using OC or HRT.

Table 24 presents the distribution of hormonal and inflammatory markers' levels for post-menopausal blood-related females (N=16), including 3 probands by PCOS status. Among these females, there were 6 PCOS cases and 10 non-PCOS females.

Among the 6 post-menopausal PCOS women, the mean age, BMI and WHR was 49.50 years (±17.17), 38.20 Kg/m^2 (±10.90)[after excluding an outlier,34.06 Kg/m^2 ± 4.51] and 0.86(±0.06). The mean testosterone, CRP and IL6 levels were respectively as follows: 1.28 ng/ml(±0.62), 9.67 mg/L(±15.40)[after excluding an outlier, 3.50mg/L±3.33] and 6.06 pg/ml(±6.87)[after excluding an outlier, 3.33 pg/ml ± 1.84]. After excluding the post-menopausal PCOS woman who was currently using HRT, these means became respectively: 1.45ng/ml(±0.51), 10.05 mg/L(±17.19)[after excluding an outlier, 2.44mg/L±2.07] and 6.72pg/ml(±7.47)[after excluding an outlier, 3.48 pg/ml ± 2.10]. The distribution of post-menopausal PCOS women by testosterone tertiles was as follows: 1(16.7%) in the lowest tertile(≤ 0.84 ng/ml), 3(50%) in the middle tertile(0.85-1.45 ng/ml) and 2(33.3%) in the highest tertile(1.46+ ng/ml). After excluding the PCOS woman who was currently using HRT, this distribution became: 0(0%) (≤ 0.84 ng/ml) tertile, 3(60%)(0.85-1.45 ng/ml) tertile and 2(40%) (1.46+ ng/ml) tertile. Their distribution by reported clinical cutoff points of testosterone (724) was as follows: 2(33.3%) in the normal cutoff point category (≤ 0.99 ng/ml), 3(50%) in the middle category (1-1.67 ng/ml) and 1(16.7%) in the highest category (1.68+ ng/ml). After excluding the PCOS woman who was currently using HRT, this distribution became: 1(20%)(≤ 0.99 ng/ml) category, 3(60%) (1-1.67 ng/ml) category and 1(20%) (1.68+ ng/ml) category. The distribution of these women by CRP tertiles was as follows:1(16.7%) in the lowest tertile(≤ 0.57 mg/L), 2(33.3%) in the middle tertile(0.58-2.45 mg/L) and 3(50%) in the highest tertile(2.46+ mg/L). After excluding the PCOS woman who was currently using HRT, this distribution became: 1(20%)(≤ 0.57 mg/L) tertile, 2(40%) (0.58-2.45 mg/L) tertile and 2(40%)(2.46+ mg/L) tertile. Their distribution by reported clinical cutoff points of CRP (725) was as follows: 2(33.3%) in the normal cutoff point category (≤ 0.99 mg/L), 1(16.7%) in the middle category (1-3 mg/L) and 3(50%) in the highest category (3.01+ mg/L). After excluding the PCOS woman who was currently using HRT, this distribution became 2(40%) (≤ 0.99 mg/L) category, 1(20%)(1-3 mg/L) category and 2(40%)(3.01 mg/L) category. Their distribution by IL6 tertiles was as follows:1(16.7%) in the lowest tertile(≤ 0.84 pg/ml), 0(0%) in the middle tertile(0.85-1.45

pg/ml) and 5(83.3%) in the highest tertile(1.46+ pg/ml). After excluding the PCOS woman who was currently using HRT, this distribution became: 1(20%)(≤ 0.84 ng/ml) tertile, 0(0%)(0.85-1.45 ng/ml) tertile and 4(80%)(1.46+ ng/ml) tertile.

Among the 10 post-menopausal non-PCOS women, the mean age, BMI and WHR was 49.50 years (±17.17) were 64.90 years (±10.70), 34.58 Kg/m^2 (±6.92) and 0.87(±0.05). The mean testosterone, CRP and IL6 levels were respectively as follows: 0.92 ng/ml(±0.34), 5.98 mg/L(±5.08) and 4.02 pg/ml(±3.31)[after excluding an outlier, 3.19 pg/ml ± 2.13] for non-PCOS women. After excluding the post-menopausal non-PCOS woman who was currently using HRT, these means became respectively: 0.95 ng/ml(±0.34), 4.87 mg/L(±3.90) and 3.61 pg/ml(±3.23)[after excluding an outlier, 2.62pg/ml ± 1.39]. The distribution of these women by testosterone tertiles was as follows: 5(50%) in the lowest tertile(≤ 0.84 ng/ml), 3(30%) in the middle tertile(0.85-1.45 ng/ml) and 2(20%) in the highest tertile(1.46+ ng/ml). After excluding the non-PCOS woman who was currently using HRT, this distribution became: 4(44%)(≤ 0.84 ng/ml) tertile, 3(33%) (0.85-1.45 ng/ml) tertile and 2(22%)(1.46+ ng/ml) tertile. Their distribution by reported clinical cutoff points of testosterone (724) was as follows: 7(70%) in the normal cutoff point category (≤ 0.99 ng/ml), 3(30%) in the middle category (1-1.67 ng/ml) and 0(0%) in the highest category (1.68+ ng/ml). After excluding the post-menopausal non-PCOS woman who was currently using HRT, this distribution became: 6(67%)(≤ 0.99 ng/ml) category, 3(33%)(1-1.67 ng/ml) category and 0(0%)(1.68+ ng/ml) category. Their distribution by CRP tertiles was as follows: 1(10%), in the lowest tertile(≤ 0.57 mg/L), 1(10%) in the middle tertile(0.58-2.45 mg/L) and 8(80%) in the highest tertile(2.46+ mg/L). After excluding the non-PCOS woman who was currently using HRT, this distribution became: 1(11%)(≤ 0.57 mg/L) tertile, 1(11%) (0.58-2.45 mg/L) tertile and 7(78%)(2.46+ mg/L) tertile. The distribution of the post-menopausal non-PCOS women by reported clinical cutoff points of CRP (725) was as follows: 1(10%) in the normal cutoff point category (≤ 0.99 mg/L), 1(10%) in the middle category (1-3 mg/L) and 8(80%) in the highest category (3.01+ mg/L). After excluding the non-PCOS woman who was currently using HRT, this distribution became 1(11%)(≤ 0.99 mg/L) category, 1(11%)(1-3 mg/L) category and 7(78%)(3.01 mg/L) category. Their distribution by IL6 tertiles was as follows: 1(10%) in the lowest tertile(≤ 0.84 pg/ml), 5(50%) in the middle tertile(0.85-1.45 pg/ml) and 4(40%) in the highest tertile(1.46+ pg/ml). After excluding the

woman who was using HRT, this distribution became: 1(11%)(≤ 0.84 ng/ml) tertile, 5(56%)(0.85-1.45 ng/ml) tertile and 3(33%)(1.46+ ng/ml) tertile.

In conclusion, our 5 PCOS and 9 non-PCOS post-menopausal women, who were not current OC and HRT users, had mean CRP levels, which are much higher than the recommended normal levels(>1mg/L) (725). PCOS post-menopausal women had higher mean testosterone levels than the recommended normal levels (>1ng/ml) as well (724). A total of 5 (100%) PCOS vs. 5(55%) non-PCOS and 4(80%) PCOS vs. 3 (33%) non-PCOS had testosterone levels in the top 2 tertiles and higher than the recommended normal levels respectively (>1ng/ml) (724). As for CRP levels, a total of 4 (80%) PCOS vs. 8(89%) non-PCOS and 3(60%) PCOS vs. 8 (89%) non-PCOS had CRP levels in the top 2 tertiles and higher than the recommended normal levels(>1mg/L) respectively (725). Moreover, 2(40%) PCOS vs. 7(78%) non-PCOS had CRP levels > 3 mg/L. CRP concentrations above 1mg/L (typical CRP concentration for healthy adults) and 3 mg/L have been associated with with increased risk of coronary heart disease and precarious outcomes after a myocardial infarct respectively (726,727 respectively). In addition, 4(80%) PCOS vs. 8(89%) non-PCOS had serum IL6 levels in the top 2 tertiles. The results demonstrate that high hormonal and inflammation levels are prevalent among our post-menopausal PCOS and non-PCOS blood-related females to PCOS women.

Table 24: Clinical Characteristics and Distribution of Hormonal and Inflammatory Markers' levels among 3 Post-menopausal Probands and their postmenopausal Female Blood-Relatives (N=16)

Hormonal or Inflammatory Marker	**PCOS Status**			
	PCOS (N=6)		**Non-PCOS (N=10)**	
	≥18 yrs			
	Mean	**S.D.**	**Mean**	**S.D.**
Age	49.50	17.17	64.90	10.70
BMI(Kg/m^2)	38.20	10.90	34.58	6.92
*BMI(Kg/m^2)**	*34.06(n=5)*	*4.51*	______	______
WHR	0.86	0.06	0.87	0.05
Testosterone(ng/ml)	1.28**(1.45)**	0.62**(0.51)**	0.92**(0.95)**	0.34**(0.34)**
C_Reactine Protein (CRP,mg/L)	9.67**(10.05)**	15.40**(17.19)**	5.98**(4.87)**	5.08**(3.90)**
*C_Reactine Protein (CRP,mg/L)**	*3.5(n=5)(2.44,n=4)*	*3.33(2.70)*	_____	_____
Interleukin_6(IL-6, pg/ml)	6.06**(6.72)**	6.87**(7.47)**	4.02**(3.61)**	3.31**(3.23)**
*Interleukin_6(IL-6, pg/ml)**	*3.33(n=5)**(3.48,n=4)***	*1.84**(2.10)***	*3.19(n=9)**(2.62 n=8)***	*2.13**(1.39)***
Testosterone Tertiles (ng/ml)	**No.**	**%**	**No.**	**%**
<= .84	1**(0)**	16.7**(0)**	5**(4)**	50**(44)**
.85 - 1.45	3**(3)**	50**(60)**	3**(3)**	30**(33)**
1.46+	2**(2)**	33.3**(40)**	2**(2)**	20**(22)**
TOTAL	6**(5)**	100**(100)**	10**(9)**	100**(100)**
Testosterone (ng/ml)	**No.**	**%**	**No.**	**%**
<= 0.99	2**(1)**	33.3**(20)**	7**(6)**	70**(67)**
1.00-1.67	3**(3)**	50**(60)**	3**(3)**	30**(33)**
1.68+	1**(1)**	16.7**(20)**	0**(0)**	0**(0)**
TOTAL	6**(5)**	100**(100)**	10**(9)**	100**(100)**

Table 24 **(Cont'd)**

Hormonal or Inflammatory Marker	**PCOS Status**			
	PCOS (N=6)		**Non-PCOS (N=10)**	
	≥18 yrs			
CRP Tertiles (mg/L)	**No.**	**%**	**No.**	**%**
<= .57	1**(1)**	16.7**(20)**	1**(1)**	10**(11)**
.58 - 2.45	2**(2)**	33.3**(40)**	1**(1)**	10**(11)**
2.46+	3**(2)**	50**(40)**	8**(7)**	80**(78)**
TOTAL	6**(5)**	100**(100)**	10**(9)**	100**(100)**
CRP (mg/L)	**No.**	**%**	**No.**	**%**
<= 0.99	2**(2)**	33.3**(40)**	1**(1)**	10**(11)**
1-3.00	1**(1)**	16.7**(20)**	1**(1)**	10**(11)**
3.01+	3**(2)**	50**(40)**	8**(7)**	80**(78)**
TOTAL	6**(5)**	100**(100)**	10**(9)**	100**(100)**
IL-6 Tertiles (pg/ml)	**No.**	**%**	**No.**	**%**
<= 1.14	1**(1)**	16.7**(20)**	1**(1)**	10**(11)**
1.15 - 2.42	0**(0)**	0**(0)**	5**(5)**	50**(56)**
2.43+	5**(4)**	83.3**(80)**	4**(3)**	40**(33)**
TOTAL	6**(5)**	100**(100)**	10**(9)**	100**(100)**

Bolded values in parentheses represent values for females not currently using OC or HRT; 2 post-menopausal were current HRT users (1PCOS & 1 not- PCOS).

Italicized variables represent values excluding outliers for the variable

5.3.2.2 Blood-Related Males

Table 25 presents the distribution of hormonal and inflammatory markers' levels for blood-related males (N=25). For these males, the mean age, BMI and WHR were 46.40 years (±21.83), 29.25 Kg/m^2 (±7.08)[after excluding an outlier, 28.23 Kg/m^2 ±5.04] and 0.94(±0.05). The mean CRP and IL6 levels were respectively as follows: 5.02 mg/L(±9.34)[after excluding 2 outliers, 2.44 mg/L±2.75) and 2.30 pg/ml(±2.27)[after excluding 2 outliers,1.93 pg/ml ±1.40]. The distribution of blood-related males by CRP tertiles was as follows: 10(40%) in the lowest tertile(≤ 0.57 mg/L), 7(28%) in the middle tertile(0.58-2.45 mg/L) and 8(32%) in the highest tertile(2.46+ mg/L). The distribution of these males by reported clinical cutoff points for CRP levels (725), was as follows:11(44%) in the normal cutoff point category (≤ 0.99 mg/L), 6(24%) in the middle category (1-3 mg/L) and 8(32%) in the highest category (3.01+ mg/L). Their distribution by IL6 tertiles was as follows:9(36%) in the lowest tertile(≤ 0.84 pg/ml), 8(32%) in the middle tertile(0.85-1.45 pg/ml) and 7(32%) in the highest tertile(1.46+ pg/ml).

In conclusion, the 25 blood-related males to our PCOS probands had mean CRP levels, which are much higher (about 5 times higher) than the recommended normal levels (>1mg/L) (725). A total of 15(60%), 14(56%) and 8(32%) had CRP levels in the top 2 tertiles, higher than the recommended normal levels (>1mg/L) (725) and >3 mg/L. These last 2 CRP levels' cutoff points have been associated with increased risk of coronary heart disease and precarious outcomes after a myocardial infarct (726,727 respectively). In addition, 16(64%) of our blood-related males had serum IL6 levels in the top 2 tertiles. The results indicate high inflammation levels among the blood-related males to our PCOS probands.

Table 25: Clinical Characteristics and Distribution of Inflammatory Markers' levels among Male Blood Relatives to Probands (N=25)

Inflammatory Marker	**Blood Related Males(N=25)**	
	≥18 yrs	
	Mean	**S.D.**
Age	46.40	21.83
BMI(Kg/m^2)	29.25	7.08
BMI(Kg/m^2)	*28.23(n=24)*	*5.04*
WHR	0.94	0.05
C_Reactine Protein (CRP,mg/L)	5.02	9.34
*C_Reactine Protein (CRP,mg/L)**	*2.44(n=23)*	*2.75*
Interleukin_6(IL-6, pg/ml)	2.82	3.42
*Interleukin_6(IL-6, pg/ml)**	*1.93(n=23)*	*1.40*
CRP Tertiles (mg/L)	**No.**	**%**
<= .57	10	40
.58 - 2.45	7	28
2.46+	8	32
TOTAL	**25**	**100**
CRP (mg/L)	**No.**	**%**
<= 0.99	11	44
1-3.00	6	24
3.01+	8	32
TOTAL	**25**	**100**
IL-6 Tertiles (pg/ml)	**No.**	**%**
<= 1.14	9	36
1.15 - 2.42	8	32
2.43+	8	32
TOTAL	**25**	**100**

**Italicized* variables represent values excluding outliers for the variable

5.3.3 Prevalence of Glucose Abnormalities and Metabolic Syndrome

In **tables 26, 28, 30 and 32** impaired fasting glucose (IFG) was defined as 110 mg/dl ≤G0≤125 mg/dl, not T2 diabetic (clinically or lab diagnosed) and not on anti-diabetic medication. Insulin resistance (IR) was defined as subjects who were detected as insulin resistant by HOMA-IR criteria and were not clinical or lab-diagnosed T2 diabetics(IR-not T2DM), clinical or/and lab-diagnosed T2 diabetic subjects(IR-T2DM), subjects who reported themselves as having a clinical diagnosis of IR, were not detected as IR by HOMA-IR criteria and were not clinical or lab-diagnosed T2 diabetics(IR-self-reported), or subjects with IFG and abnormal β cell function who were not detected as insulin resistant by HOMA-IR criteria(IR-IFG & abnormal β cell function). In our study, we had 2 self-reported IR cases who were PCOS females, did not have IFG or T2DM and were not detected by HOMA-IR as IR and 1 male subject with IFG and abnormal β cell function, who was not detected as insulin resistant by HOMA-IR criteria. T2 diabetes (T2DM) was defined as cases diagnosed clinically or/and according to our fasting glucose lab results (G0≥126 mg/dl). IFG /IR/T2DM was defined as subjects who had any of the glucose abnormalities (IFG or/and IR-not T2DM, or T2DM). For the purpose of this latter definition, the 2-self reported cases as IR fall into the IR-not T2DM category and the male subject with IFG and abnormal β cell function falls into the IFG and IR-not T2diabetic category.

5.3.3.1 Probands & Blood-Related Females

Tables 26 & 27 present the distribution of the PCOS probands and their blood-related females by the results of the fasting blood specimen and clinic visit measurements, and the number of components of metabolic syndrome respectively.

Among the 9 PCOS probands 1 (~11%) had IFG, 6(~67%) had IR; 2 (~22%) were T2Diabetics, 6(~67%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 6(~67%) satisfied the metabolic syndrome (MS) ATP III diagnostic criteria (28). The distribution of the 6 probands who had MS was as follows: 3 (~33%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS and the remaining 3

(~33%) had 4 components or more of these criteria. In addition, 2 (~22%) of the remaining 3 PCOS probands had 1-2 components and only 1(~11%) had 0 components **(Tables 26 & 27).**

Among the 5 mothers of the PCOS probands, 1(20%) had PCOS. The PCOS mother had T2DM and therefore falls into the categories of IR-T2DM and IFG or/and IR-not T2DM or T2DM. This mother also had metabolic syndrome. Among the group of 4 non-PCOS mothers of our probands, none had IFG, 2 (~50%) had T2DM and therefore fall into the categories of IR-T2DM and IFG or/and IR-not T2DM or T2DM and 2 (50%) non-PCOS mothers had metabolic syndrome. In total, 3 (~60%) of the mothers of our PCOS probands had T2DM and therefore falls into the categories of IR-T2DM and IFG or/and IR-not T2DM or T2DM and 3 (60%) had MS. The distribution of the mothers by the number of components of MS was as follows: 1 (~20%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS, 2 (~40%) had 4 components or more and the remaining 2 mothers (~40%) of our PCOS probands had 1-2 components **(Tables 26 & 27).**

Among the 12 sisters of the PCOS probands, 2(~17%) had PCOS. These 2 PCOS sisters had none of the glucose abnormalities or metabolic syndrome. Among the 10 non-PCOS sisters, none had IFG, 3(30%) had IR; 2 (~20%) were T2Diabetics, 3(30%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM), and all 3 IR sisters (30%) had metabolic syndrome as well. The distribution of the sisters of the probands by number of MS components was as follows: 1 (~8%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS and the remaining 2 with MS (~17%) had 4 components or more of these criteria. Among the remaining 9 sisters, one PCOS sister (~8%) had 1-2 components and 8(~67%) had 0 components **(Tables 26 & 27).**

Among the 9 daughters of the PCOS probands, 5(~56%) had PCOS and 4(44.4%) ≤ 17 years. The distribution of the results of the fasting blood specimen and clinic visit measurements for PCOS (n=3) vs. non-PCOS (n=1) adolescent daughters was as follows: none of the adolescent daughters had IFG or MS, all had IR, 1(~33%) PCOS daughter vs. 0% of the non-PCOS daughters had T2DM and all adolescent daughters had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM). The distribution of adolescent daughters by number of MS components was as follows: 3 (~75%) satisfied 1-2 components of the MS ATP III diagnostic criteria and the remaining 1 (~25%) had 0 components **(Tables 26 & 27).** Among

the 5 adult daughters of our PCOS probands, 2 had PCOS. None of the 3 non-PCOS daughters had any of the glucose abnormalities or metabolic syndrome. The distribution of the results of the fasting blood specimen and clinic visit measurements for PCOS adult daughters (n=2) was as follows: 1 (50%) had IFG, 2(100%) had IR, none had T2DM, 2(~100%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 1 (50%) had MS. The distribution of the adult daughters of the probands by number of MS components was as follows: 1(~20%) satisfied 4 components of the MS ATP III diagnostic criteria; 1 (~20%) satisfied 2 components of the MS ATP III diagnostic criteria and the remaining 3 (~60%) had 0 components **(Tables 26 & 27).**

In summary, among the 9 daughters of our PCOS probands, 1(11%) had IFG, 6 (~67%) had IR, 1(11%) had T2DM, 6(~67%) had one or more of the glucose abnormalities (IFG or/and IR-noT2DM or T2DM) and 1(11%) had MS, who satisfied 4 components of the MS ATP III diagnostic criteria. Of the remaining 8 daughters, 4(44%) had 1-2 components and the remaining 4 (44%) had 0 components **(Tables 26 & 27).**

Among the 33 other female blood-relatives of the PCOS probands, defined as 1st,2nd or 3rd cousin ; aunt; grandma ; granddaughter or niece, 3 (~9%) had PCOS and were ≥ 18 years old and 12(36%) ≤ 17 years. Among the group of the 12 adolescent non-PCOS other female-blood relatives, 1(~8%) had IFG, 7 (~58%) had IR, 0(0%) had T2DM, 7 (~58%) had IFG or/and IR or T2DM and 1(~8%) had MS, who satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS. Of the remaining 11 non-PCOS other female-blood relatives, 8(~67%) had 1-2 components of the MS ATP III diagnostic criteria and 3(25%) had 0 components**(Tables26&27).**

Among the group of the 21 adult other female-blood relatives of the probands, the distribution of the results of the fasting blood specimen and clinic visit measurements for PCOS (n=3) vs. non-PCOS (n=18) females was as follows: 0% vs. 2(~11%) had IFG, all 3(100%) vs. 7 (~39%) had IR, 0% vs. 2(~11%) had T2DM, all 3(100%) vs. 8(~44%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and all the 3 (100%) adult PCOS vs. 5(~28%) of the adult non-PCOS other blood-related females had metabolic syndrome. The distribution of MS components among the adult other blood-related females to our PCOS probands was as follows: 6 (~29%) satisfied 3 components of the MS ATP III diagnostic criteria,

sufficient to be diagnosed with MS; 2(~10%) had 4 components, 10(~48%) had 1-2 components and the remaining 3 (14%) had 0 components**(Tables 26 & 27).**

In summary, among the 33 other female blood-relatives of the PCOS probands, 3(~9%) had IFG, 17 (~52%) had IR, 2(~6%) had T2DM, 18(~55%) had one or more of the glucose abnormalities (IFG or/and IR-noT2DM or T2DM) and 9 (27%) had MS. The distribution of MS conditions among the other female blood-relatives of our PCOS probands was as follows: 7 (21%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS; 2 (~6%) had 4+ components, 18(~55%) had 1-2 components and the remaining 6 (~18%) had 0 components **(Tables 26 & 27).**

Among the total 68 blood-related females, including the 9 PCOS probands, 20(~29%) had PCOS and 16(~24%) ≤ 17 years. The distribution of the results of the fasting blood specimen and clinic visit measurements for PCOS (n=3) vs. non-PCOS (n=13) adolescent female blood-relatives of the PCOS probands was as follows: 0% vs. 1(~8%) had IFG, all 3(100%) vs. 8 (~62%) had IR, 1(~33.3%) vs. 0% had T2DM, all 3(100%) vs. 8(~62%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 0 (0%) adolescent PCOS vs. 1(~8%) of the adolescent non-PCOS female blood-relatives had metabolic syndrome. The distribution of MS components among the adolescent female blood-relatives of our PCOS probands was as follows: 1(~6%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS; 11 (69%) had 1-2 components and the remaining 4 (25%) had 0 components **(Tables 26 & 27).**

Among the 52(76%) adult female blood relatives, including the 9 PCOS probands, the distribution of the results of the fasting blood specimen and clinic visit measurements for PCOS (n=17) vs. non-PCOS (n=35) adult female blood-relatives was as follows: 2 (~12%) vs. 2(~6%) had IFG; 12(~71%) vs. 12(~34%) had IR, 3(~18%) vs. 6(~17%) had T2DM, 12(~71%) vs. 13(~37%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 11(~65%) of the adult PCOS probands & female blood- relatives vs. 10(~29%) of the non-PCOS adult female blood-relatives had MS. The distribution of MS components among the 52 adult PCOS probands and their female blood-relatives was as follows: 11 (~21%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS;

10(~19%) had 4 components, 16(31%) had 1-2 components and the remaining 15 (29%) had 0 components **(Tables 26 & 27).**

Table 26: Distribution of Impaired Fasting Glucose (IFG), Insulin Resistance (IR), T2DM and Metabolic Syndrome (MS) among Probands and their Blood-Related Females (N=68)

Characteristic	Probands(N=9)		Mothers(N=5)				Sisters(N=12)				Daughters(N=9)							
			Non PCOS(n=4)		PCOS(n=1)		Non PCOS(n=10)		PCOS(n=2)		Non PCOS(n=4)				PCOS(n=5)			
	≥18 yrs		≥18 yrs				≥18 yrs				≤ 17 yrs (n=1)		≥ 18 yrs(n=3)		≤ 17 yrs (n=3)		≥ 18 yrs(n=2)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
IFG**																		
Yes	1	11.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	50
No	8	88.9	4	100	1	100	10	100	2	100	1	100	3	100	3	100	1	50
TOTAL	**9**	**100**	**4**	**100**	**1**	**100**	**10**	**100**	**2**	**100**	**1**	**100**	**3**	**100**	**3**	**100**	**2**	**100**
IR																		
IR_No T2DM**	3	33.3	0	0	0	0	1	10	0	0	1	100	0	0	1	33.3	2	100
IR_T2DM**	2	22.2	2	50	1	100	2	20	0	0	0	0	0	0	1	33.3	0	0
IR-Self Report**	1	11.1	0	0	0	0	0	0	0	0	0	0	0	0	1	33.3	0	0
Yes	6	66.7	2	50	1	100	3	30	0	0	1	100	0	0	3	100	2	100
No	3	33.3	2	50	0	0	7	70	2	100	0	0	3	100	0	0	0	0
TOTAL	**9**	**100**	**4**	**100**	**1**	**100**	**10**	**100**	**2**	**100**	**1**	**100**	**3**	**100**	**3**	**100**	**2**	**100**
T2DM**																		
Yes	2	22.2	2	50	1	100	2	20	0	0	0	0	0	0	1	33.3	0	0
No	7	77.8	2	50	0	0	8	80	2	100	1	100	3	100	2	66.7	2	100
TOTAL	**9**	**100**	**4**	**100**	**1**	**100**	**10**	**100**	**2**	**100**	**1**	**100**	**3**	**100**	**3**	**100**	**2**	**100**
IFG/IR/T2DM**																		
Yes	6	66.7	2	50	1	100	3	30	0	0	1	100	0	0	3	100	2	100
No	3	33.3	2	50	0	0	7	70	2	100	0	0	3	100	0	0	0	0
TOTAL	**9**	**100**	**4**	**100**	**1**	**100**	**10**	**100**	**2**	**100**	**1**	**100**	**3**	**100**	**3**	**100**	**2**	**100**
Metabolic Syndrome***																		
Yes	6	66.7	2	50	1	100	3	30	0	0	0	0	0	0	0	0	1	50
No	3	33.3	2	50	0	0	7	70	2	100	1	100	3	100	3	100	1	50
TOTAL	**9**	**100**	**4**	**100**	**1**	**100**	**10**	**100**	**2**	**100**	**1**	**100**	**3**	**100**	**3**	**100**	**2**	**100**

Table 26 **(Cont'd)**

Characteristic	Other Blood-Related Female (N=33)*						Probands and TOTAL Female Blood-Relatives (N=68)*							
	Non PCOS(n=30)				PCOS(n=3)		Non PCOS(n=48)				PCOS(n=20)			
	≤ 17 yrs (n=12)		≥ 18 yrs(n=18)		≥ 18 yrs		≤ 17 yrs (n=13)		≥ 18 yrs(n=35)		≤ 17 yrs (n=3)		≥ 18 yrs(n=17)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
IFG**														
Yes	1	8.3	2	11.1	0	0	1	7.7	2	5.7	0	0	2	11.8
No	11	91.7	16	88.9	3	100	12	92.3	33	94	3	100	15	88.2
TOTAL	**12**	**100**	**18**	**100**	**3**	**100**	**13**	**100**	**35**	**100**	3	**100**	**17**	**100**
IR														
IR_No T2DM**	7	58.3	5	27.8	3	100	8	61.5	6	17	1	33.3	8	47.1
IR_T2DM**	0	0	2	11.1	0	0	0	0	6	17	1	33.3	3	17.6
IR-Self Reported**	0	0	0	0	0	0	0	0	0	0	1	33.3	1	5.9
Yes	7	58.3	7	38.9	3	100	8	61.5	12	34	3	100	12	70.6
No	5	41.7	11	61.1	0	0	5	38.5	23	66	0	0	5	29.4
TOTAL	**12**	**100**	**18**	**100**	**3**	**100**	**13**	**100**	**35**	**100**	**3**	**100**	**17**	**100**
T2DM**														
Yes	0	0	2	11.1	0	0	0	0	6	17	1	33.3	3	17.6
No	12	100	16	88.9	3	100	13	100	29	83	2	66.7	14	82.4
TOTAL	**12**	**100**	**18**	**100**	**3**	**100**	**13**	**100**	**35**	**100**	**3**	**100**	**17**	**100**
IFG/IR/T2DM**														
Yes	7	58.3	8	44.4	3	100	8	61.5	13	37	3	100	12	70.6
No	5	41.7	10	55.6	0	0	5	38.5	22	63	0	0	5	29.4
TOTAL	**12**	**100**	**18**	**100**	**3**	**100**	**13**	**100**	**35**	**100**	**3**	**100**	**17**	**100**
Metabolic Syndrome**														
Yes	1	8	5	27.8	3	100	1	8	10	29	0	0	11	64.7
No	11	92	13	72.2	0	0	12	92	25	71	3	100	6	35.3
TOTAL	**12**	**100**	**18**	**100**	**3**	**100**	**13**	**100**	**35**	**100**	**3**	**100**	**17**	**100**

*** Other Blood-Related females** include 1st, 2nd or 3rd cousin, niece, aunt, grandma and granddaughter; **Probands and total female relatives**(N=68;this is excluding 4 Not-Blood related females).
** **IFG** is defined as 110≤G0≤125 mg/dl, were not T2diabetics and not on anti-diabetic medications; **IR-No T2DM** are subjects who were detected as IR by HOMA-IR criteria (>3.9 for 18yrs+;>2.85 for 17yrs;>3.10 for 16 yrs; >3.35 for 15 yrs; >3.61 for 14 yrs) AND were not T2 diabetics. **IR-T2DM** are T2 diabetic subjects who were either detected by HOMA-IR criteria as IR or not. **IR-self reported** are subjects who self reported themselves as doctor-diagnosed with IR and were on Metformin for this purpose AND who were not detected by HOMA-IR criteria as IR. **T2DM**-This includes both clinically diagnosed T2DM cases as well as cases identified according to our G0 lab results; T2DM was defined as G0 ≥ 126 mg/dl; **IFG/IR/T2DM** are subjects who had any of the glucose abnormalities (IFG only, IR+IFG, IR only or T2DM).
***__MS__ was defined according to ATPIII Diagnostic Criteria in Adults and modified for age in Adolescents.

Table 27: Number of Metabolic Syndrome (MS) Components among Probands and their Female Blood-Relatives (N=68)*

# of MS Components	Probands(N=9)		Mothers(N=5)		Sisters(N=12)		Daughters(N=9)				Other Blood-Related Females (N=33)**				Probands&Female Blood-Relatives (N=68)**			
	≥18yrs		≥18yrs		≥18yrs		≤17yrs(N=4)		≥18yrs(N=5)		≤17yrs(n=12)		≥18yrs(n=21)		≤17yrs(n=16)		≥18yrs(n=52)	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
0	1	11.1	0	0	8	66.7	1	25	3	60	3	25	3	14.3	4	25	15	29
1-2	2	22.2	2	40	1	8.3	3	75	1	20	8	67	10	47.6	11	69	16	31
3	3	33.3	1	20	1	8.3	0	0	0	0	1	8	6	28.6	1	6	11	21
4+	3	33.3	2	40	2	16.7	0	0	1	20	0	0	2	9.5	0	0	10	19
TOTAL	**9**	**100**	**5**	**100**	**12**	**100**	**4**	**100**	**5**	**100**	**12**	**100**	**21**	**100**	**16**	**100**	**52**	**100**

***MS** was defined according to ATPIII Diagnostic Criteria in Adults and modified for age in Adolescents.

****Other Blood-Related females** include 1st, 2nd or 3rd cousin, niece, aunt, grandma and granddaughter. **Probands and Female Blood-Relatives N=68** (excluding the 4 Not-Blood related females).

5.3.3.2 Blood-Related Males

Tables 28 and 29 present the distribution of the blood-related males (all≥ 18yrs) to the PCOS probands by the results of the fasting blood specimen and clinic visit measurements, and the number of components of metabolic syndrome respectively.

Among the 6 fathers of the probands, the distribution of the results of the fasting blood specimen and clinic visit measurements was as follows: 2(~33%) had IFG, 5(~83%) had IR, 3(50%) had T2DM, 5(~83%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 3(50%) had MS. The distribution of MS components among the 6 fathers of our PCOS probands was as follows: 2(~33%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS, 1(~17%) had 4+ components and the remaining 3(50%) had 1-2 components (**Tables 28 and 29**).

Among the 8 brothers of the probands, the distribution of the results of the fasting blood specimen and clinic visit measurements was as follows: 0% had IFG, 2(25%) had IR, 1(12.5%) had T2DM, 2(25%) had one or more of the glucose abnormalities(IFG or/and IR-not T2DM or T2DM) and 1(12.5%) had MS. The distribution of MS components among the 8 brothers of the PCOS probands was as follows: 1(12.5%) had 4+ components of the MS ATP III diagnostic criteria, 2(25%) had 1-2 components and the remaining 5(62.5%) had 0 components (**Tables 28 and 29**).

The only participating son of the probands had neither of the glucose abnormalities nor MS (**Tables 28 and 29**).

Among the 10 other blood-related males of the probands, defined as, 1st, 2nd or 3rd cousins, uncles, or nephews, the distribution of the results of the fasting blood specimen and clinic visit measurements was as follows: 1(10%) had IFG, 5(50%) had IR, 1(10%) had T2DM, 5(50%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 1(10%) had MS, who satisfied 3 components of the MS ATP III diagnostic criteria. However, 7(70%) had 1-2 components and the remaining 2(20%) had 0 components (**Tables 28 and 29**).

Table 28: Distribution of Impaired Fasting Glucose (IFG), Insulin Resistance (IR), T2DM and Metabolic Syndrome (MS) among Male Blood-Relatives to Probands (N=25)

Characteristic	**Fathers(N=6)**		**Brothers(N=8)**		**Sons(N=1)**		**Other Blood-Related Males(N=10)***		**TOTAL Male Blood-Relatives(N=25)***	
	≥18 yrs		**≥18 yrs**		**≥18 yrs**		**≥18 yrs**		**≥18 yrs**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
IFG**										
Yes	2	33	0	0	0	0	1	10	3	12
No	4	67	8	100	1	100	9	90	22	88
TOTAL	**6**	**100**	**8**	**100**	**1**	**100**	**10**	**100**	**25**	**100**
IR										
IR_No T2DM**	1	17	1	12.5	0	0	4	40	6	24
IR_T2DM**	3	50	1	12.5	0	0	1	10	5	20
IR-IFG & Abnormal βCell**	1	17	0	0	0	0	0	0	1	4
Yes	5	83	2	25	0	0	5	50	12	48
No	1	17	6	75	1	100	5	50	13	52
TOTAL	**6**	**100**	**8**	**100**	**1**	**100**	**10**	**100**	**25**	**100**
T2DM**										
Yes	3	50	1	12.5	0	0	1	10	5	20
No	3	50	7	87.5	1	100	9	90	20	80
TOTAL	**6**	**100**	**8**	**100**	**1**	**100**	**10**	**100**	**25**	**100**
IFG/IR/T2DM**										
Yes	5	83	2	25	0	0	5	50	12	48
No	1	17	6	75	1	100	5	50	13	52
TOTAL	**6**	**100**	**8**	**100**	**1**	**100**	**10**	**100**	**25**	**100**

Table 28 **(Cont'd)**

Characteristic	Fathers(N=6)		Brothers(N=8)		Sons(N=1)		Other Blood-Related Males(N=10)*		TOTAL Male Blood-Relatives(N=25)*	
	≥18 yrs		≥18 yrs		≥18 yrs		≥18 yrs		≥18 yrs	
MS* **	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Yes	3	50	1	12.5	0	0	1	10	5	20
No	3	50	7	87.5	1	100	9	90	20	80
TOTAL	**6**	**100**	**8**	**100**	**1**	**100**	**10**	**100**	**25**	**100**

* **Other Blood-Related males** include 1st, 2nd or 3rd cousin, nephew or uncle; **Total Male Blood-Relatives** N=25 (excluding 4 Not-Blood related males).

** **IFG** is defined as 110≤G0≤125 mg/dl, were not T2diabetics and not on anti-diabetic medications; **IR-No T2DM** are subjects who were detected as IR by HOMA-IR criteria (>3.9 for 18yrs+)AND were not T2 diabetics. **IR-T2DM** are T2 diabetic subjects who were either detected by HOMA-IR criteria as IR or not; IR**-IFG & Abnormal β Cell** are subjects categorized as IR based on IFG together with abnormal β cell function; **T2DM** is defined as clinically diagnosed T2DM cases as well as cases identified according to our G0 lab results; T2DM was defined as G0 ≥ 126 mg/dl**;** **IFG/IR/T2DM** are subjects who had any of the glucose abnormalities (IFG only, IR+IFG, IR only or T2DM).

*** **MS** was defined according to ATPIII Diagnostic Criteria in Adults

Table 29: Number of Metabolic Syndrome (MS) Components among Male Blood-Relatives to Probands (N=25)*

Number of MS Components	Fathers(N=6)		Brothers(N=8)		Sons(N=1)		Other Male Blood Relatives (N=10)**		Total Male Blood Relatives (N=25)**	
	≥18 yrs		≥18 yrs		≥18 yrs		≥18 yrs		≥18 yrs	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
0	0	0	5	63	1	100	2	20	8	32
1-2	3	50	2	25	0	0	7	70	12	48
3	2	33	0	0	0	0	1	10	3	12
4+	1	17	1	13	0	0	0	0	2	8
TOTAL	**6**	**100**	**8**	**100**	**1**	**100**	**10**	**100**	**25**	**100**

***MS** was defined according to ATPIII Diagnostic Criteria in Adults

****Other Male Blood-Relatives** include 1st, 2nd or 3rd cousin, nephew or uncle; Total N=25 (excluding the 4 Not-Blood related males)

5.3.3.3 Conclusions

The distribution of the results of the fasting blood specimen and clinic visit measurements and the number of components of MS for PCOS probands and their blood-related females, blood related males, total blood relatives as well as blood-related adolescents are summarized below.

Among the total 68 blood-related females, including the 9 PCOS probands, 5(~7%) had IFG, 35 (~51%) had IR, 10(~15%) had T2DM, 36(~53%) had one or more of the glucose abnormalities (IFG or/and IR-noT2DM or T2DM) and 22 (32%) had MS. The distribution of MS conditions among the PCOS probands and their female blood-relatives was as follows: 12 (18%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS; 10 (15%) had 4+ components, 27(40%) had 1-2 components and the remaining 19 (28%) had 0 components **(Tables 30 & 31).**

Among the total 25 male blood-relatives of our PCOS probands, 3(12%) had IFG, 12(48%) had IR, 5(20%) had T2DM, 12(48%) had one or more of the glucose abnormalities (IFG or/and IR-noT2DM or T2DM) and 5(20%) had MS. The distribution of MS components among the male blood-relatives to PCOS probands was as follows: 3 (12%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS; 2 (8%) had 4+ components, 12(48%) had 1-2 components and the remaining 8 (32%) had 0 components **(Tables 30 & 31).**

Among the total 93 participants, including the probands and total blood relatives, 8(9%) had IFG, 47(51%) had IR, 15(16%) had T2DM, 48(52%) had one or more of the glucose abnormalities (IFG or/and IR-noT2DM or T2DM) and 27(29%) had MS. The distribution of MS components among the blood-relatives to PCOS probands was as follows:15(16%) satisfied 3 components of the MS ATP III diagnostic criteria, sufficient to be diagnosed with MS; 12(13%) had 4+ components, 39(42%) had 1-2 components and the remaining 27(29%) had 0 component. It is noteworthy that 66 (~71%) had at least 1 component of the MS ATP III diagnostic criteria **(Tables 30 & 31).**

Among the 16 adolescent blood-relatives, 1(~6%) had IFG, 11(~69%) had IR, 1(~6%) had T2DM, 11(~69%) had one or more of the glucose abnormalities (IFG or/and IR-noT2DM or T2DM) and 1(6%) had MS, who satisfied 3 components of the MS ATP III diagnostic

criteria, sufficient to be diagnosed with MS. However, 11(69%) of the adolescent blood-relatives had 1-2 components and the remaining 4 (25%) had 0 components. It is noteworthy that 12 adolescents out of 16 total (75%) had at least 1 component of the MS ATP III diagnostic criteria **(Tables 30 & 31).**

Table 30: Distribution of Impaired Fasting Glucose (IFG), Insulin Resistance (IR), T2DM and Metabolic Syndrome (MS) among Probands and their Participating Blood-Relatives (N=93)

Characteristic	Probands & Female Blood-Relatives (N=68)*						Male Blood-Relatives (N=25) *		Probands&Total Blood Relatives (N=93)*	
	By Adult Status				Total					
	≤ 17 yrs (n=16)		≥ 18 yrs (n=52)				≥ 18 yrs			
	No.	%	No.	%	No.	%	No.	%	No.	%
IFG**										
Yes	1	6.25	4	7.7	5	7	3	12	8	9
No	15	93.75	48	92.3	63	93	22	88	85	91
TOTAL	**16**	**100**	**52**	**100**	**68**	**100**	**25**	**100**	**93**	**100**
IR										
IR_No T2DM**	9	56.25	14	26.9	23	34	6	24	29	31
IR_T2DM**	1	6.25	9	17.3	10	15	5	20	15	16
IR-Self Reported**	1	6.25	1	2	2	3	0	0	2	2
IR-IFG & Abnormal β Cell**	0	0	0	0	0	0	1	4	1	1
Yes	11	68.75	24	46.2	35	51	12	48	47	51
No	5	31.25	28	53.8	33	49	13	52	46	49
TOTAL	**16**	**100**	**52**	**100**	**68**	**100**	**25**	**100**	**93**	**100**
T2DM**										
Yes	1	6.25	9	17.3	10	15	5	20	15	16
No	15	93.75	43	82.7	58	85	20	80	78	84
TOTAL	**16**	**100**	**52**	**100**	**68**	**100**	**25**	**100**	**93**	**100**
IFG/IR/T2DM**										
Yes	11	68.75	25	48	36	53	12	48	48	52
No	5	31.25	27	52	32	47	13	52	45	48
TOTAL	**16**	**100**	**52**	**100**	**68**	**100**	**25**	**100**	**93**	**100**

Table 30 **(Cont'd)**

Characteristic	Probands & Female Blood-Relatives (N=68)*						Male Blood-Relatives (N=25) *		Probands&Total Blood Relatives (N=93)*	
	By Adult Status				Total					
	≤ 17 yrs (n=16)		≥ 18 yrs(n=52)				≥ 18 yrs			
	No.	%	No.	%	No.	%	No.	%	No.	%
Metabolic Syndrome* **										
Yes	1	6	21	40.4	22	32	5	20	27	29
No	15	94	31	59.6	46	68	20	80	66	71
TOTAL	**16**	**100**	**52**	**100**	**68**	**100**	**25**	**100**	**93**	**100**

* **Probands & Female Blood-Relatives** (N=68; this is excluding the 4 Not-Blood related females); **Male Blood- Relatives** N=25 (excluding the 4 Not-Blood related males). **Probands and Total Blood-Relatives** (N=93; this is excluding the 8 not-blood related males and females)

** **IFG** is defined as 110≤G0≤125 mg/dl, were not T2diabetics and not on anti-diabetic medications; IR-**No T2DM** are subjects who were detected as IR by HOMA-IR criteria (>3.9 for 18yrs+) AND were not T2 diabetics. **IR-T2DM** are T2 diabetic subjects who were either detected by HOMA-IR criteria as IR or not; **IR-IFG & Abnormal β Cell** are subjects categorized as IR based on impaired G0 together with abnormal β cell function; **T2DM** is defined as clinically diagnosed T2DM cases as well as cases identified according to our G0 lab results; T2DM was defined as G0 ≥ 126 mg/dl**; IFG/IR/T2DM** are subjects who had any of the glucose abnormalities (IFG only, IR+IFG, IR only or T2DM);

*** **MS** was defined according to ATPIII Diagnostic Criteria in Adults and modified for age in Adolescents.

Table 31: Number of MS Components among Probands and their Participating Blood-Relatives (N=93)*

Number of MS Components	Probands&Female-Blood Relatives(N=68)**						Male Blood Relatives(N=25)**		TOTAL (N=93)**	
	≤17yrs(n=16)		≥18yrs(n=52)		Total(N=68)		≥18 yrs			
	No.	%	No.	%	No.	%	No.	%	No.	%
0	4	25	15	29	19	28	8	32	27	29
1-2	11	69	16	31	27	40	12	48	39	42
3	1	6	11	21	12	18	3	12	15	16
4+	0	0	10	19	10	15	2	8	12	13
TOTAL	**16**	**100**	**52**	**100**	**68**	**100**	**25**	**100**	**93**	**100**

***MS** was defined according to ATPIII Diagnostic Criteria in Adults and modified for age in Adolescents.
** **Female Blood-Relatives** include 1st, 2nd or 3rd cousin, niece, aunt, grandma and granddaughter; **Male Blood-Relatives** include 1st, 2nd or 3rd cousin, nephew or uncle; **Total N=93** (excluding the 8 Not-Blood related males and females).

5.3.3.4 Blood-Related Males and Females by Degree of Relationship to Proband

Tables 32 presents the distribution of the blood relatives of the 9 PCOS probands ,defined by degree of relation to proband, by the results of the fasting blood specimen and clinic visit measurements. 1st Degree relative was defined by a child, a parent or a sibling; 2nd degree relative by a nephew/niece, paternal or maternal uncle/aunt, grandparent or grandchild; 3rd degree relative by 1st cousin, 4^{th+} degree relative by a 2nd or 3rd cousin.

Among the 41 1st degree relatives of the PCOS probands, 4(~10%) were ≤ 17 years. The distribution of the results of the fasting blood specimen and clinic visit measurements among the 1st degree relatives was as follows: 3(~7%) had IFG (0% adolescents; 3(~8%) adults), 19(~46%) had IR (4(100%) adolescents; 15(40.5%) adults), 10(~24%) had T2DM (1(25%) adolescents; 9(~24%) adults), 19(~46%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) (4(100%) adolescents and 15(40.5%) adults) and 11(~27%) of the 1st degree relatives had MS (0(0%) adolescents; 11(~30%) adults).

Among the 18 2nd degree relatives of the probands, 8(~44%) were ≤ 17 years. The distribution of the results of the fasting blood specimen and clinic visit measurements among the 2nd degree relatives was as follows: 4(~22%) had IFG (1(12.5%) adolescents; 3(30%) adults), 12(~67%) had IR (5(62.5%) adolescents; 7(70%) adults), 2(~11%) had T2DM (0(0%) adolescents; 2(20%) adults),13(~72%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) (5(62.5%) adolescents; 8(80%) adults) and 4(~22%) of the 2nd degree relatives had MS (0(0%) adolescents; 4(40% adults).

All 12 3rd degree relatives of our PCOS probands were ≥ 18 years. The distribution of the results of the fasting blood specimen and clinic visit measurements among the 12 3rd degree relatives was as follows: 0(0%) had IFG, 7(~58%) had IR, 1(~8%) had T2DM, 7(~58%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 5(~42%) of the 3rd degree relatives had MS.

Among the 13 4^{th+} degree relatives of the probands, 4(31%) were ≤ 17 years. The distribution of the results of the fasting blood specimen and clinic visit measurements among the 4^{th+}degree relatives was as follows: 0(0%) had IFG, 3(~23%) had IR (2(50%) adolescents;

1(11%) adults), 0(0%) had T2DM, 3(~23%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) (2(50%) adolescents; 1(11%) adults) and 1(8%) of the 4th degree relatives had MS (1(25%) adolescents; 0(0%) adults).

For small sample size limitation and comparison purposes, we collapsed 1st and 2nd degree relatives into one group (N=59) and 3rd and 4^{th+} degree relatives (n=25) into another group. The distribution of the results of the fasting blood specimen and clinic visit measurements among the 1st and 2nd degree relatives' group vs. the 3rd and 4^{th+} degree relatives' group was as follows: 7(12%)vs.0(0%) had IFG, 31(~53%)vs.10(40%) had IR, 12(20%)vs.1(10%) had T2DM, 32(~54%)vs.10(40%) had one or more of the glucose abnormalities (IFG or/and IR-not T2DM or T2DM) and 15(~25%) vs. 6(24%) had MS. The results suggest that the prevalence of the glucose abnormalities and metabolic syndrome decreases as the blood-relationship to the proband gets farther.

Table 32: Distribution of Impaired Fasting Glucose (IFG), Insulin Resistance (IR), Metabolic Syndrome (MS) and PCOS Status by Degree of Relation to Proband and Adult Status

Characteristic	1st Degree Relatives (N=41)*						2nd Degree Relatives(N=18)*						3rd Degree Relatives(N=12)*		4th+ Degree Relatives (N=13)*					
	≤ 17 yrs (n=4)		≥ 18 yrs(n=37)		Total(N=41)		≤ 17 yrs (n=8)		≥ 18 yrs(n=10)		Total(N=18)		≥ 18 yrs		≤ 17 yrs (n=4)		≥ 18 yrs(n=9)		Total (n=13)	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
IFG*																				
Yes	0	0	3	8.1	3	7.3	1	12.5	3	30	4	22	0	0	0	0	0	0	0	0
No	4	100	34	91.9	38	92.7	7	87.5	7	70	14	78	12	100	4	100	9	100	13	100
TOTAL	**4**	**100**	**37**	**100**	**41**	**100**	**8**	**100**	**10**	**100**	**18**	**100**	**12**	**100**	**4**	**100**	**9**	**100**	**13**	**100**
IR																				
IR_No T2DM**	2	50	5	13.5	7	17.1	5	62.5	5	50	10	56	6	50	2	50	1	11	3	23
IR_T2DM**	1	25	9	24.3	10	24.4	0	0	2	20	2	11	1	8.3	0	0	0	0	0	0
IR-Self reported**	1	25	0	0	1	2.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IR-IFG & Abnormal β Cell**	0	0	1	2.7	1	2.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Yes	4	100	15	40.5	19	46.3	5	62.5	7	70	12	67	7	58.3	2	50	1	11	3	23
No	0	100	22	59.5	22	53.7	3	37.5	3	30	6	33	5	41.7	2	50	8	89	10	77
TOTAL	**4**	**100**	**37**	**100**	**41**	**100**	**8**	**100**	**10**	**100**	**18**	**100**	**12**	**100**	**4**	**100**	**9**	**100**	**13**	**100**
T2DM**																				
Yes	1	25	9	24.3	10	24.4	0	0	2	20	2	11	1	8.3	0	0	0	0	0	0
No	3	75	28	75.7	31	75.6	8	100	8	80	16	89	11	91.7	4	100	9	100	13	100
TOTAL	**4**	**100**	**37**	**100**	**41**	**100**	**8**	**100**	**10**	**100**	**18**	**100**	**12**	**100**	**4**	**100**	**9**	**100**	**13**	**100**
IFG/IR/T2DM**																				
Yes	4	100	15	40.5	19	46.3	5	62.5	8	80	13	72	7	58.3	2	50	1	11	3	23
No	0	0	22	59.5	22	53.7	3	37.5	2	20	5	28	5	41.7	2	50	8	89	10	77
TOTAL	**4**	**100**	**37**	**100**	**41**	**100**	**8**	**100**	**10**	**100**	**18**	**100**	**12**	**100**	**4**	**100**	**9**	**100**	**13**	**100**

Table 32 **(Cont'd)**

	1st Degree Relatives (N=41)*						**2nd Degree Relatives(N=18)***						**3rd Degree Relatives(N=12)***		**4th+ Degree Relatives (N=13)***					
	≤ 17 yrs (n=4)		**≥ 18 yrs(n=37)**		**Total(N=41)**		**≤ 17 yrs (n=8)**		**≥ 18 yrs(n=10)**		**Total(N=18)**		**≥ 18 yrs**		**≤ 17 yrs (n=4)**		**≥ 18 yrs(n=9)**		**Total (n=13)**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Metabolic Syndrome* **																				
Yes	0	0	11	29.7	11	27	0	0	4	40	4	22	5	41.7	1	25	0	0	1	8
No	4	100	26	70.3	30	73	8	100	6	60	14	78	7	58.3	3	75	9	100	12	92
TOTAL	**4**	**100**	**37**	**100**	**41**	**100**	**8**	**100**	**10**	**100**	**18**	**100**	**12**	**100**	**4**	**100**	**9**	**100**	**13**	**100**

***1st Degree relative** is defined by a child, a parent or a sibling; **2nd degree relative** by nephew/niece, paternal or maternal uncle/aunt, grandparent or grandchild;**3rd degree** relative by 1st cousin, **4th+ degree relative** by a 2nd or 3rd cousin.

****IFG** is defined as 110≤G0≤125 mg/dl, were not T2diabetics and not on anti-diabetic medications; **IR-No T2DM** are subjects who were detected as IR by HOMA-IR criteria (>3.9 for 18yrs+;>2.85 for 17yrs;>3.10 for 16 yrs; >3.35 for 15 yrs; >3.61 for 14 yrs) AND were not T2 diabetics. **IR-T2DM** are T2 diabetic subjects who were either detected by HOMA-IR criteria as IR or clinically diagnosed cases. **IR-self reported** are subjects who were self reported doctor-diagnosed with IR and were on Metformin for this purpose AND who were not detected by HOMA-IR criteria as IR. **IR-IFG & Abnormal β Cell** are subjects categorized as IR based on impaired G0 together with abnormal β cell function. **T2DM** includes both clinically diagnosed T2DM cases as well as cases identified according to our G0 lab results. T2DM was defined as G0 ≥ 126 mg/dl**. IFG/IR/T2DM** are subjects who had any of the glucose abnormalities (IFG only, IR+IFG, IR only or T2DM).

*** **MS** was defined according to ATPIII Diagnostic Criteria in Adults and modified for age in Adolescents.

5.4 LINKAGE RESULTS

There was no evidence of linkage of any of our studied dichotomous and quantitative traits with either the IL6 G-174C SNP's or the PPARλ P12A SNP's locus **(Table 33)**.

Table 33: Results of Linkage Analyses of the Loci of the IL6 G-174C & PPARλ P12A SNPs to Different Dichotomous and Quantitative Traits (N=101)

Trait	SNP	LOD Score
Dichotomous Traits		
PCOS/Insulin Resistance	IL6 G-174C	0.13
	PPARλ P12A	-0.02
Insulin Resistance	IL6 G-174C	0.12
	PPARλ P12A	-0.02
Metabolic Syndrome	IL6 G-174C	0.02
	PPARλ P12A	0.14
Quantitative Traits		
Waist	IL6 G-174C	0
	PPARλ P12A	0
ln_Triglycerides	IL6 G-174C	0
	PPARλ P12A	0
ln_HDL	IL6 G-174C	0.28
	PPARλ P12A	0
ln_SBP	IL6 G-174C	0
	PPARλ P12A	0
DBP*	IL6 G-174C	0
	PPARλ P12A	0
Fasting Glucose (G0)*	IL6 G-174C	0.35
	PPARλ P12A	0
ln_IL6	IL6 G-174C	0
	PPARλ P12A	0
ln_CRP	IL6 G-174C	0.16
	PPARλ P12A	0
ln_HOMAIR	IL6 G-174C	0
	PPARλ P12A	0.1

* Both DBP and G0 were used as the variable excluding outliers

5.5 ASSOCIATION RESULTS

Waist was found to be normally distributed in our population of PCOS families, however, TG, HDL, SBP, DBP, G0, HOMA-IR and IL6/CRP/testosterone serum levels were found to be not-normally distributed and therefore were used in analysis, except for DBP and G0, as the natural logarithm (ln) transformation of the variable. Using the ln transformation of CRP serum levels resulted in 3 missing values because 3 participants had values of 0 mg/L for CRP serum level. DBP and G0 were used in analysis after excluding the outliers (DBP, 2 outliers; G0, 5 outliers) because no transformation helped in normalizing the variable. For Males' analysis, waist was used instead of BMI (except when waist was the dependent variable) because waist was univariately significantly associated with each studied dependent variable whereas BMI was not. In addition, waist was consistently more significantly associated than BMI with the dependent variables. PCOS status was not univariately significantly associated with HDL, SBP, DBP and G0 in total analysis. In females, PCOS status was not significantly univariately associated with testosterone as well. After adjusting for age and BMI and using a cutoff p-value of ≤ 0.1, PCOS status was found to significantly associate with HDL in total analysis (p=0.05) and borderline significantly associate with HDL and SBP in females (p-value=0.1) and therefore PCOS status was included in the multivariate regression analysis of HDL in total and female analyses and SBP in female analysis. There was only 1 person with the Ala12Ala genotype of the P12A PPARλ SNP in our study population. Therefore, we collapsed the Ala12Ala genotype category with the Pro12Ala genotype category and had 2 categories total for analyses (Ala-X category and the Pro12Pro category- reference category).

5.5.1 Dichotomous Traits

5.5.1.1 Total Analysis (N=101)

5.5.1.1.1 Metabolic Syndrome (MS)

For MS, the following variables were entered in a stepwise fashion: ever HRT use, menopause status, CRP/ IL6 serum levels and physical activity (Kcal/week).

5.5.1.1.1.1 IL6 G-174C (N=101)

IL6 SNP-BMI interaction was assessed and found to be borderline significant for IL6 CG genotype of this SNP (p-value~0.07). Before adjustment for the IL6SNP-BMI interaction, IL6 CG genotype of the IL6 SNP was not significantly associated with MS (p-value=0.218) but BMI was**.** These p-values became borderline significant for IL6CG genotype (p-value~0.06; OR=0.000) and not significant for BMI (p-value=0.11), after adjusting for this interaction**.** However, including the interaction term in the model resulted in very unstable and big CI due to the resulting extremely small size from testing this interaction. Therefore, our small sample size makes us reluctant to accept this result; instead we found that it is more sensible to restrict the parameters included in our model and exclude this interaction term. In the final model, age, gender, IR and BMI were significantly positively associated with MS whereas, race and menopause status were significantly/borderline significantly negatively associated with MS respectively. Adjusting for IL6 SNP, age, gender, race, IR, PCOS status, BMI and menopause status (when applicable), the odds of having MS was found to be ~ 10% higher for every year increase in age and ~34 % higher for every 1 Kg/m^2 increase in BMI. In addition, females were found to be at ~30 times higher risk of MS than males; African Americans were at ~98% lower risk of MS than Caucasians, insulin resistant subjects were found to be at ~ 7 times higher risk of MS than non-IR subjects and post-menopausal women were found to be at 89 % lower risk of MS than pre-menopausal women (**Table 34**).

Table 34: Results of Multiple logistic Regression of Metabolic Syndrome and G-174C IL6 SNP in Total Sample (N=101)

Independent Variable	OR	p-value	95% CI
IL6G-174C	______		_______
IL6 CG	0.337	0.218	0.06,1.903
IL6 GG	1.189	0.877	0.133,10.653
Age	**1.102**	**0.002**	**1.038,1.170**
Gender	**29.89**	**0.012**	**2.134,418.745**
Race	**0.023**	**0.008**	**0.001,0.378**
IR	**6.566**	**0.024**	**1.274,33.839**
PCOS Status	4.317	0.144	0.607,30.674
BMI	**1.341**	**0.001**	**1.134,1.584**
Menopause Status	**0.111**	**0.060**	**0.011,1.097**

5.5.1.1.1.2 PPARλ P12A (N=101)

Interactions between PPARλ SNP and each of IR, PCOS and BMI were tested and found to be not significant. It is noteworthy to mention that the effect of PPARλ SNP on MS became borderline significant (p-value was 0.17 and became 0.073) at the time BMI entered the regression model (data not shown). In the final model, age, race, IR and BMI were found to significantly associate with MS. The P12A PPARλ SNP and gender were also found to borderline significantly associate with MS (p-values=0.07 and 0.06 respectively). Adjusting for IL6 SNP, age, gender, race, IR, PCOS status and BMI (when applicable), the odds of having MS was found to be ~ 6% higher for every year increase in age and ~28 % higher for every 1 Kg/m^2 increase in BMI. In addition, subjects with at least 1 Ala12 allele of the P12A PPARλ SNP were found to be at ~ 91% lower risk of MS than subjects with the Pro12Pro genotype, females were found to be at ~6 times higher risk of MS than males; African Americans were at ~ 97% lower risk of MS than Caucasians and insulin resistant subjects were found to be at ~ 5 times higher risk of MS than non-IR subjects **Table 35**).

Table 35: Results of Multiple logistic Regression of Metabolic Syndrome and P12A PPARλ SNP in Total Sample (N=101)

Independent Variable	OR	p-value	95% CI
PPARλ P12A	**0.094**	**0.073**	0.007-1.243
Age	**1.058**	**0.006**	**1.016-1.102**
Gender	**5.917**	**0.060**	**0.927-37.765**
Race	**0.030**	**0.005**	**0.003-0.351**
IR	**5.391**	**0.035**	**1.126-25.815**
PCOS Status	4.378	0.105	0.734-26.132
BMI	**1.275**	**0.001**	**1.107-1.469**

Since the p-value for each of the IL6 SNP was > 0.1 in its final model and the p-value for PPARλ SNP was >0.05 in its final model, there was a need to neither assess gene-gene interaction nor adjust for a SNP in the other SNP's final model.

FBAT showed no association of either the IL6 or the PPARλ SNP with Metabolic Syndrome in the total sample.

5.5.1.1.2 Insulin Resistance (IR)

For IR, the following variables were entered in a stepwise fashion: ever smoking, current smoking, IL6 serum level, HDL and TG; smoking was defined by smoking cigarettes or/&pipes or/&cigars).

5.5.1.1.2.1 IL6 G-174C (N=101)

No potential IL6 SNP interactions were identified. In the final model, BMI, ever smoking and HDL were significantly associated with IR. Moreover, PCOS status was found to borderline significantly associate with IR (p-value~0.08). Adjusting for IL6 SNP, age, gender, race, PCOS status, BMI, ever smoking and HDL (when applicable), the odds of having IR was found to be ~ 15% higher for every 1 Kg/m^2 increase in BMI and ~ 6% lower for every 1 mg/dl increase in HDL serum level. PCOS females were found to be at ~4 times higher risk of IR than non-PCOS females and ever smokers were found to be at ~ 71% lower risk of IR than non-smokers (**Table 36**).

Table 36: Results of Multiple logistic Regression of Insulin Resistance and G-174C IL6 SNP in Total Sample (N=101)

Independent Variable	OR	p-value	95% CI
IL6G-174C	_____	0.835	______
IL6 CG	0.736	0.591	0.240-2.254
IL6 GG	1.055	0.943	0.243-4.589
Age	1.005	0.755	0.977-1.033
Gender	0.802	0.735	0.222-2.890
Race	1.387	0.661	0.322-5.977
PCOS Status	**4.127**	**0.082**	**0.834-20.419**
BMI	**1.148**	**0.009**	**1.035-1.272**
Ever smoking	**0.295**	**0.036**	**0.094-0.925**
HDL	**0.942**	**0.026**	**0.893-0.993**

5.5.1.1.2.2 PPARλ P12A (N=101)

No potential PPARλ SNP interactions were identified. According to the final model, BMI, ever smoking and HDL were significantly associated with IR. Moreover, PCOS status was found to borderline significantly associate with IR (p-value~0.07). Adjusting for PPARλ SNP, age, gender, race, PCOS status and BMI, ever smoking and HDL (when applicable), the odds of having IR was found to be ~ 15% higher for every 1 Kg/m^2 increase in BMI and ~ 6% lower for every 1 mg/dl increase in HDL serum level. PCOS females were found to be at ~4 times higher risk of IR than non-PCOS females and ever smokers were found to be at ~ 76% lower risk of IR than non-smokers (**Table 37**).

Table 37: Results of Multiple logistic Regression of Insulin Resistance and P12A PPARλ SNP in Total Sample (N=101)

Independent Variable	OR	p-value	95% CI
PPARλ P12A	0.431	0.275	0.095-1.954
Age	1.003	0.829	0.975-1.032
Gender	0.668	0.525	0.193-2.314
Race	1.227	0.784	0.283-5.313
PCOS Status	**4.322**	**0.072**	**0.879-21.248**
BMI	**1.151**	**0.008**	**1.038-1.277**
Ever smoking	**0.244**	**0.020**	**0.074-0.799**
HDL	**0.943**	**0.026**	**0.896-0.993**

Since the p-value for each of the IL6 SNP and PPARλ SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor adjust for a SNP in the other SNP's final model. No potential gene-environment interactions were identified as well.

FBAT showed no association of either the IL6 or the PPARλ SNP with Insulin resistance in the total sample.

5.5.1.2 Subgroup Analysis- Females (N=72)

5.5.1.2.1 Metabolic Syndrome (MS)

For MS, the following variables were entered in a stepwise fashion: ever HRT use, menopause status, CRP/IL6 serum levels and physical activity (measured in Kcal/week) were entered in a stepwise fashion.

5.5.1.2.1.1 IL6 G-174C (N=72)

IL6 SNP-BMI interaction was assessed and found to be not significant. In the final model, age, race, IR, BMI were found to be significantly associated with MS. Moreover, menopause status was found to borderline significantly associate with IR(p-value~0.05). Adjusting for IL6 SNP, age, race, IR, PCOS status, BMI and menopause status (when applicable), among females the odds of having MS is ~ 12% higher for every year increase in age and ~46 % higher for every 1 Kg/m^2 increase in BMI. In addition, African American, insulin resistant and postmenopausal females were found to be at ~98% lower risk, ~ 10 times higher risk and ~95% lower risk of MS than Caucasian, non-IR and pre-menopausal females respectively **(Table 38).**

Table 38: Results of Multiple logistic Regression of Metabolic Syndrome and G-174C IL6 SNP in Females (N=72)

Independent Variable	OR	p-value	95% CI
IL6G-174C	____	0.491	_____
IL6 CG	0.276	0.234	0.033-2.302
IL6 GG	0.551	0.745	0.015-19.920
Age	**1.118**	**0.013**	**1.024-1.222**
Race	**0.021**	**0.022**	**0.001-0.570**
IR	**9.691**	**0.025**	**1.324-70.901**
PCOS Status	4.892	0.201	0.429-55.763
BMI	**1.455**	**0.003**	**1.140-1.858**
Menopause Status	**0.049**	**0.053**	**0.002-1.044**

5.5.1.2.1.2 PPARλ P12A (N=72)

PPARλ SNP-BMI interaction was assessed and found to be not significant. It is noteworthy to mention that the effect of PPARλ SNP on MS became borderline significant (p-value was 0.323 and became 0.096) at the time BMI entered the regression model. In the final model, race, IR and BMI were found to be significantly associated with MS. Moreover, the PPARλ SNP was found to borderline significantly associate with MS in females (p-value=0.096). Adjusting for PPARλ SNP, age, race, IR, PCOS status and BMI (when applicable), among females the odds of having MS is ~38 % higher for every 1 Kg/m^2 increase in BMI. In addition, females with at least 1 Ala12 allele of the P12A PPARλ SNP, African American females and insulin resistant females were found to be at ~ 93% lower risk, ~97% lower risk and ~ 6.5 times higher risk of MS than females carrying the Pro12Pro genotype, Caucasian females and non-IR females respectively **(Table 39).**

Table 39: Results of Multiple logistic Regression of Metabolic Syndrome and P12A PPARλ in Females (N=72)

Independent Variable	OR	p-value	95% CI
PPARλ P12A	**0.074**	**0.096**	**0.003-1.596**
Age	1.044	0.100	0.992-1.100
Race	**0.027**	**0.015**	**0.001-0.496**
IR	**6.523**	**0.047**	**1.022-41.640**
PCOS Status	3.898	0.172	0.552-27.501
BMI	**1.381**	**0.004**	**1.110-1.720**

Since the p-value for the IL6 SNP in its final model was > 0.1 and the p-value for PPARλ SNP was >0.05 in its final model, there was a need to neither assess gene-gene interaction nor adjust for a SNP in the other SNP's final model.

5.5.1.2.2 Insulin Resistance (IR)

For IR, the following variables were entered in a stepwise fashion: ever smoking, current smoking, CRP/IL6 serum levels, HDL and TG; smoking was defined by smoking cigarettes or/&pipes or/&cigars).

5.5.1.2.2.1 IL6 G-174C (N=72)

No potential IL6 SNP interactions were identified. According to the final model, PCOS status, ever smoking and HDL were borderline significantly associated with IR. Adjusting for IL6 SNP, age, race, PCOS status, BMI, ever smoking and HDL (when applicable), among females the odds of having IR was found to ~ 6% lower for every 1 mg/dl increase in HDL serum level. PCOS females were found to be at ~6 times higher risk of IR than non-PCOS females and female ever smokers were found to be at ~ 73% lower risk of IR than female never smokers (**Table 40**).

Table 40: Results of Multiple logistic Regression of Insulin Resistance and G-174C IL6 SNP in Females (N=72)

Independent Variable	OR	p-value	95% CI
IL6G-174C	_____	0.862	______
IL6 CG	0.953	0.944	0.250-3.638
IL6 GG	0.588	0.600	0.081-4.282
Age	0.992	0.698	0.954-1.032
Race	2.722	0.346	0.339-21.883
PCOS Status	**5.573**	**0.052**	**0.987-31.459**
BMI	1.106	0.149	0.965-1.268
Ever smoking	**0.266**	**0.056**	**0.068-1.035**
HDL	**0.939**	**0.054**	**0.880-1.001**

5.5.1.2.2.2 PPARλ P12A (N=72)

PPARλ SNP and ever smoking interaction was assessed and found to be not significant. In the final model, BMI, ever smoking and TG were found to be significantly associated with IR. Moreover, PCOS status was found to borderline significantly associate with IR (p-value~0.09). Adjusting for IL6 SNP, age, race, PCOS status, BMI, ever smoking and TG (when applicable), among females the odds of having IR was found to be ~ 1% higher for every 1 mg/dl increase in TG serum level and ~ 16% higher for every 1 Kg/m^2 increase in BMI. PCOS females and ever smokers females were found to be at ~ 4 times higher risk and ~ 91% lower risk of IR than non-PCOS females and female never-smokers respectively (**Table 41**).

Table 41: Results of Multiple logistic Regression of Insulin Resistance and P12A PPARλ SNP in Females (N=72)

Independent Variable	OR	p-value	95% CI
PPARλ P12A	0.214	0.129	0.029-1.568
Age	0.969	0.115	0.932-1.008
Race	2.658	0.365	0.321-21.992
PCOS Status	**4.152**	**0.093**	**0.790-21.826**
BMI	**1.159**	**0.026**	**1.018-1.318**
Ever smoking	**0.088**	**0.004**	**0.017-0.468**
TG	**1.010**	**0.037**	**1.001-1.020**

Since the p-value for each of the IL6 SNP and PPARλ SNP in its final model was > 0.1, there was a need to neither assess gene-gene interaction nor adjust for a SNP in the other SNP's final model.

5.5.1.3 Subgroup Analysis- Males (N=29)

5.5.1.3.1 Metabolic Syndrome (MS)

No variables were univariately significantly associated with MS other than age, waist and IR, which were all forced into the model as specified a priori and therefore no variables were entered in a stepwise fashion in the MS regression analysis in males.

We were not able to successfully carry out PPARλ analyses on MS. A huge Standard error resulted with an upper bound of ∞ of the 95% CI, when entering PPARλ SNP as the only covariate. This is probably because of the absence of Males with the "Ala-X category"

genotype that had MS. Gene-gene interaction or adjustment for either SNP in the final model of the other SNP, therefore, were not applicable in males' analyses of MS.

5.5.1.3.1.1 IL6 G-174C (N=27)

IL6 SNP-waist interaction was assessed and found to be not significant. In the final model, among males no significant associations with MS were found **(Table 42).**

Table 42: Results of Multiple logistic Regression of Metabolic Syndrome and G-174C IL6 SNP in Males (N=27)

Independent Variable	**OR**	**p-value**	**95% CI**
IL6G-174C	____	0.603	_____
IL6 CG	5.116	0.405	0.109-239.145
IL6 GG	4.040	0.439	0.118-138.218
Age	1.061	0.218	0.965-1.167
IR	3.741	0.477	0.098-142.156
Waist	1.072	0.174	0.970-1.185

5.5.1.3.2 Insulin Resistance (IR)

For IR, only HDL was entered in a stepwise fashion.

5.5.1.3.2.1 IL6 G-174C (N=27)

IL6 SNP-waist interaction was assessed and found to be not significant. In the final model, among males waist was significantly associated with IR. Adjusting for IL6 SNP, and age, among males the odds of having IR was found to be ~ 31% higher for every 1 cm increase in waist (**Table 43**).

Table 43: Results of Multiple logistic Regression of Insulin Resistance and G-174C IL6 SNP in Males (N=27)

Independent Variable	**OR**	**p-value**	**95% CI**
IL6G-174C	____	0.445	_____
IL6 CG	0.070	0.372	0.000-24.087
IL6 GG	4.858	0.336	0.195-121.310
Age	0.967	0.586	0.896-1.064
Waist	**1.307**	**0.035**	**1.019-1.676**

5.5.1.3.2.2 PPARλ P12A (N=27)

PPARλ SNP-age and PPARλ SNP-waist interactions were assessed and found to be not significant. In the final model, among males waist was significantly associated with IR and PPARλ SNP was borderline significantly associated with IR. Adjusting for PPARλ SNP and age, among males the odds of having IR was found to be ~ 41% higher for every 1 cm increase in waist. Adjusting for age and waist, the odds of having IR was found to be ~ 93 times higher for males with at least 1 Ala12 allele compared to males with the Pro12Pro genotype (**Table 44).**

Table 44: Results of Multiple logistic Regression of Insulin Resistance and P12A PPARλ SNP in Males (N=27)

Independent Variable	OR	p-value	95% CI
PPARλ P12A	**93.416**	**0.057**	**0.882-9895.530**
Age	1.023	0.602	0.940-1.112
Waist	**1.413**	**0.041**	**1.015-1.966**

Since the p-value for each of the IL6 SNP in its final model was > 0.1, there was not a need to assess gene-gene interaction. Adjustment for the effect of one SNP in the final model of the other SNP was not needed as well since the p-value of each of the IL6 and PPARλ SNPs in its final model was > 0.05.However, an attempt was made to explore the effect of adjusting for PPARλ SNP in the IL6 final model; the model became so unstable with extremely large OR's and CI with an upper bound of ∞, so that it was more sensible to present the IL6 SNP-IR model without adjusting for the PPARλ SNP.

5.5.2 Quantitative Traits

5.5.2.1 Total Analysis (N=101)

5.5.2.1.1 Waist (cm)

For waist, the following variables were entered in a stepwise fashion: ever smoking, current smoking, current OC use, menopause status, HDL, LDL, TG, CRP/IL6 serum levels, SBP and DBP; smoking was defined by smoking cigarettes or/&pipes or/&cigars).

5.5.2.1.1.1 IL6 G-174C SNP (N=99)

Potential IL6 SNP interactions with ever smoking, TG, current OC, IL6 serum level and BMI were assessed and found to be not significant. It is noteworthy to mention that the effect of IL6 GG genotype on waist became significant (p-value was 0.120 and became 0.046) at the time ever smoking variable entered the regression model. Moreover, it became more significant (p-value was 0.043 and became 0.025) at the time IL6 serum level variable entered the regression model.

In the final **regression** model, IL6 GG genotype, gender, IR, BMI, ever smoking, TG, current OC use and IL6 serum level were found to significantly associate with waist. Adjusting for IL6 SNP, age, gender, race, IR, PCOS status, BMI, ever smoking, TG, current OC use and IL6 serum level (when applicable), the decrease in waist was found to be ~ 3.7 cm, ~10 cm and ~ 5 cm for a subject with the IL6 GG genotype compared to a subject with the CC genotype, for a female compared to a male and for a female who currently uses OC compared to a non-current OC user respectively. On the other hand, the increase in waist was found to be ~4.6 cm, ~1.7 cm, ~ 3.3 cm, 0.02 cm and 0.46 cm for an insulin resistant subject compared to a non-IR subject, for a subject whose BMI is 1 kg/m^2 higher, for a subject who ever smoked compared to a subject who never smoked, for a subject whose TG serum levels are 1 mg/dl higher than another subject's and for a subject whose serum IL6 serum level are 1 pg/ml higher than another subject's respectively (**Table45**).

The findings of the **VARIANCE COMPONENTS TEST** were pretty well consistent with the regression results, except for IL6GG genotype and IL6 serum level. As opposed to the regression findings (p-value for IL6GG genotype IL6 SNP=0.025), IL6 GG genotype of the IL6 SNP was found to be not significantly associated with waist. Moreover, IL6 serum level was found to borderline associate with waist (p-value ~0.06) rather than significantly associate with waist, as per the regression findings (p-value=0.048). In summary the variance components test showed that gender, IR, BMI, ever smoking, TG and current OC use were significantly associated with waist. Moreover, IL6 serum level was found to borderline associate with waist (p-value ~0.06). Adjusting for gender, IR, BMI, ever smoking, TG, current OC use and IL6 serum level (when applicable), the decrease in waist was found to be ~11 cm and ~ 4 cm for a female compared to a male and for a female who currently uses OC compared

to a non-current OC user respectively. On the other hand, the increase in waist was found to be ~4.8 cm, ~1.8 cm, ~ 2.7 cm, 0.02 cm and ~ 0.32 cm for an insulin resistant subject compared to a non-IR subject, for a subject whose BMI is 1 kg/m^2 is higher than another subject's, for a subject who ever smoked compared to a subject who never smoked, for a subject whose TG serum levels are 1 mg/dl higher than another subject's and for a subject whose serum IL6 serum levels are 1 pg/ml higher than another subject's respectively. The residual heritability for waist was found to be significant and equal to 58%, meaning that 58% of the total variation in waist is explained by other genetic factors, adjusting for gender, IR, BMI, ever smoking, TG, current OC use and IL6 serum level. Moreover, the proportion of variance in waist explained by gender, IR, BMI, ever smoking, TG, current OC use and IL6 serum level is ~ 92% **(Table 45)**.

Table 45: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of Waist and G-174C IL6 SNP in Total Sample (N=99)

Regression					VARIANCE COMPONENTS TEST H2r=0.58±0.16;p-value=0.00001 $\sigma^2_{\Sigma\beta}$=0.915		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-1.853	1.293	0.155	-4.423,0.717	——	——	0.511
IL6 GG	**-3.664**	**1.604**	**0.025**	**-6.852, -0.475**	——	——	0.266
Age	0.017	0.034	0.621	-0.051,0.084	——	——	——
Gender	**-10.296**	**1.450**	**0.000**	**-13.178,-7.414**	**-10.556**	**1.142**	**1.153×10^{-13}**
Race	0.577	1.674	0.731	-2.751,3.905	——	——	——
IR	**4.550**	**1.335**	**0.001**	**1.896,7.204**	**4.807**	**1.114**	**0.000051**
PCOS Status	1.326	1.805	0.465	-2.263,4.915	——	——	——
BMI	**1.670**	**0.108**	**0.000**	**1.455,1.885**	**1.751**	**0.0879**	**1.075×10^{-35}**
Ever Smoking	**3.286**	**1.297**	**0.013**	**0.708,5.864**	**2.711**	**1.100**	**0.00097**
TG	**0.02**	**0.007**	**0.005**	**0.006,0.035**	**0.019**	**0.006**	**0.00073**
Current OC use	**-5.027**	**2.193**	**0.023**	**-9.432, -0.712**	**-4.353**	**1.648**	**0.0076**
IL6 serum level(pg/ml)	**0.457**	**0.228**	**0.048**	**0.004,0.910**	**0.320**	**0.184**	**0.0615**

5.5.2.1.1.2 PPARλ P12A SNP (N=99)

No Potential PPARλ SNP interactions were identified. In the final model, gender, IR, BMI, IL6 GG genotype, ever smoking, TG and current OC use were found to significantly associate with waist. It is worth mentioning that ever smoking, TG and current OC use entered the model, even after adjusting for the effect of both the IL6 and PPARλ SNPs on waist. Adjusting for IL6 SNP, age, gender, race, IR, PCOS status, BMI ever smoking, TG and current OC use (when

applicable), the decrease in waist was found to be ~10 cm, ~ 3.50 cm, and ~ 5 cm for a female compared to a male, for a subject with the IL6 GG genotype compared to a subject with the CC genotype, and for a female who currently uses OC compared to a non-current OC user respectively. On the other hand, the increase in waist was found to be ~4 cm, ~1.8 cm, ~ 3 cm and ~0.02 cm for an insulin resistant subject compared to a non-IR subject, for a subject whose BMI is 1 kg/m^2 higher than another subject's, for a subject who ever smoked compared to a subject who never smoked and for a subject whose TG serum levels are 1 mg/dl higher than another subject's respectively (**Table 46**).

The findings of the **VARIANCE COMPONENTS TEST** were pretty well consistent with the regression results, except for IL6GG genotype. As opposed to the regression findings (p-value for IL6GG genotype IL6 SNP=0.037), IL6 GG genotype of the IL6 SNP was found to be not significantly associated with waist. In the final model, gender, IR, BMI, ever smoking, TG and current OC use were found to significantly associate with waist. Adjusting for gender, IR, BMI, ever smoking, TG and current OC use (when applicable), the decrease in waist was found to be ~11 cm and ~4 cm for a female compared to a male and for a female who currently uses OC compared to a non-current OC user respectively. On the other hand, the increase in waist was found to be ~5 cm, ~1.8 cm, ~ 2.5 cm and ~0.02 cm for an insulin resistant subject compared to a non-IR subject, for a subject whose BMI is 1 kg/m^2 higher than another subject's, for a subject who ever smoked compared to a subject who never smoked and for a subject whose TG serum levels are 1 mg/dl higher than another subject's respectively The residual heritability for waist was found to be significant and equal to 57%, meaning that 57% of the total variation in waist is explained by other genetic factors, adjusting for gender, IR, BMI, ever smoking, TG and current OC use. Moreover, the proportion of variance in waist explained by gender, IR, BMI, ever smoking, TG and current OC use is ~ 91%. (**Table 46**). Comparing Tables 23-a and 23-b, it seems that gender, IR, BMI, ever smoking, TG and current OC use explain 91.2% and IL6 serum level explains 0.3% of the total variation in waist.

Table 46: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of Waist and Pro12Ala PPARλ SNP in Total Sample (N=99)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.57±0.15;p-value=0.000005 $\sigma^2_{\Sigma\beta}$ =0.912		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-1.095	1.750	0.533	-4.574,2.384	——	——	0.987
Age	0.034	0.034	0.311	-0.033,-0.101	——	——	——
Gender	**-10.430**	**1.480**	**0.000**	**-13.372,-7.489**	**-10.748**	**1.155**	**$9x10^{-14}$**
Race	0.690	1.736	0.692	-2.762,4.142	——	——	——
IR	**4.195**	**1.357**	**0.003**	**1.496,6.893**	**4.562**	**1.123**	**0.00015**
PCOS Status	1.670	1.840	0.366	-1.987,5.327	——	——	——
BMI	**1.750**	**0.103**	**0.000**	**1.545,1.954**	**1.816**	**0.080**	**$2x10^{-40}$**
IL6 CG	-1.867	1.321	0.161	-4.493,0.759	——	——	0.582
IL6 GG	**-3.488**	**1.648**	**0.037**	**-6.764,-0.212**	——	——	0.403
Ever smoking	**2.899**	**1.326**	**0.031**	**0.264,5.535**	**2.495**	**1.112**	**0.021**
TG	**0.018**	**0.007**	**0.015**	**0.004,0.033**	**0.019**	**0.006**	**0.001**
Current OC use	**-5.129**	**2.244**	**0.025**	**-9.590,-0.667**	**-4.211**	**1.666**	**0.011**

Since the p-value for each of the PPARλ SNP was > 0.1 in its final model, there was no need to assess gene-gene interaction. Adjustment for the effect of IL6 SNP in the final model of the PPARλ SNP was performed since the p-value of the IL6 and PPARλ SNP in its final model was < 0.05.

5.5.2.1.2 Ln-Triglycerides (ln-TG, mg/dl)

For ln-TG, the following variables were entered in a stepwise fashion: ever smoking, current smoking, current drinking, ever OC use, current OC use, ever HRT use, SBP and DBP; smoking was defined by smoking cigarettes or/&pipes or/&cigars.

5.5.2.1.2.1 IL6 G-174C SNP (N=101)

No Potential IL6 SNP interactions were identified. It is noteworthy to mention that the effect of IR on ln-TG became not significant (p-value was 0.045 and became 0.129) at the time PCOS status variable entered the regression model. In the final model, age, race, BMI, ever smoking and current OC use were found to significantly associate with ln-TG. Adjusting for IL6 SNP, age, gender, race, IR, PCOS status, BMI, ever smoking, TG, and current OC use (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.02 mg/dl, ~ 0.29 mg/dl and

~ 0.48 mg/dl for a subject who is 1 year older than another subject, for a subject whose BMI is 1 kg/m^2 higher than another subject's, for a subject who ever smoked compared to a subject who never smoked and for a female who currently uses OC compared to a non-current OC user respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.48 mg/dl for an African American subject compared to a Caucasian subject (**Table 47)**.

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. The results of this test showed that age, race, BMI, ever smoking and current OC use were significantly associated with ln-TG. Adjusting for age, race, BMI, ever smoking and current OC use (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.02 mg/dl, ~ 0.23 mg/dl and ~ 0.45 mg/dl for a subject who is 1 year older, for a subject whose BMI is 1 kg/m^2 higher, for a subject who ever smoked compared to a subject who never smoked and for a female who currently uses OC compared to a non-current OC user respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.44 mg/dl for an African American subject compared to a Caucasian subject. The residual heritability for ln-TG was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in ln-TG, adjusting for age, race, BMI, ever smoking and current OC use. Moreover, the proportion of variance in ln-TG explained by age, race, BMI, ever smoking and current OC use is ~ 28% (**Table 47**).

Table 47: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-TG and G-174C IL6 SNP in Total Sample (N=101)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.00; p-value=0.50 $\sigma^2_{\Sigma\beta}$=0.28		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.069	0.122	0.575	-0.311,0.173	——	——	0.477
IL6 GG	-0.138	0.153	0.372	-0.442,0.167	——	——	0.310
Age	**0.007**	**0.003**	**0.033**	**0.001,0.013**	**0.007**	**0.003**	**0.021**
Gender	0.008	0.136	0.955	-0.262,0.277	——	——	——
Race	**-0.479**	**0.152**	**0.002**	**-0.780,-0.177**	**-0.439**	**0.139**	**0.001**
IR	0.188	0.123	0.130	-0.056,0.433	——	——	——
PCOS Status	-0.086	0.172	0.620	-0.428,0.257	——	——	——
BMI	**0.019**	**0.009**	**0.044**	**0.001,0.038**	**0.023**	**0.007**	**0.001**
Ever Smoking	**0.287**	**0.119**	**0.018**	**0.050,0.523**	**0.228**	**0.104**	**0.020**
Current OC use	**0.475**	**0.206**	**0.024**	**0.065,0.886**	**0.448**	**0.174**	**0.015**

5.5.2.1.2.2 PPARλ P12A SNP (N=101)

No Potential PPARλ SNP interactions were identified. It is noteworthy to mention that the effect of IR on ln-TG became not significant (p-value was 0.045 and became 0.131) at the time PCOS status variable entered the regression model. In the final model, age, race, BMI, ever smoking and current OC use were found to significantly associate with ln-TG. Adjusting for PPARλ SNP, age, gender, race, IR, PCOS status, BMI ever smoking, and current OC use (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.02 mg/dl, ~ 0.25 mg/dl and ~ 0.48 mg/dl for a subject who is 1 year older, for a subject whose BMI is 1 kg/m^2 higher, for a subject who ever smoked compared to a subject who never smoked and for a female who currently uses OC compared to a non-current OC user respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.46 mg/dl for an African American subject compared to a Caucasian subject (**Table 48**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. This test showed that age, race, BMI, ever smoking and current OC use were significantly associated with ln-TG. Adjusting for age, race, BMI, ever smoking and current OC use (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.02 mg/dl, ~ 0.23 mg/dl and ~ 0.45 mg/dl for a subject who is 1 year older, for a subject whose BMI is 1 kg/m^2 higher, for a subject who ever smoked compared to a subject who never smoked and for a female who currently uses OC compared to a non-current OC user respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.44 mg/dl for an African American subject compared to a Caucasian subject. The residual heritability for ln-TG was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in ln-TG, adjusting for age, race, BMI, ever smoking and current OC use. Moreover, the proportion of variance in ln-TG explained by age, race, BMI, ever smoking and current OC use is ~ 28% (**Table 48**).

Table 48: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-TG and Pro12Ala PPARλ SNP in Total Sample (N=101)

Regression					VARIANCE COMPONENTS TEST H2r=0.00; p-value=0.50 $\sigma^2_{\Sigma\beta}$=0.28		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.112	0.161	0.488	-0.433,0.208	——	——	0.368
Age	**0.006**	**0.003**	**0.034**	**0.001,0.012**	**0.007**	**0.003**	**0.022**
Gender	-0.006	0.131	0.961	-0.266,0.253	——	——	——
Race	**-0.464**	**0.149**	**0.003**	**-0.760,-0.167**	**-0.439**	**0.139**	**0.001**
IR	0.186	0.123	0.134	-0.058,0.431	——	——	——
PCOS Status	-0.078	0.172	0.652	-0,419,0.264	——	——	——
BMI	**0.019**	**0.009**	**0.045**	**0.000,0.038**	**0.023**	**0.007**	**0.002**
Ever Smoking	**0.253**	**0.118**	**0.035**	**0.018,0.488**	**0.228**	**0.104**	**0.044**
Current OC use	**0.482**	**0.205**	**0.021**	**0.074,0.890**	**0.447**	**0.174**	**0.013**

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction or to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.1.3 Ln-HDL,mg/dl

For ln-HDL, the following variables were entered in a stepwise fashion: current drinking, ever OC use and current OC use.

5.5.2.1.3.1 IL6 G-174C SNP (N=101)

IL6 SNP interactions with race, IR, BMI and current drinking were tested and found to be not significant. It is noteworthy to mention that the effect of IL6 GG genotype on ln-HDL became borderline significant (p-value was 0.142 and became 0.09) at the time race variable entered the regression model. This also applied to BMI when it entered the regression model (p-value was 0.124 and became 0.09). In the final model, gender, race, IR, PCOS status, BMI and current drinking were found to significantly associate with ln-HDL. Moreover, age was found to borderline significantly associate with ln-HDL (p-value ~0.09). Adjusting for IL6 SNP, age, gender, race, IR, PCOS status, BMI and current drinking (when applicable), the increase in ln-HDL was found to be ~ 0.002 mg/dl , ~0.13 mg/dl, ~0.18 mg/dl, ~0.13 mg/dl and~ 0.09 mg/dl

for a subject who is 1 year older than another subject, for a female compared to a male, for an African American subject compared to a Caucasian subject, for a PCOS female compared to a non-PCOS female and for a subject who currently drinks compared to a subject who does not currently drink respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.13 mg/dl and ~ 0.01 mg/dl for an insulin resistant subject compared to a non-insulin resistant and for a subject whose BMI is 1 kg/m^2 higher respectively (**Table 49**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. The results of this test showed that age, gender, race, IR, PCOS status, BMI and current drinking were significantly associated with ln-HDL. Adjusting for age, gender, race, IR, PCOS status, BMI and current drinking (when applicable), the increase in ln-HDL was found to be ~ 0.002 mg/dl , ~0.13 mg/dl, ~0.19 mg/dl, ~0.13 mg/dl and~ 0.1 mg/dl for a subject who is 1 year older than another subject, for a female compared to a male, for an African American subject compared to a Caucasian subject, for a PCOS female compared to a non-PCOS female and for a subject who currently drinks compared to a subject who does not currently drink respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.13 mg/dl and ~ 0.01 mg/dl for an insulin resistant subject compared to a non-insulin resistant and for a subject whose BMI is 1 kg/m^2 higher respectively. The residual heritability for ln-HDL was found to be significant and equal to 42%, meaning that 42% of the total variation in ln-HDL is explained by other genetic factors, adjusting for age, gender, race, IR, PCOS status, BMI and current drinking. Moreover, the proportion of variance in ln-HDL which is explained by age, gender, race, IR, PCOS status, BMI and current drinking is ~ 29% **(Table 49).**

Table 49: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HDL and G-174C IL6 SNP in Total Sample (N=101)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.42±0.18; p value=0.001 $\sigma^2_{\Sigma\beta}$=0.29		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.048	0.050	0.333	-0.146,0.050	——	——	0.234
IL6 GG	0.094	0.062	0.134	-0.029,0.217	——	——	0.727
Age	**0.002**	**0.001**	**0.088**	**0.000,0.005**	**0.002**	**0.001**	**0.024**
Gender	**0.131**	**0.053**	**0.015**	**0.026,0.236**	**0.129**	**0.047**	**0.004**
Race	**0.179**	**0.061**	**0.004**	**0.057,0.301**	**0.186**	**0.074**	**0.028**
IR	**-0.131**	**0.049**	**0.009**	**-0.229,-0.034**	**-0.128**	**0.045**	**0.003**
PCOS Status	**0.133**	**0.064**	**0.04**	**0.006,0.260**	**0.134**	**0.058**	**0.027**
BMI	**-0.010**	**0.004**	**0.009**	**-0.017,-0.003**	**-0.012**	**0.004**	**0.002**
Current Drinking	**0.091**	**0.045**	**0.044**	**0.003,0.180**	**0.099**	**0.040**	**0.014**

5.5.2.1.3.2 PPARλ P12A SNP (N=101)

No Potential PPARλ SNP interactions were identified. In the final model, gender, race, IR, PCOS status, BMI and current drinking were found to significantly associate with ln-HDL. Adjusting for IL6 SNP, age, gender, race, IR, PCOS status, BMI and current drinking (when applicable), the increase in ln-HDL was found to be ~0.11 mg/dl, ~0.17 mg/dl, ~0.14 mg/dl and~ 0.09 mg/dl for a female compared to a male, for an African American subject compared to a Caucasian subject, for a PCOS female compared to a non-PCOS female and for a subject who currently drinks compared to a subject who does not currently drink respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.13 mg/dl and ~ 0.01 mg/dl for an insulin resistant subject compared to a non-insulin resistant and for a subject whose BMI is 1 kg/m^2 higher respectively (**Table 50**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. This test similarly showed that gender, race, IR, PCOS status, BMI and current drinking were significantly associated with ln-.HDL. Adjusting for gender, race, IR, PCOS status, BMI and current drinking (when applicable), the increase in ln-HDL was found to be ~0.11 mg/dl, ~0.17 mg/dl, ~0.11 mg/dl and~ 0.11 mg/dl for a female compared to a male, for an African American subject compared to a Caucasian subject, for a PCOS female compared to a non-PCOS female and for a subject who currently drinks compared to a subject who does not currently drink respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.13 mg/dl and ~ 0.01 mg/dl for an insulin resistant subject compared to a non-insulin resistant and for a subject whose BMI is 1 kg/m^2 higher respectively. The residual heritability for ln-HDL was found to be significant and equal to 36%, meaning that 36% of the total variation in ln-HDL is explained by other genetic factors, adjusting for gender, race, IR, PCOS status, BMI and current drinking. Moreover, the proportion of variance in ln-HDL which is explained by gender, race, IR, PCOS status, BMI and current drinking is ~ 27% **(Table 50).** Comparing the variance components test results of Tables 49 & 50 showed that age explains 2% of the variation in ln-HDL.

Table 50: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HDL and Pro12Ala PPARλ SNP in Total Sample (N=101)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.36±0.17;p value = 0.005 $\sigma^2_{\Sigma\beta}$=0.27		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.003	0.067	0.962	-0.137,0.131	——	——	0.594
Age	0.002	0.001	0.133	-0.001,0.004	——	——	——
Gender	**0.109**	**0.052**	**0.040**	**0.005,0.213**	**0.109**	**0.047**	**0.024**
Race	**0.169**	**0.063**	**0.008**	**0.045,0.293**	**0.169**	**0.073**	**0.023**
IR	**-0.132**	**0.050**	**0.010**	**-0.231,-0.033**	**-0.134**	**0.046**	**0.005**
PCOS Status	**0.135**	**0.065**	**0.041**	**0.006,0.265**	**0.114**	**0.059**	**0.060**
BMI	**-0.010**	**0.004**	**0.007**	**-0.018,-0.003**	**-0.010**	**0.003**	**0.006**
Current drinking	**0.094**	**0.046**	**0.043**	**0.003,0.185**	**0.107**	**0.041**	**0.010**

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction or to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.1.4 Ln-Systolic Blood Pressure (ln-SBP,mm)

For ln-SBP, the following variables were entered in a stepwise fashion: ever smoking, current smoking, current drinking, current OC use, ever HRT, current HRT use, menopause status, LDL, TG and physical activity.

5.5.2.1.4.1 IL6 G-174C SNP (N=101)

IL6 SNP interactions with race, IR, BMI and current drinkinging were tested and found to be not significant. In the final model, age, current drinking, current smoking and menopause status were found to significantly associate with ln-SBP. Adjusting for IL6 SNP, age, gender, race, BMI, current drinking, current smoking and menopause status (when applicable), the increase in ln-SBP was found to be 0.001 mm, ~0.05 mm, ~0.06 mm and ~0.08 mm, for a subject who is 1 year older, for a subject who currently drinks compared to a subject who does not currently

drink, for a current smoker compared to a non-current smoker and for a post-menopausal woman compared to a pre-menopausal woman respectively (**Table 51**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. This test similarly showed that age, current drinking, current smoking and menopause status were significantly associated with ln-SBP. Adjusting for age, current drinking, current smoking and menopause status (when applicable), the increase in ln-SBP was found to be 0.002 mm, ~0.04 mm, ~0.07 mm and ~0.07 mm, for a subject who is 1 year older, for a subject who currently drinks compared to a subject who does not currently drink, for a current smoker compared to a non-current smoker and for a postmenopausal woman compared to a pre-menopausal woman respectively. The residual heritability for ln-SBP was found to be borderline significant and equal to 25% (p-value=0.06), meaning that 25% of the genetic variation in ln-SBP is explained by other genetic factors, adjusting for age, current drinking, current smoking and menopause status. Moreover, the proportion of variance in ln-SBP which is explained by age, current drinking, current smoking and menopause status was found to be ~ 36% **(Table 51).**

Table 51: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-SBP and G-174C IL6 SNP in Total Sample (N=101)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.25±0.20; p value = 0.06 $\sigma^2_{\Sigma\beta}$ =0.36		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	0.000	0.022	0.987	-0.044,0.043	——	——	0.407
IL6 GG	0.027	0.028	0.335	-0.028,0.081	——	——	0.995
Age	**0.001**	**0.001**	**0.030**	**0.000,0.003**	**0.002**	**0.0005**	**0.001**
Gender	-0.031	0.026	0.222	-0.082,0.019	——	——	——
Race	0.038	0.027	0.163	-0.016,0.092	——	——	——
BMI	0.001	0.001	0.461	-0.002,0.004	——	——	——
Current drinking	**0.052**	**0.021**	**0.016**	**0.010,0.094**	**0.044**	**0.020**	**0.025**
Current smoking	**0.059**	**0.025**	**0.020**	**0.010,0.109**	**0.074**	**0.023**	**0.002**
Menopause Status	**0.076**	**0.032**	**0.020**	**0.012,0.141**	**0.066**	**0.026**	**0.010**

5.5.2.1.4.2 PPARλ P12A SNP(N=101)

PPARλ SNP interaction with BMI was tested and found to be not significant. It is noteworthy to mention that the unadjusted effect of the PPARλ SNP on ln-SBP was significant (p-value = 0.047) and lost significance at the time race entered the regression model . In the final model, age, current drinking, current smoking and menopause status were found to significantly associate with ln-SBP. Adjusting for IL6 SNP, age, gender, race, BMI, current drinking, current smoking and menopause status (when applicable), the increase in ln-SBP was found to be 0.001 mm, ~0.05 mm, ~0.06 mm and ~0.08 mm, for a subject who is 1 year older, for a subject who currently drinks compared to a subject who does not currently drink, for a current smoker compared to a non-current smoker and for a postmenopausal woman compared to a pre-menopausal woman respectively (**Table 52**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. This test similarly showed that age, current drinking, current smoking and menopause status were significantly associated with ln-SBP. Adjusting for age, current drinking, current smoking and menopause status (when applicable), the increase in ln-SBP was found to be 0.002 mm, ~0.04 mm, ~0.07 mm and ~0.07 mm, for a subject who is 1 year older, for a subject who currently drinks compared to a subject who does not currently drink, for a current smoker compared to a non-current smoker and for a post-menopausal woman compared to a pre-menopausal woman respectively. The residual heritability for ln-SBP was found to be borderline significant and equal to 25%, meaning that 25% of the total variation in ln-HDL is explained by other genetic factors, adjusting for age, current drinking, current smoking and menopause status. Moreover, the proportion of variance in ln-SBP which is explained by age, current drinking, current smoking and menopause status was found to be ~ 36% **(Table 52).**

Table 52: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-SBP and Pro12Ala PPARλ SNP in Total Sample (N=101)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.25±0.20; p value = 0.06 $\sigma^2_{\Sigma\beta}=0.36$		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.043	0.029	0.143	-0.102,0.015	——	——	0.267
Age	**0.001**	**0.001**	**0.036**	**0.000,0.003**	**0.002**	**0.0005**	**0.002**
Gender	-0.035	0.025	0.161	-0.084,0.014	——	——	——
Race	0.028	0.027	0.291	-0.025,0.082	——	——	——
BMI	0.001	0.001	0.504	-0.002,0.004	——	——	——
Current drinking	**0.050**	**0.021**	**0.019**	**0.009,0.092**	**0.044**	**0.020**	**0.032**
Current smoking	**0.056**	**0.025**	**0.026**	**0.007,0.105**	**0.074**	**0.023**	**0.002**
Menopause Status	**0.079**	**0.032**	**0.015**	**0.016,0.143**	**0.066**	**0.026**	**0.011**

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction or to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.1.5 Diastolic Blood Pressure (DBP, mm)

For DBP, the following variables were entered in a stepwise fashion: ever smoking, current smoking, current drinking, current OC use, current HRT use, LDL, TG and physical activity.

5.5.2.1.5.1 IL6 G-174C SNP (N=99)

IL6 SNP interactions with race, gender, BMI and current drinking were tested and found to be not significant. It is noteworthy to mention that the effect of the IL6 CG genotype on DBP became borderline significant (p-value was 0.209 and became 0.08) at the time gender variable entered the regression model. One step further, it became significant when race variable entered the regression model (p-value became 0.043). Then it became borderline significant when BMI

entered the model (p-value=0.067) and lost its significance when current drinking variable entered the model (p-value=0.12). Moreover, race became not significant after adjusting for PPARλ SNP (p-value was 0.028, became 0.082). It is also worth mentioning that current drinking entered the model after adjusting for the effects of both the PPARλ and IL6 SNPs on DBP. In the final model, gender, BMI, PPARλ SNP and current drinking were found to significantly associate with DBP. Moreover, race was found to borderline significantly associate with DBP (p-value =0.08). Adjusting for IL6 and PPARλ SNPs, age, gender, race, BMI and current drinking (when applicable), the decrease in DBP was found to be ~ 4 mm, 4.6 mm for a female compared to male and for a subject with at least one Ala12 allele of the the PPARλ SNP compared to a subject with the wild genotype(Pro12Pro) respectively. On the other hand, the increase in DBP was found to be ~3mm,~ 0.3mm and ~5.5mm, for an African American subject compared to a Caucasian, for a subject whose BMI is 1 kg/m^2 higher and for a subject who currently drinks compared to a subject who does not currently drink respectively**(Table 53**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. An exception is that this test showed that the IL6 CG genotype of the IL6 SNP is borderline significantly associated with DBP(p-value~0.09), as opposed to the regression results which showed no significant association (p-value~0.14). In the final model, gender, race, BMI, PPARλ SNP and current drinking were found to significantly associate with DBP. Moreover, the IL6 CG genotype of the IL6 SNP was found to borderline significantly associate with DBP (p-value~0.09). Adjusting for IL6 CG genotype, gender, race, BMI , PPARλ SNP and current drinking (when applicable), the decrease in DBP was found to be ~ 4.3 mm and ~4.7 mm for a female compared to male and for a subject with at least one Ala12 allele of the the PPARλ SNP compared to a subject with the wild genotype(Pro12Pro) respectively. On the other hand, the increase in DBP was found to be ~2 mm, ~3 mm, ~ 0.4 mm and ~ 5.7 mm, for a subject with the IL6 CG genotype compared to a subject with the wild genotype (CC), for an African American subject compared to a Caucasian, for a subject whose BMI is 1 kg/m^2 higher and for a subject who currently drinks compared to a subject who does not currently drink respectively. The residual heritability for DBP was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a

genetic model in explaining the variation in DBP, adjusting for IL6CG genotype of the IL6 SNP, gender, race, BMI, PPARλ SNP and current drinking. Moreover, the proportion of variance in DBP which is explained by IL6 CG genotype, gender, race, BMI, PPARλ SNP and current drinking is ~ 44% **(Table 53).** Excluding the IL6 SNP and running the model including only gender, race, BMI, PPARλ SNP and current drinking and then comparing $\sigma^2_{\Sigma\beta}$ from this model ($\sigma^2_{\Sigma\beta}$ =0.41) and the $\sigma^2_{\Sigma\beta}$ from the model shown in **Table 53** ($\sigma^2_{\Sigma\beta}$ =0.44), showed that gender, race, BMI , PPARλ SNP and current drinking explain 41% variation in DBP and IL6CG genotype of the IL6 SNP explains an additional 3%.

Table 53: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of DBP and G-174C IL6 SNP in Total Sample (N=99)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.00 p value = 0.50 $\sigma^2_{\Sigma\beta}$ =0.44		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	2.232	1.487	0.137	-0.723,5.187	**2.183**	**1.321**	**0.091**
IL6 GG	0.707	1.858	0.704	-2.984,4.398	——	——	0.662
Age	0.047	0.037	0.213	-0.027,0.121	——	——	——
Gender	**-3.827**	**1.515**	**0.013**	**-6.836,-0.818**	**-4.268**	**1.419**	**0.003**
Race	**3.182**	**1.809**	**0.082**	**0.411,6.776**	**2.907**	**1.707**	**0.081**
BMI	**0.309**	**0.095**	**0.002**	**0.121,0.497**	**0.345**	**0.087**	**0.0001**
PPARλ P12A	**-4.616**	**1.985**	**0.022**	**-8.559,-0.673**	**-4.699**	**1.891**	**0.018**
Current drinking	**5.479**	**1.346**	**0.000**	**2.804,8.153**	**5.710**	**1.283**	**0.00003**

5.5.2.1.5.2 PPARλ P12A SNP (N=99)

No potential PPARλ SNP interactions were identified. It is noteworthy to mention that the unadjusted effect of the PPARλ SNP on DBP was significant (p-value = 0.002). In the final model, PPARλ SNP, gender, BMI and current drinking were found to statistically significantly associate with DBP. Moreover, race was found to borderline significantly associate with DBP (p-value= 0.099). Adjusting for PPARλ SNP, age, gender, race, BMI and current drinking (when applicable), the decrease in DBP was found to be 4.7 mm and 3.3 mm for a subject with at least one Ala12 allele of the the PPARλ SNP compared to a subject with the wild genotype(Pro12Pro) and for a female compared to a male respectively . On the other hand, the increase in DBP was found to be ~3 mm, ~ 0.3 mm and 5.7 mm, for an African American subject compared to a Caucasian, for a subject whose BMI is 1 kg/m^2 higher and for a subject

who currently drinks compared to a subject who does not currently drink respectively (**Table 54**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. An exception is that this test showed that race was not significantly associated with DBP, as opposed to the regression results which showed a borderline significant association. In the final model, PPARλ SNP, gender, BMI, and current drinking were found to significantly associate with DBP. Adjusting for PPARλ SNP, gender, BMI and current drinking (when applicable), the decrease in DBP was found to be ~5 mm and 4 mm for a subject with at least one Ala12 allele of the the PPARλ SNP compared to a subject with the wild genotype(Pro12Pro) and for a female compared to a male respectively . On the other hand, the increase in DBP was found to be ~ 0.4 mm and 5.6 mm, for a subject whose BMI is 1 kg/m^2 higher and for a subject who currently drinks compared to a subject who does not currently drink respectively. The residual heritability for DBP was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model, adjusting for PPARλ SNP, gender, BMI, and current drinking. Moreover, the proportion of variance in DBP which is explained by PPARλ SNP, gender, BMI, and current drinking is ~ 41% **(Table 54).** Excluding the PPARλ P12A SNP and running the model including gender, BMI and current drinking only and then comparing $\sigma^2_{\Sigma\beta}$ from this model ($\sigma^2_{\Sigma\beta}$ =0.36) and the $\sigma^2_{\Sigma\beta}$ from the model shown in **Table 54** ($\sigma^2_{\Sigma\beta}$ =0.41), showed that gender, BMI and current drinking explain 36% of the variation in G0 and PPAR P12A SNP explains an additional 5%.

Table 54: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of DBP and Pro12Ala PPARλ SNP in Total Sample (N=99)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.00 p value = 0.50 $\sigma^2_{\Sigma\beta}$=0.41		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	**-4.708**	**1.969**	**0.019**	**-8.618,-0.797**	**-5.254**	**1.909**	**0.016**
Age	0.053	0.037	0.161	-0.021,0.126	———	———	———
Gender	**-3.305**	**1.473**	**0.027**	**-6.231,-0.380**	**-3.854**	**1.419**	**0.009**
Race	**2.960**	**1.779**	**0.099**	**-0.572,6.492**	———	———	0.107
BMI	**0.323**	**0.094**	**0.001**	**0.135,0.510**	**0.410**	**0.084**	**0.00006**
Current drinking	**5.652**	**1.341**	**0.000**	**2.989,8.314**	**5.580**	**1.302**	**0.00002**

Though the p-values for both the IL6CG genotype of the IL6 SNP and the PPARλ SNP were < 0.1 in their final models, we were not able to test the IL6 SNP PPARλ interaction because of the extremely small sample size in one IL6SNP-PPARλ SNP crosstab cells. Adjustment for the effect of PPARλ SNP in the final model of the IL6 SNP was performed since the p-value of the PPARλ SNP in its final model was < 0.05.

5.5.2.1.6 Fasting Glucose (G0, mg/dl)

For G0, the following variables were entered in a stepwise fashion: ever HRT, menopause status, CRP/ IL6 serum levels, SBP and TG.

5.5.2.1.6.1 IL6 G-174C SNP (N=96)

No potential IL6 SNP interactions were identified. In the final model, age, race and PPARλ SNP were found to significantly associate with G0. BMI was found to borderline significantly associate with fasting glucose (p-value=0.07). Adjusting for IL6 and PPARλ SNPs, age, gender, race and BMI (when applicable), the increase in G0 was found to be ~0.4 mg/dl and 0.3 mg/dl for a subject who is 1 year older and for a subject whose BMI is 1 kg/m^2 higher respectively. On the other hand, the decrease in G0 was found to be ~ 11 mg/dl and 8 mg/dl for an African American subject compared to a Caucasian and for a subject with at least one Ala12

allele of the PPARλ SNP compared to a subject with the wild genotype (Pro12Pro) respectively (**Table 55**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. PPARλ SNP was borderline significantly associated, rather than significantly associated with G0. In the final model, age and race were found to significantly associate with G0. BMI and PPARλ SNP were found to borderline significantly associate with fasting glucose (p-value=0.07 and 0.08 respectively). Adjusting for age, race BMI and PPARλ SNP (when applicable), the increase in G0 was found to be ~0.4 mg/dl and 0.3 mg/dl for a subject who is 1 year older and for a subject whose BMI is 1 kg/m^2 higher respectively. On the other hand, the decrease in G0 was found to be ~ 10 mg/dl and 7 mg/dl for an African American subject compared to a Caucasian and for a subject with at least one Ala12 allele of the the PPARλ SNP compared to a subject with the wild genotype (Pro12Pro) respectively. The residual heritability for G0 was found to be not significant, meaning that there is no genetic contribution to G0. Moreover, the proportion of variance in G0 which is explained by age, race, BMI and PPARλ SNP was found to be ~ 43% **(Table 55).**

Table 55: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of G0 and G-174C IL6 SNP in Total Sample (N=96)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.26±0.24 p value = 0.10 $\sigma^2_{\Sigma\beta}$=0.43		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	2.474	2.725	0.366	-7.889,2.914	——	——	0.167
IL6 GG	-3.688	3.432	0.286	-10.508,3.133	——	——	0.481
Age	**0.413**	**0.067**	**0.000**	**0.280,0.546**	**0.410**	**0.060**	**$3x10^{-10}$**
Gender	-1.049	2.826	0.711	-6.666,4.567	——	——	——
Race	**-11.067**	**3.402**	**0.002**	**-17.828,-4.306**	**-10.346**	**3.857**	**0.012**
BMI	**0.317**	**0.174**	**0.071**	**-0.028,0.662**	**0.292**	**0.169**	**0.08**
PPARλ P12A	**-8.444**	**3.568**	**0.020**	**-15.534,-1.354**	**-7.007**	**3.775**	**0.08**

5.5.2.1.6.2 PPARλ P12A SNP(N=96)

PPARλ SNP interaction with age was tested and found to be not significant. It is noteworthy to mention that the effect of the PPARλ SNP on G0 became borderline significant (p-value was 0.121, became 0.08) at the time age entered the regression model and became significant (p-

value=0.028) when race entered the model. We were not able to test for PPARλ SNP and race interaction because of the absence of African Americans in the Ala-X category. In the final model, PPARλ SNP, age and race were found to significantly associate with G0. BMI was found to borderline significantly associate with fasting glucose (p-value=0.08). Adjusting for PPARλ SNP, age, gender, race and BMI (when applicable), the decrease in G0 was found to be ~8 mg/dl and 10.5 mg/dl for a subject with at least one Ala12 allele of the PPARλ SNP compared to a subject with the wild genotype(Pro12Pro) and for an African American compared to a Caucasian respectively. On the other hand, the increase in G0 was found to be ~0.41 mg/dl and ~ 0.31 mg/dl for a subject who is 1 year older and for a subject whose BMI is 1 kg/m^2 higher respectively (**Table 56**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results; PPARλ SNP was found to borderline significantly associate rather than significantly associate with G0. In the final model, age and race were found to significantly and BMI was found to borderline significantly associate with G0. Adjusting for PPARλ SNP, age, race and BMI (when applicable), the decrease in G0 was found to be ~7 mg/dl and ~10 mg/dl for a subject with at least one Ala12 allele of the PPARλ SNP compared to a subject with the wild genotype(Pro12Pro) and for an African American compared to a Caucasian respectively. On the other hand, the increase in G0 was found to be ~0.41 mg/dl and ~ 0.30 mg/dl for a subject who is 1 year older and for a subject whose BMI is 1 kg/m^2 higher respectively. The residual heritability for G0 was found to be not significant, meaning that there is no genetic contribution to G0. Moreover, the proportion of variance in G0 which is explained by age, race, BMI and PPARλ SNP was found to be ~ 43% **(Table 56).** Excluding the PPARλ P12A SNP and running the model including age, race and BMI only and then comparing $\sigma^2_{\Sigma\beta}$ from this model ($\sigma^2_{\Sigma\beta}$ =0.39) and the $\sigma^2_{\Sigma\beta}$ from the model shown in **Table 56** ($\sigma^2_{\Sigma\beta}$ =0.43), showed that age, race and BMI explains 39% of the variation in G0 and PPAR P12A SNP explains an additional 4 %.

Table 56: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of G0 and Pro12Ala PPARλ SNP in Total Sample (N=96)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.26±0.24 p value = 0.10 $\sigma^2_{\Sigma\beta}$=0.43		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	**-7.865**	**3.519**	**0.028**	**-14.857,-0.873**	**-7.007**	**3.775**	**0.070**
Age	**0.405**	**0.066**	**0.000**	**0.275,0.536**	**0.410**	**0.060**	**$8x10^{-10}$**
Gender	-1.308	2.720	0.632	-6.713,4.096	____	_____	____
Race	**-10.454**	**3.346**	**0.002**	**-17.101,-3.807**	**-10.345**	**3.857**	**0.014**
BMI	**0.311**	**0.173**	**0.075**	**-0.032,0.654**	**0.292**	**0.169**	**0.086**

Since the p-value for the IL6 SNP was > 0.1 in its final model, there was no need to assess gene-gene interaction. Adjustment for the effect of PPARλ SNP in the final model of the IL6 SNP was performed since the p-value of the PPARλ SNP in its final model was < 0.05.

5.5.2.1.7 Ln-Interleukin 6 (ln-IL6 pg/ml)

For ln-IL6 serum level, the following variables were entered in a stepwise fashion: menopause status, CRP serum level, DBP, LDL and physical activity (Kcal/Week).

5.5.2.1.7.1 IL6 G-174C SNP (N=101)

IL6 SNP interactions with race, fasting insulin and C-reactive protein level (CRP) were tested and found to be not significant. It is noteworthy to mention that the effect of the IL6 SNP on ln-IL6 serum level became borderline significant (p-value was 0.132, became ~ 0.07) at the time fasting insulin entered the regression model and became more significant (p-value=0.06) when CRP serum level entered the model. In the final model, race, BMI and CRP serum level were found to significantly associate with ln-IL6 serum level. IL6 GG genotype of the IL6 SNP was found to borderline significantly associate with IL6 serum level as well (p-value=0.056). Adjusting for IL6 SNP, age, gender, race, fasting insulin, PCOS status, BMI and CRP serum level (when applicable), the increase in ln-IL6 serum level was found to be 0.28 pg/ml, ~0.61 pg/ml, ~0.02 pg/ml and ~ 0.06 pg/ml for a subject with the GG genotype of the IL6 SNP compared to a subject with the wild genotype(CC), for an African American compared to a

Caucasian, for a subject whose BMI is 1 kg/m^2 higher and for a subject whose CRP serum levels are 1 mg/L higher than another subject's respectively (**Table 57**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. In the final model, IL6 GG genotype of the IL6 SNP (as opposed to borderline significance in the regression model), race, BMI and CRP serum level were found to significantly associate with ln-IL6 serum level. Adjusting for IL6 GG genotype of the IL6 SNP, race, BMI and CRP serum level (when applicable), the increase in ln-IL6 serum level was found to be 0.25 pg/ml, ~0.57 pg/ml, ~0.03 pg/ml and ~ 0.06 pg/ml for a subject with the GG genotype of the IL6 SNP compared to a subject with the wild genotype(CC), for an African American compared to a Caucasian, for a subject whose BMI is 1 kg/m^2 higher and for a subject whose CRP serum levels are 1 mg/L higher respectively. The residual heritability for ln-IL6 was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in ln-IL6, adjusting for IL6 GG genotype of the IL6 SNP, race, BMI and CRP serum level. Moreover, the proportion of variance in ln-IL6 which is explained by the IL6 GG genotype of the IL6 SNP, race, BMI and CRP serum level is ~ 61% **(Table 57).** Excluding the IL6 SNP and running the model including race, BMI and CRP serum level only and then comparing $\sigma^2_{\Sigma\beta}$ from this model ($\sigma^2_{\Sigma\beta}$ =0.59) and the $\sigma^2_{\Sigma\beta}$ from the model shown in **Table 57** ($\sigma^2_{\Sigma\beta}$ =0.61), showed that race, BMI and CRP serum level explains 59% variation in ln-IL6 and IL6 GG genotype of the IL6 SNP explains an additional 2%.

Table 57: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-IL6 and G-174C IL6 SNP in Total Sample (N=101)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.00 p value = 0.50 $\sigma^2_{\Sigma\beta}$ =0.61		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	0.039	0.116	0.734	-0.191,0.270	____	_____	0.552
IL6 GG	**0.280**	**0.145**	**0.056**	**-0.007,0.568**	**0.250**	**0.129**	**0.045**
Age	0.003	0.003	0.353	-0.003,0.009	____	_____	____
Gender	0.099	0.124	0.424	-0.146,0.345	____	_____	____
Race	**0.607**	**0.150**	**0.000**	**0.309,0.904**	**0.574**	**0.134**	**0.0001**
Fasting Insulin	-0.003	0.006	0.591	-0.014,0.008	____	_____	____
PCOS status	0.128	0.148	0.390	-0.166,0.422	____	_____	____
BMI	**0.024**	**0.010**	**0.023**	**0.003,0.044**	**0.026**	**0.008**	**0.001**
CRP(mg/L)	**0.061**	**0.009**	**0.000**	**0.044,0.078**	**0.061**	**0.008**	**$4x10^{-12}$**

5.5.2.1.7.2 PPARλ P12A SNP (N=101)

No potential PPARλ SNP interactions were identified. In the final model, race, BMI and CRP serum level were found to significantly associate with ln-IL6 serum level. Adjusting for PPARλ SNP, age, gender, race, fasting insulin, PCOS status, BMI and CRP serum level (when applicable), the increase in ln-IL6 serum level was found to be ~0.54pg/ml, ~0.03 pg/ml and ~0.06 pg/ml for an African American compared to a Caucasian, for a subject whose BMI is 1 kg/m^2 higher and for a subject whose CRP serum levels are 1 mg/L higher than another subject's respectively (**Table 58**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, race, BMI and CRP serum level were found to significantly associate with ln-IL6 serum level. Adjusting for race, BMI and CRP serum level (when applicable), the increase in ln-IL6 serum level was found to be ~0.54pg/ml, ~0.03 pg/ml and ~0.06 pg/ml for an African American compared to a Caucasian, for a subject whose BMI is 1 kg/m^2 higher and for a subject whose CRP serum level are 1 mg/L higher than another subject's respectively. The residual heritability for ln-IL6 was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in ln-IL6, adjusting for race, BMI, and CRP serum level. Moreover, the

proportion of variance in ln-IL6 which is explained by race, BMI and CRP serum level was found to be ~ 59% **(Table 58).**

Table 58: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-IL6 and Pro12Ala PPARλ SNP in Total Sample (N=101)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.00 p value = 0.50 $\sigma^2_{\Sigma\beta}$ =0.59		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.133	0.155	0.390	-0.441,0.174	____	_____	0.393
Age	0.003	0.003	0.392	-0.003,0.009	____	_____	____
Gender	0.088	0.122	0.470	-0.153,0.330	____	_____	____
Race	**0.535**	**0.151**	**0.001**	**0.236,0.834**	**0.540**	**0.135**	**0.0007**
Fasting Insulin	-0.004	0.006	0.463	-0.015,0.007	____	_____	____
PCOS status	0.124	0.149	0.408	-0.172,0.421	____	_____	____
BMI	**0.025**	**0.010**	**0.020**	**0.004,0.045**	**0.025**	**0.008**	**0.002**
CRP(mg/L)	**0.062**	**0.009**	**0.000**	**0.044,0.079**	**0.062**	**0.008**	**$5x10^{-12}$**

Since the p-value for the PPARλ SNP was > 0.1 in its final model, there was no need to assess gene-gene interaction. Adjustment for the effect of either SNP was unnecessary since the p-value of each in its final regression model was > 0.05.

5.5.2.1.8 Ln-C Reactive Protein (ln-CRP mg/L)

For ln-CRP serum level, the following variables were entered in a stepwise fashion: current drinking, ever OC use, ever HRT use, menopause status, IL6 serum level, SBP, DBP, LDL, TG and physical activity(Kcal/Week).

5.5.2.1.8.1 IL6 G-174C SNP (N=98)

No potential IL6 SNP -environment interactions were identified. In the final model, age, fasting insulin, BMI, IL6 serum level and ever OC use were found to significantly associate with ln-CRP serum level. Gender and race were found to borderline significantly associate with ln-CRP serum level as well (p-value ~ 0.07 and ~0.09 respectively). Adjusting for IL6 SNP, age, gender, race, fasting insulin, PCOS status, BMI, IL6 serum level and ever OC use (when

applicable), the increase in ln-CRP serum level was found to be ~ 0.02 mg/L,~ 0.10 mg/L, ~0.25 mg/L and ~0.74 mg/L for a subject who is 1 year older, for a subject whose BMI is 1 kg/m^2 higher, for a subject whose IL6 serum levels are 1 pg/ml higher than another subject's and for a female who ever used OC compared to a female who never used OC respectively. On the other hand the decrease in ln-CRP serum level was found to be ~ 0.66 mg/L, 0.66 mg/L and 0.03 mg/L for a female compared to a male, for an African American compared to a Caucasian and for a subject, whose fasting insulin level is 1 μU/ml higher respectively (**Table 59**).

The findings of the **VARIANCE COMPONENTS TEST** were pretty well consistent with the regression results. In the final model, age, fasting insulin, BMI, IL6 serum level and ever OC use were found to significantly associate with ln-CRP serum level. Gender was also found to borderline significantly associate with ln-CRP serum level as well (p-value ~ 0.05). However, race was found to be not significantly associated with ln-CRP, as opposed to the "borderline significance" regression result for this variable (p-value ~0.09). Adjusting for age,gender, fasting insulin, BMI, IL6 serum level and ever OC use (when applicable), the increase in ln-CRP serum level was found to be ~ 0.01 mg/L,~ 0.09 mg/L, ~0.26 mg/L and ~0.92 mg/L for a subject who is 1 year older, for a subject whose BMI is 1 kg/m^2 higher, for a subject whose IL6 serum level are 1 pg/ml higher than another subject's and for a female who ever used OC compared to a female who never used OC respectively. On the other hand the decrease in ln-CRP serum level was found to be ~ 0.62 mg/L and 0.03 mg/L for a female compared to a male and for a subject whose fasting insulin level is 1 μU/ml higher than another subject's respectively. The residual heritability for ln-CRP was found to be significant and equal to 62%, meaning that ~62% of the total variation in ln-CRP is due to other genetic factors, adjusting for age, gender, fasting insulin, BMI, IL6 serum level and ever OC use. Moreover, the proportion of variance in ln-IL6 which is explained by the age, gender, fasting insulin, BMI, IL6 serum level and ever OC use was found to be ~ 50% **(Table 59).**

Table 59: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-CRP and G-174C IL6 SNP in Total Sample (N=98)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.62±0.24 p value =0.01 $\sigma^2_{\Sigma\beta}$ =0.50		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.037	0.297	0.900	-0.628,0.553	____	_____	0.161
IL6 GG	-0.358	0.376	0.345	-1.106,0.391	____	_____	0.129
Age	**0.017**	**0.008**	**0.040**	**0.001,0.032**	**0.014**	**0.007**	**0.040**
Gender	**-0.661**	**0.358**	**0.068**	**-1.373,0.051**	**-0.621**	**0.315**	**0.056**
Race	**-0.664**	**0.390**	**0.092**	**-1.439,0.111**	____	_____	0.121
Fasting Insulin	**-0.032**	**0.015**	**0.036**	**-0.062,-0.002**	**-0.027**	**0.014**	**0.018**
PCOS status	0.479	0.389	0.221	-0.294,1.252	____	_____	____
BMI	**0.102**	**0.026**	**0.000**	**0.050,0.154**	**0.085**	**0.023**	**0.0002**
IL6(pg/ml)	**0.251**	**0.057**	**0.000**	**0.138,0.365**	**0.263**	**0.052**	**$5x10^{-7}$**
Ever OC use	**0.741**	**0.338**	**0.031**	**0.068,1.413**	**0.916**	**0.274**	**0.001**

5.5.2.1.8.2 PPARλ P12A SNP(N=98)

No potential PPARλ SNP-environment interactions were identified. In the final model, age, fasting insulin, BMI, IL6 serum level and ever OC use were found to significantly associate with ln-CRP serum level. Gender and race were found to borderline significantly associate with ln-CRP serum level as well (p-values=0.07 and 0.088 respectively). Adjusting for PPARλ SNP, age, gender, race, fasting insulin, PCOS status, BMI, IL6 serum level and ever OC use (when applicable), the increase in ln-CRP serum level was found to be ~ 0.02 mg/L,~ 0.10 mg/L, ~0.24 mg/L and ~0.79 mg/L for a subject who is 1 year older, for a subject whose BMI is 1 kg/m^2 higher, for a subject whose IL6 serum levels are 1 pg/ml higher than another subject's and for a female who ever used OC compared to a female who never used OC respectively. On the other hand the decrease in ln-CRP serum level was found to be ~0.65mg/L, 0.66 mg/L and 0.03 mg/L for a female compared to a male, an African American compared to a Caucasian and for a subject whose fasting insulin level is 1 µU/ml higher (**Table 60**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. As opposed to the results of the regression model, age was borderline significantly vs. significantly, gender was significantly vs. borderline significantly and race was

not significant vs. borderline significantly associated with ln-CRP. In the final model gender, fasting insulin, BMI, IL6 serum level and ever OC use were found to significantly associate with ln-CRP serum level. Age was found to borderline significantly associate with CRP serum level as well (p-value=0.08). Adjusting for age, gender, fasting insulin, BMI, IL6 serum level and ever OC use (when applicable), the increase in ln-CRP serum level was found to be ~ 0.01 mg/L,~ 0.09 mg/L, ~0.26 mg/L and ~0.92 mg/L for a subject who is 1 year older, for a subject whose BMI is 1 kg/m^2 higher, for a subject whose IL6 serum levels are 1 pg/ml higher than another subject's and for a female who ever used OC compared to a female who never used OC respectively . On the other hand the decrease in ln-CRP serum level was found to be ~ 0.62 mg/L and 0.03 mg/L for a female compared to a male and for a subject, whose fasting insulin level is 1 μU/ml higher than another subject's. The residual heritability for ln-CRP was found to be significant and equal to 62%, meaning that ~62% of the total variation in ln-CRP is due to other genetic factors, adjusting for age, gender, fasting insulin, BMI, IL6 serum level and ever OC use. Moreover, the proportion of variance in ln-IL6 which is explained by the age, gender, fasting insulin, BMI, IL6 serum level and ever OC use was found to be ~ 50% **(Table 60).**

Table 60: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-CRP and Pro12Ala PPARλ SNP in Total Sample (N=98)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.62±0.24 p value = 0.01 $\sigma^2_{\Sigma\beta}$ =0.50		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.503	0.382	0.191	-1.262,0.256	____	_____	0.274
Age	**0.017**	**0.008**	**0.037**	**0.001,0.032**	**0.014**	**0.007**	**0.078**
Gender	**-0.645**	**0.352**	**0.070**	**-1.345,0.055**	**-0.620**	**0.315**	**0.049**
Race	**-0.657**	**0.381**	**0.088**	**-1.414,0.101**	____	_____	0.138
Fasting Insulin	**-0.030**	**0.015**	**0.046**	**-0.059,-0.001**	**-0.027**	**0.014**	**0.024**
PCOS status	0.465	0.383	0.228	-0.296,1.225	____	_____	_____
BMI	**0.100**	**0.026**	**0.000**	**0.049,0.151**	**0.085**	**0.023**	**0.0001**
IL6(pg/ml)	**0.241**	**0.055**	**0.000**	**0.131,0.351**	**0.263**	**0.052**	**$2.5x10^{-6}$**
Ever OC use	**0.792**	**0.330**	**0.019**	**0.135,1.448**	**0.916**	**0.273**	**0.001**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor to adjust for the effect of either SNP in the other SNP's final model.

5.5.2.1.9 Ln-HOMA-IR

For ln-HOMA-IR, the following variables were entered in a stepwise fashion: menopause status, CRP serum level, IL6 serum level, SBP, DBP, LDL, HDL and TG. Current anti-diabetic medication use was forced into both the regression and variance components models.

5.5.2.1.9.1 IL6 G-174C SNP (N=101)

No potential IL6 SNP interactions were identified. In the final model, BMI, current anti-diabetic medication use and HDL serum level were found to significantly associate with ln-HOMA-IR. Race was found to borderline significantly associate with ln-HOMA-IR as well (p-value ~ 0.05). Adjusting for IL6 SNP, age, gender, race, PCOS status, BMI, anti-diabetic medication use and HDL serum level (when applicable), the increase in HOMA-IR was found to be ~0.04 μU/ml* mmol/L and ~0.32 μU/ml* mmol/L for a subject whose BMI is 1 kg/m^2 higher and for a subject who currently takes anti-diabetic meds compared to a current non-user. On the other hand, the decrease in ln-HOMA-IR was found to be ~0.24 μU/ml* mmol/L and ~0.01 μU/ml* mmol/L for an African American compared to a Caucasian and for a subject whose HDL serum levels are 1 mg/dl higher than another subject's respectively (**Table 61**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. As opposed to the results of the regression model, race was not significant rather than borderline significantly associated with ln-HOMA-IR. In the final model, BMI, current anti-diabetic medication use and HDL serum level were found to significantly associate with ln-HOMA-IR. Adjusting for BMI, current anti-diabetic medication use and HDL serum level(when applicable), the increase in HOMA-IR was found to be ~0.04 μU/ml* mmol/L and ~0.35 μU/ml* mmol/L for a subject whose BMI is 1 kg/m^2 higher and for a subject who currently takes anti-diabetic meds compared to a current non-user. On the other hand, the decrease in ln-HOMA-IR was found to be 0.01 μU/ml* mmol/L for a subject whose HDL serum level are 1 mg/dl higher than another subject's respectively. The residual

heritability for ln-HOMA-IR was found to be significant and equal to 48%, meaning that ~48% of the total variation in ln-HOMA-IR is due to other genetic factors, adjusting for BMI, current anti-diabetic medication use and HDL serum level. Moreover, the proportion of variance in ln-HOMA-IR which is explained by the BMI, current anti-diabetic medication use and HDL serum level was found to be 47% **(Table 61).**

Table 61: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HOMA-IR and G-174C IL6 SNP in Total Sample (N=101)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.48±0.16 p value =0.0005 $\sigma^2_{\Sigma\beta}$=0.47		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.048	0.094	0.606	-0.234,0.138	_______	______	0.289
IL6 GG	-0.001	0.119	0.993	-0.238,0.235	_______	______	0.954
Age	-4.1×10^{-5}	0.002	0.987	-0.005,0.005	_______	______	_______
Gender	0.109	0.102	0.288	-0.094,0.312	_______	______	_______
Race	**-0.238**	**0.121**	**0.053**	**-0.479,0.003**	_______	______	0.142
PCOS status	-0.094	0.130	0.473	-0.352,0.165	_______	______	_______
BMI	**0.042**	**0.007**	**0.000**	**0.028,0.056**	**0.035**	**0.006**	**7×10^{-9}**
Current Anti-diabetic Meds use	**0.321**	**0.130**	**0.015**	**0.063,0.579**	**0.353**	**0.110**	**0.002**
HDL(mg/dl)	**-0.009**	**0.004**	**0.016**	**-0.016,-0.002**	**-0.010**	**0.003**	**0.004**

5.5.2.1.9.2 PPARλ P12A SNP(N=101)

No potential PPARλ SNP interactions were identified. In the final model, race, BMI, current anti-diabetic medication use and HDL serum level were found to significantly associate with ln-HOMA-IR. Adjusting for PPARλ SNP, age, gender, race, PCOS status, BMI, current anti-diabetic medication use and HDL serum level (when applicable), the increase in ln-HOMA-IR was found to be ~0.04 µU/ml* mmol/L and ~0.32 µU/ml* mmol/L for a subject whose BMI is 1 kg/m^2 higher and for a subject who currently takes anti-diabetic meds compared to a current non-user. On the other hand, the decrease in ln-HOMA-IR was found to be ~0.26 µU/ml* mmol/L and ~0.01 µU/ml* mmol/L for an African American compared to a Caucasian and for a subject whose HDL serum level are 1 mg/dl higher than another subject's respectively (**Table 62**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. As opposed to the results of the regression model, race was not significant rather than borderline significantly associated with ln-HOMA-IR. In the final model, BMI, current anti-diabetic medication use and HDL serum level were found to significantly associate with ln-HOMA-IR. Adjusting for BMI, current anti-diabetic medication use and HDL serum level(when applicable), the increase in HOMA-IR was found to be ~0.04 μU/ml* mmol/L and ~0.35 μU/ml* mmol/L for a subject whose BMI is 1 kg/m^2 higher and for a subject who currently takes anti-diabetic meds compared to a current non-user. On the other hand, the decrease in ln-HOMA-IR was found to be 0.01 μU/ml* mmol/L for a subject whose HDL serum level is 1 mg/dl higher respectively. The residual heritability for ln-HOMA-IR was found to be significant and equal to 48%, meaning that ~48% of the total variation in ln-HOMA-IR is due to other genetic factors, adjusting for BMI, current anti-diabetic medication use and HDL serum level. Moreover, the proportion of variance in ln-HOMA-IR which is explained by the BMI, current anti-diabetic medication use and HDL serum level was found to be 47% **(Table 62).**

Table 62: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HOMA-IR and Pro12Ala PPARλ SNP in Total Sample (N=101)

Regression					VARIANCE COMPONENTS TEST		
					H2r=48±0.16 p value =0.0005 $\sigma^2_{\Sigma\beta}$=0.47		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.198	0.122	0.106	-0.440,0.043	_____	_____	0.222
Age	0.000	0.002	0.898	-0.005,0.004	_____	_____	_____
Gender	0.101	0.097	0.300	-0.091,0.294	_____	_____	_____
Race	**-0.263**	**0.118**	**0.028**	**-0.497,-0.030**	_____	_____	0.121
PCOS status	-0.090	0.128	0.485	-0.343,0.164	_____	_____	_____
BMI	**0.041**	**0.007**	**0.000**	**0.027,0.055**	**0.035**	**0.006**	**$1x10^{-8}$**
Current Anti-diabetic Meds use	**0.324**	**0.127**	**0.013**	**0.071,0.576**	**0.353**	**0.110**	**0.002**
HDL(mg/dl)	**-0.009**	**0.004**	**0.013**	**-0.016,-0.002**	**-0.010**	**0.003**	**0.005**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor to adjust for the effect of either SNP in the other SNP's final model.

5.5.2.2 Subgroup Analysis -Females (N=72)

5.5.2.2.1 Waist (cm)

For waist, the following variables were entered in a stepwise fashion: ever smoking, ever OC use, current OC use, menopause status, HDL, LDL, TG, CRP/IL6 serum levels, SBP and DBP; smoking was defined by smoking cigarettes or/&pipes or/&cigars).

5.5.2.2.1.1 IL6 G-174C SNP (N=72)

Potential IL6 SNP interactions with ever smoking was assessed and found to be not significant. It is noteworthy to mention that the effect of IL6 GG genotype on waist became borderline significant (p-value was 0.16 and became 0.06) at the time "ever smoking" variable entered the regression model. In the final model, IR, BMI and ever smoking were found to significantly associate with waist. Moreover, the IL6 GG genotype was found to borderline significantly associate with waist in females (p-value ~0.06). Adjusting for IL6 SNP, age, race, IR, PCOS status, BMI and ever smoking (when applicable), the decrease in waist was found to be ~ 4 cm for a female with the IL6 GG genotype compared to a female with the CC genotype. On the other hand, the increase in waist was found to be ~ 5 cm, ~1.8 cm and ~ 4.5 cm for an insulin resistant female compared to a non-IR female, for a female whose BMI is 1 kg/m^2 higher and for a female who ever smoked compared to another who never smoked respectively(**Table 63**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. As opposed to the results of the regression model, IL6GG genotype was found to be not significantly rather than borderline significantly associated with waist. In the final model, IR, BMI and ever smoking variable were found to be significantly associated with waist. Adjusting for IR, BMI and ever smoking (when applicable), the increase in waist was found to be ~ 5 cm, ~1.9 cm and ~ 4 cm for an insulin resistant female compared to a non-IR female, for a female whose BMI is 1 kg/m^2 higher and for a female who ever smoked compared to another who never smoked respectively. The residual heritability for waist was found to be significant and equal to 53%, meaning that ~53% of the total variation in waist is due to other genetic factors, adjusting for IR, BMI and ever smoking. Moreover, the proportion

of variance in waist which is explained by the IR, BMI and ever smoking was found to be ~ 89% **(Table 63).**

Table 63: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of Waist and G-174C IL6 SNP in Females (N=72)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.53±0.21 p value=0.002 $\sigma^2_{\Sigma\beta} = 0.89$		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.927	1.613	0.568	-4.149-2.296	____	____	0.934
IL6 GG	**-4.211**	**2.215**	**0.062**	**-8.637-0.215**	____	____	0.289
Age	0.029	0.044	0.510	-0.059-0.118	____	____	_____
Race	1.286	2.219	0.564	-3.148-5.720	____	____	_____
IR	**5.161**	**1.686**	**0.003**	**1.792-8.530**	**5.244**	**1.474**	**0.0006**
PCOS Status	0.044	1.892	0.982	-3.738-3.825	____	____	_____
BMI	**1.796**	**0.129**	**0.000**	**1.538-2.055**	**1.868**	**0.098**	**$4x10^{-30}$**
Ever smoking	**4.499**	**1.532**	**0.005**	**1.438-7.560**	**3.944**	**1.372**	**0.004**

5.5.2.2.1.2 PPARλ P12A SNP (N=72)

No Potential PPARλ SNP interactions were identified. In the final model, IR, BMI and ever smoking were found to significantly associate with waist. Adjusting for PPARλ SNP, age, race, IR, PCOS status, BMI and ever smoking (when applicable), the increase in waist was found to be ~ 5.5 cm, ~1.8 cm and ~ 4 cm for an insulin resistant female compared to a non-IR female, for a female whose BMI is 1 kg/m^2 higher and for a female who ever smoked compared to another who never smoked respectively (**Table 64**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, IR, BMI and ever smoking were found to be significantly associated with waist. Adjusting for IR, BMI and ever smoking (when applicable), the increase in waist was found to be ~ 5 cm, ~1.9 cm and ~ 4 cm for an insulin resistant female compared to a non-IR female, for a female whose BMI is 1 kg/m^2 higher and for a female who ever smoked compared to another who never smoked respectively. The residual heritability for waist was found to be significant and equal to 53%, meaning that ~53% of the genetic variation in waist is due to other genetic factors, adjusting for IR, BMI and ever smoking. Moreover, the proportion of variance in waist which is explained by the IR, BMI and ever smoking was found to be ~ 89% **(Table 64).**

Table 64: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of Waist and Pro12Ala PPARλ SNP in Females (N=72)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.53±0.21 p-value=0.002 $\sigma^2_{\Sigma\beta}$ = 0.89		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	0.179	2.152	0.934	-4.121,4.478	____	____	0.990
Age	0.042	0.045	0.350	-0.047,0.131	____	____	____
Race	2.126	2.218	0.341	-2.305,6.558	____	____	____
IR	**5.507**	**1.738**	**0.002**	**2.034,8.980**	**5.244**	**1.474**	**0.0008**
PCOS Status	0.344	1.915	0.858	-3.481,4.170	____	____	____
BMI	**1.753**	**0.129**	**0.000**	**1.495,2.012**	**1.868**	**0.098**	**$3x10^{-30}$**
Ever Smoking	**4.145**	**1.555**	**0.010**	**1.040,7.251**	**3.944**	**1.372**	**0.005**

Since the p-value for the PPARλ SNP was > 0.1 in its final model, there was no need to assess gene-gene interaction. Adjustment for the effect of either SNP in the final model of the other SNP was unnecessary as well, since the p-value for each of the the PPARλ and IL6 SNPs in its final model was > 0.05.

5.5.2.2.2 Ln-Triglycerides (ln-TG,mgdl)

For ln-TG, the following variables were entered in a stepwise fashion: ever smoking, current drinking, ever OC use, current OC use, ever HRT use, menopause status, SBP, DBP and physical activity (Kcal/week); smoking was defined by smoking cigarettes or/&pipes or/&cigars.

5.5.2.2.2.1 IL6 G-174C SNP (N=72)

No Potential IL6 SNP interactions were identified. In the final model, age, race, IR, current OC use and ever smoking were found to significantly associate with ln-TG. Adjusting for IL6 SNP, age, race, IR, PCOS status, BMI, current OC use and ever smoking (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.36 mg/dl, ~ 0.52 mg/dl and ~ 0.33 mg/dl for a female who is 1 year older, for an insulin resistant female compared to a non- IR female, for a female who currently uses OC compared to a non-current OC user and for a female who

ever smoked compared to another female who never smoked respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.46 mg/dl for an African American female compared to a Caucasian female (**Table 65**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, age, race, IR, current OC use and ever smoking were found to significantly associate with ln-TG. Adjusting for age, race, IR, current OC use and ever smoking (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.41 mg/dl, ~ 0.46 mg/dl and ~ 0.33 mg/dl for a female who is 1 year older, for an insulin resistant female compared to a non-IR female, for a female who currently uses OC compared to a non-current OC user and for a female who ever smoked compared to another female who never smoked respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.33 mg/dl for an African American female compared to a Caucasian female. The residual heritability for ln-TG was found to be not significant, meaning that there is no genetic contribution to ln-TG. Moreover, the proportion of variance in ln-TG which is found to be explained by the age, race, IR, current OC use and ever smoking is ~ 38% **(Table 65).**

Table 65: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-TG and G-174C IL6 SNP in Females (N=72)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.08±0.18 p-value=0.325 $\sigma^2_{\Sigma\beta}$ = 0.38		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.096	0.127	0.453	-0.351,0.158	____	____	0.528
IL6 GG	-0.125	0.176	0.478	-0.477,0.226	____	____	0.679
Age	**0.009**	**0.004**	**0.016**	**0.002,0.016**	**0.011**	**0.003**	**0.0006**
Race	**-0.462**	**0.175**	**0.010**	**-0.812,-0.112**	**-0.331**	**0.150**	**0.025**
IR	**0.357**	**0.134**	**0.010**	**0.089,0.626**	**0.411**	**0.111**	**0.0004**
PCOS Status	-0.134	0.168	0.426	-0.469,0.201	____	____	____
BMI	0.015	0.010	0.159	-0.006,0.036	____	____	____
Current OC use	**0.520**	**0.189**	**0.008**	**0.142,0.899**	**0.463**	**0.158**	**0.006**
Ever Smoking	**0.332**	**0.121**	**0.008**	**0.090,0.574**	**0.333**	**0.111**	**0.003**

5.5.2.2.2.2 PPARλ P12A SNP (N=72)

No Potential PPARλ SNP interactions were identified. In the final model, age, race, IR, current OC use and ever smoking were found to significantly associate with ln-TG. Adjusting for PPARλ SNP, age, race, IR, PCOS status, BMI, current OC use and ever smoking (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.38 mg/dl, ~ 0.54 mg/dl and

~ 0.32 mg/dl for a female who is 1 year older, for an insulin resistant female compared to a non-IR female, for a female who currently uses OC compared to a non-current OC user and for a female who ever smoked compared to another female who never smoked respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.42 mg/dl for an African American female compared to a Caucasian female (**Table 66**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, age, race, IR, current OC use and ever smoking were found to significantly associate with ln-TG. Adjusting for age, race, IR, current OC use and ever smoking (when applicable), the increase in ln-TG was found to be ~ 0.01 mg/dl, ~0.41 mg/dl, ~ 0.46 mg/dl and ~ 0.33 mg/dl for a female who is 1 year older, for an insulin resistant female compared to a non- IR female, for a female who currently uses OC compared to a non-current OC user and for a female who ever smoked compared to another female who never smoked respectively. On the other hand, the decrease in ln-TG was found to be ~ 0.33 mg/dl for an African American female compared to a Caucasian female. The residual heritability for ln-TG was found to be not significant, meaning that there is no genetic contribution to ln-TG. Moreover, the proportion of variance in ln-TG which is found to be explained by the age, race, IR, current OC use and ever smoking is ~ 38% **(Table 66).**

Table 66: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-TG and Pro12 Ala PPARλ SNP in Females (N=72)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.08±0.18 p-value=0.325 $\sigma^2_{\Sigma\beta}$ = 0.38		
Independent variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	0.062	0.167	0.711	-0.271,0.396	____	____	0.652
Age	**0.009**	**0.003**	**0.012**	**0.002,0.016**	**0.011**	**0.003**	**0.0005**
Race	**-0.415**	**0.171**	**0.018**	**-0.757,-0.073**	**-0.331**	**0.150**	**0.039**
IR	**0.376**	**0.135**	**0.007**	**0.106,0.646**	**0.411**	**0.111**	**0.0003**
PCOS Status	-0.128	0.167	0.447	-0.461,0.206	____	____	____
BMI	0.013	0.010	0.209	-0.007,0.034	____	____	____
Current OC use	**0.541**	**0.189**	**0.006**	**0.164,0.919**	**0.463**	**0.159**	**0.004**
Ever Smoking	**0.319**	**0.120**	**0.010**	**0.079,0.559**	**0.333**	**0.111**	**0.003**

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction or to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.2.3 Ln-HDL,mg/dl

For ln-HDL, the following variables were entered in a stepwise fashion: current drinking, current OC use, ever smoking and current smoking; smoking was defined by smoking cigarettes or/&pipes or/&cigars.

5.5.2.2.3.1 IL6 G-174C SNP (N=72)

No Potential IL6 SNP interactions were identified. In the final model, age, race, IR, PCOS status and BMI were found to significantly associate with ln-HDL. Adjusting for IL6 SNP, age, race, IR, PCOS status and BMI (when applicable), the increase in ln-HDL was found to be 0.004 mg/dl, ~0.20mg/dl and ~0.18 mg/dl for a female who is 1 year older than another female, for an African American female compared to a Caucasian female and for a PCOS female compared to a non-PCOS female respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.15 mg/dl and ~ 0.01 mg/dl for an insulin resistant subject female compared to a non-IR female and for a female whose BMI is 1 kg/m^2 higher respectively (**Table 67**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, age, race, IR, PCOS status and BMI were found to significantly associate with ln-HDL. Adjusting for age, race, IR, PCOS status and BMI (when applicable), the increase in ln-HDL was found to be 0.005 mg/dl, ~0.23 mg/dl and ~0.20 mg/dl for a female who is 1 year older than another female, for an African American female compared to a Caucasian female and for a PCOS female compared to a non-PCOS female respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.15 mg/dl and ~ 0.02 mg/dl for an insulin resistant subject female compared to a non-IR female and for a female whose BMI is 1 kg/m^2 higher respectively. The residual heritability for ln-HDL was found to be significant and equal to 60%, meaning that ~60% of the total variation in ln-HDL is due to other genetic factors, adjusting for age, race, IR, PCOS status and BMI. Moreover, the proportion of variance in ln-HDL which is found to be explained by the age, race, IR, PCOS status and BMI is ~ 27% **(Table 67).**

Table 67: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HDL and G-174C IL6 SNP in Females (N=72)

Regression					VARIANCE COMPONENTS TEST H2r=0.60±0.23 p-value=0.003 $\sigma^2_{\Sigma\beta}$ = 0.27		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	0.004	0.057	0.942	-0.110,0.119	____	____	0.890
IL6 GG	0.099	0.079	0.217	-0.059,0.256	____	____	0.868
Age	**0.004**	**0.002**	**0.010**	**0.001,0.007**	**0.005**	**0.001**	**0.001**
Race	**0.196**	**0.079**	**0.016**	**0.038,0.355**	**0.225**	**0.09**	**0.019**
IR	**-0.150**	**0.058**	**0.013**	**-0.266,-0.033**	**-0.150**	**0.05**	**0.005**
PCOS Status	**0.177**	**0.068**	**0.011**	**0.042,0.312**	**0.195**	**0.06**	**0.002**
BMI	**-0.012**	**0.005**	**0.010**	**-0.022,-0.003**	**-0.017**	**0.005**	**0.0005**

5.5.2.2.3.2 PPARλ P12A SNP(N=72)

No Potential PPARλ SNP interactions were identified. In the final model, age, race, IR, PCOS status and BMI were found to significantly associate with ln-HDL. Adjusting for PPARλ SNP, age, race, IR, PCOS status and BMI (when applicable), the increase in ln-HDL was found to be 0.004 mg/dl, ~0.17mg/dl and ~0.17 mg/dl for a female who is 1 year older than another female, for an African American female compared to a Caucasian female and for a PCOS female compared to a non-PCOS female respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.17 mg/dl and ~ 0.01 mg/dl for an insulin resistant female compared to a non-IR female and for a female whose BMI is 1 kg/m^2 higher respectively (**Table 68**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, age, race, IR, PCOS status and BMI were found to significantly associate with ln-HDL. Adjusting for age, race, IR, PCOS status and BMI (when applicable), the increase in ln-HDL was found to be 0.005 mg/dl, ~0.23 mg/dl and ~0.20 mg/dl for a female who is 1 year older than another female, for an African American female compared to a Caucasian female and for a PCOS female compared to a non-PCOS female respectively. On the other hand, the decrease in ln-HDL was found to be ~ 0.15 mg/dl and ~ 0.02 mg/dl for an insulin resistant subject female compared to a non-IR female and for a female

whose BMI is 1 kg/m^2 higher respectively. The residual heritability for ln-HDL was found to be significant and equal to 60%, meaning that ~60% of the total variation in ln-HDL is due to other genetic factors, adjusting for age, race, IR, PCOS status and BMI. Moreover, the proportion of variance in ln-HDL which is found to be explained by the age, race, IR, PCOS status and BMI was ~ 27% **(Table 68).**

Table 68: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HDL and Pro12Ala PPARλ SNP in Females (N=72)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.60±0.23 p-value = 0.003 $\sigma^2_{\Sigma\beta}$ = 0.27		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.062	0.075	0.416	-0.212,0.089			0.915
Age	**0.004**	**0.002**	**0.017**	**0.001,0.007**	**0.005**	**0.001**	**0.001**
Race	**0.172**	**0.078**	**0.032**	**0.015,0.328**	**0.225**	**0.09**	**0.018**
IR	**-0.166**	**0.058**	**0.006**	**-0.283,-0.049**	**-0.150**	**0.05**	**0.005**
PCOS Status	**0.174**	**0.067**	**0.012**	**0.04,0.308**	**0.195**	**0.06**	**0.002**
BMI	**-0.011**	**0.005**	**0.015**	**-0.02,-0.002**	**-0.017**	**0.005**	**0.0004**

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.2.4 Ln-Systolic Blood Pressure (ln-SBP,mm)

For ln-SBP, the following variables were entered in a stepwise fashion: ever smoking, current smoking, current drinking, ever HRT, current HRT use, menopause status, LDL, TG, CRP serum level, and physical activity (Kcal/Week); smoking was defined by smoking cigarettes or/&pipes or/&cigars.

5.5.2.2.4.1 IL6 G-174C SNP (N=72)

IL6 SNP interactions with age, PCOS status and BMI were tested and found to be not significant. It is noteworthy to mention that the unadjusted effect of the IL6 CG genotype of the IL6 SNP, compared to the wild genotype(CC), on ln-SBP was borderline significant (p-value=0.085). This significance was lost once age entered the model (p-value became 0.265 for the IL6 CG genotype). However, the IL6 GG genotype became borderline significant again,

after adjusting for age, race, PCOS and BMI (p-value unadjusted = 0.295; became ~ 0.08) and significant after adjusting for current smoking (p-value=0.000). In the final model, age, and current smoking were found to significantly associate with ln-SBP. Moreover PCOS status was found to be borderline significantly associated with ln-SBP (p-value=0.067). Adjusting for IL6 SNP, age, race, PCOS status, BMI and current smoking (when applicable), the increase in ln-SBP was found to be 0.004 mm, 0.05 mm and 0.08 mm, for a female who is 1 year older than another female, for a PCOS female compared to a non-PCOS female and for a current female smoker compared to a non-current female smoker respectively (**Table 69**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, age, and current smoking were found to significantly associate with ln-SBP. Moreover PCOS status was found to be borderline significantly associate with ln-SBP (p-value~0.06). Adjusting for age, PCOS status, and current smoking (when applicable), the increase in ln-SBP was found to be 0.004 mm ,0.04 mm and 0.09 mm, for a female who is 1 year older than another female, for a PCOS female compared to a non-PCOS female and for a female current smoker compared to a female non-current smoker respectively. The residual heritability for ln-SBP was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in ln-SBP, adjusting for age, PCOS status, and current smoking. Moreover, the proportion of variance in ln-SBP, which is found to be explained by the age, PCOS status, and current smoking, is ~ 46% **(Table 69).**

Table 69: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-SBP and G-174C IL6 SNP in Females (N=72)

Regression					VARIANCE COMPONENTS TEST H2r=0 p-value =0.50 $\sigma^2_{\Sigma\beta}$ = 0.46		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	0.021	0.023	0.356	-0.025,0.068	_____	_____	0.444
IL6 GG	0.043	0.032	0.182	-0.020,0.105	_____	_____	0.258
Age	**0.004**	**0.001**	**0.000**	**0.002,0.005**	**0.004**	**0.0005**	**$6x10^{-9}$**
Race	0.037	0.032	0.256	-0.027,0.10	_____	_____	_____
PCOS status	**0.049**	**0.026**	**0.067**	**-0.004,0.102**	**0.039**	**0.021**	**0.065**
BMI	-0.001	0.002	0.527	-0.005,0.002	_____	_____	_____
Current smoking	**0.081**	**0.026**	**0.003**	**0.029,0.134**	**0.092**	**0.024**	**0.0006**

5.5.2.2.4.2 PPARλ P12A SNP (N=72)

No potential PPARλ SNP interactions were identified. In the final model, age, and current smoking were found to significantly associate with ln-SBP. Moreover PCOS status was found to be borderline significantly associate with ln-SBP (p-value=0.099). Adjusting for PPARλ SNP, age, race, PCOS status, BMI and current smoking (when applicable), the increase in ln-SBP was found to be 0.004 mm, ~0.04 mm and ~0.09 mm, for a female who is 1 year older than another female, for a PCOS female compared to a non-PCOS female and for a female current smoker compared to a female non-current smoker respectively (**Table 70**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, age, and current smoking were found to significantly associate with ln-SBP. Moreover PCOS status was found to be borderline significantly associated with ln-SBP (p-value~0.07). Adjusting for age, PCOS status, and current smoking (when applicable), the increase in ln-SBP was found to be 0.004 mm ,0.04 mm and 0.09 mm, for a female who is 1 year older than another female, for a PCOS female compared to a non-PCOS female and for a female current smoker compared to a female non-current smoker respectively. The residual heritability for ln-SBP was found to be significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in ln-SBP, adjusting for age, PCOS status, and current smoking. Moreover, the

proportion of variance in ln-SBP, which is found to be explained by the age, PCOS status, and current smoking, is ~ 46% **(Table 70).**

Table 70: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-SBP and Pro12Ala PPARλ SNP in Females (N=72)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0 p-value =0.50 $\sigma^2_{\Sigma\beta}$ = 0.46		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.007	0.030	0.810	-0.068,0.053	_____	_____	0.691
Age	**0.004**	**0.001**	**0.000**	**0.002,0.005**	**0.004**	**0.0005**	**$4x10^{-9}$**
Race	0.024	0.031	0.449	-0.039,0.086	_____	_____	_____
PCOS status	**0.044**	**0.026**	**0.099**	**-0.009,0.097**	**0.039**	**0.021**	**0.069**
BMI	-0.001	0.002	0.725	-0.004,0.003	_____	_____	_____
Current smoking	**0.088**	**0.026**	**0.001**	**0.035,0.140**	**0.092**	**0.242**	**0.0005**

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction or to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.2.5 Diastolic Blood Pressure (DBP, mm)

For DBP, the following variables were entered in a stepwise fashion: ever smoking, ever OC use, current smoking, current drinking, current HRT use, menopause status, CRP/IL6 serum levels, LDL, TG and physical activity(Kcal/Week); smoking was defined by smoking cigarettes or/&pipes or/&cigars.

5.5.2.2.5.1 IL6 G-174C SNP (N=71)

IL6 SNP interactions with age, race, gender, BMI and current drinking were tested and found to be not significant. It is noteworthy to mention that the unadjusted effect of the IL6 CG genotype on DBP was borderline significant (p-value was 0.079) and then lost significance when age variable entered the regression model (p-value became 0.170). One step further, it became borderline significant at the time race variable entered the regression model (p-value

became (0.099) and became not significant when BMI entered the model (p-value=0.165). On the other hand, the IL6 GG genotype became borderline significant after adjusting for age and race (p-value was 0.171 and became 0.06) and then became less significant when BMI variable entered the model (p-value became 0.098). In the final model, BMI and current drinking were found to significantly associate with DBP. Moreover, race was found to borderline significantly associate with DBP (p-value=0.097). Adjusting for IL6 SNP, age, race, BMI and current drinking (when applicable), the increase in DBP was found to be ~ 3.8 mm, ~ 0.29 mm, ~6.2 mm, for an African American female compared to a Caucasian female, for a female whose BMI is 1 kg/m^2 higher and for a female who currently drinks compared to another female who does not currently drink respectively (**Table 71)**.

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. In the final model, BMI and current drinking were found to significantly associate with DBP. However, race was found not be significantly associated rather than borderline significantly associated with DBP. Adjusting for BMI and current drinking (when applicable), the increase in DBP was found to be ~ 0.44 mm and ~6.7 mm for a female whose BMI is 1 kg/m^2 higher and for a female who currently drinks compared to another female who does not currently drink respectively. The residual heritability for DBP was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in DBP, adjusting for BMI and current drinking. Moreover, the proportion of variance in DBP, which is found to be explained by BMI and current drinking is ~ 35% **(Table 71).**

Table 71: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of DBP and G-174C IL6 SNP in Females (N=71)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0 p-value =0.50 $\sigma^2_{\Sigma\beta} = 0.35$		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	1.738	1.768	0.329	-1.795,5.270	____	____	0.273
IL6 GG	3.677	2.388	0.129	-1.094,8.448	____	____	0.147
Age	0.071	0.049	0.149	-0.026,0.168	____	____	____
Race	**3.808**	**2.260**	**0.097**	**-0.707,8.324**	____	____	0.121
BMI	**0.294**	**0.117**	**0.014**	**0.061,0.527**	**0.435**	**0.097**	**0.0008**
Current drinking	**6.188**	**1.644**	**0.000**	**2.904,9.473**	**6.677**	**1.552**	**0.00004**

5.5.2.2.5.2 PPARλ P12A SNP (N=71

No potential PPARλ SNP interactions were identified. It is noteworthy to mention that the unadjusted effect of the PPARλ SNP on DBP was borderline significant (p-value = 0.086), became more significant after adjusting for age (p-value=0.076) and then lost significance after race variable entered the model (p-value became 0.145). In the final model, BMI and current drinking were found to significantly associate with DBP. Adjusting for PPARλ SNP, age, race, BMI and current drinking (when applicable), the increase in DBP was found to be ~ 0.32 mm and ~6.2 mm for a female whose BMI is 1 kg/m^2 higher and for a female who currently drinks compared to another female who does not currently drink respectively (**Table 72**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, BMI and current drinking were found to significantly associate with DBP. Adjusting for BMI and current drinking (when applicable), the increase in DBP was found to be ~ 0.44 mm, ~6.7 mm for a female whose BMI is 1 kg/m^2 higher and for a female who currently drinks compared to another female who does not currently drink respectively. The residual heritability for DBP was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in DBP, adjusting for BMI and current drinking. Moreover, the proportion of variance in DBP, which is found to be explained by BMI and current drinking, is ~ 35% **(Table 72).**

Table 72: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of DBP and Pro12Ala PPARλ SNP (N=71)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0 p-value =0.50 $\sigma^2_{\Sigma\beta} = 0.35$		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-2.671	2.311	0.252	-7.286,1.943	_____	_____	0.166
Age	0.067	0.048	0.172	-0.030,0.163	_____	_____	_____
Race	2.631	2.267	0.250	-1.897,7.160	_____	_____	_____
BMI	**0.323**	**0.115**	**0.007**	**0.093,0.553**	**0.435**	**0.097**	**0.00002**
Current drinking	**6.197**	**1.645**	**0.000**	**2.911,9.483**	**6.678**	**1.552**	**0.00008**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor adjust for the effect of either SNP in the final model of the other SNP.

5.5.2.2.6. Fasting Glucose (G0, mg/dl)

For G0, the following variables were entered in a stepwise fashion: ever HRT, menopause status, CRP/IL6 serum levels, SBP and TG.

5.5.2.2.6.1 IL6 G-174C SNP (N=69)

No potential IL6 SNP interactions were identified. In the final model, age, race and PPARλ SNP were found to significantly associate with G0. Adjusting for IL6 and PPARλ SNPs, age, race and BMI (when applicable), the increase in G0 was found to be ~0.4 mg/dl for a female who is 1 year older. On the other hand, the decrease in G0 was found to be ~ 10 mg/dl and ~11.5 mg/dl for an African American female compared to a Caucasian female and for a female with at least one Ala12 allele of the PPARλ SNP compared to a female with the wild genotype (Pro12Pro) respectively (**Table 73**).

The findings of the **VARIANCE COMPONENTS TEST** were not consistent with the regression results. In the final model, only age was found to significantly associate with G0, as opposed to regression results which showed age, race and PPARλ SNP to be significantly

associated with G0. The increase in DBP was found to be ~ 0.42 mm for a female who is 1 year older than another female. The residual heritability for G0 was found to be significant and equal to 75%, meaning that ~75% of the total variation in G0 is due to other genetic factors, adjusting for age. Moreover the proportion of variance in G0, which is found to be explained by age was found to be ~ 35% **(Table 73).**

Table 73: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of Fasting Glucose and G-174C IL6 SNP (N=69)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.75±0.27 p-value =0.001 $\sigma^2_{\Sigma\beta}$ = 0.35		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-2.081	3.154	0.512	-8.387,4.224	____	____	0.231
IL6 GG	-2.019	4.499	0.655	-11.013,6.976	____	____	0.791
Age	**0.436**	**0.086**	**0.000**	**0.264,0.609**	**0.424**	**0.073**	**$3x10^{-8}$**
Race	**-10.009**	**4.186**	**0.020**	**-18.375,-1.642**	____	____	0.131
BMI	0.223	0.207	0.287	-0.192,0.637	____	____	____
PPARλ P12A	**-11.471**	**4.162**	**0.008**	**-19.791,-3.152**	____	____	0.110

5.5.2.2.6.2 PPARλ P12A SNP(N=69)

PPARλ SNP interactions with age was tested and found to be not significant. It is noteworthy to mention that the unadjusted effect of the PPARλ SNP on G0 was borderline significant (p-value ~0.06), became significant after adjusting for age(p-value=0.023) and became highly significant after adjusting for race (p-value=0.008). We were not able to test for PPARλ-race interaction because of the absence of African Americans in the Ala-X category. In the final model, age, race and PPARλ SNP were found to significantly associate with G0. Adjusting for PPARλ SNP, age, race and BMI (when applicable), the decrease in G0 was found to be ~ 11 mg/dl and 9.5 mg/dl for a female with at least one Ala12 allele of the the PPARλ SNP compared to a female with the wild genotype (Pro12Pro) and for an African American female compared to a Caucasian female respectively. On the other hand, the increase in G0 was found to be ~0.4 mg/dl for a female who is 1 year older (**Table 37-b**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. In the final model and similar to the final regression model, age was

found to significantly associate with G0. In contrast to the results of the regression model, the PPARλ SNP was found to borderline significantly (p-value~0.07) rather than significantly associated with G0. Moreover, race was found to be not significantly associated with G0 as opposed to significant association with G0 found in the regression model. The decrease in G0 was found to be ~6.7 mg/dl for a female with at least one Ala12 allele of the the PPARλ SNP compared to a female with the wild genotype (Pro12Pro). On the other hand, the increase in G0 was found to be ~0.43 mg/dl for a female who is 1 year older than another female. The residual heritability for G0 was found to be significant and equal to 64%, meaning that ~64% of the total variation in G0 is due to other genetic factors, adjusting for age and the PPARλ SNP. Moreover the proportion of variance in G0 which is found to be explained by the PPARλ SNP and age, was ~ 39% **(Table 74).** Comparing $\sigma^2_{\Sigma ß}$ from the IL6 SNP model ($\sigma^2_{\Sigma ß}$ =0.35), where age was the only significant covariate (**Table 73**) and the $\sigma^2_{\Sigma ß}$ from the model shown in **Table 74** ($\sigma^2_{\Sigma ß}$ =0.39), showed that age explains 35% of the variation in G0 and the PPARλ SNP explains an additional 4%.

Table 74: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of Fasting Glucose and Pro12 Ala PPARλ SNP in Females (N=69)

Regression					VARIANCE COMPONENTS TEST H2r=0.64±0.30 p-value =0.001 $\sigma^2_{\Sigma ß}$ = 0.39		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	**-11.148**	**4.028**	**0.007**	**-19.194,-3.102**	**-6.684**	**4.666**	**0.072**
Age	**0.433**	**0.083**	**0.000**	**0.264,0.600**	**0.431**	**0.073**	**$5x10^{-8}$**
Race	**-9.509**	**4.054**	**0.022**	**-17.608,-1.409**	____	____	0.140
BMI	0.199	0.202	0.328	-0.204,0.603	____	_____	_____

Since the p-value for the IL6 SNP was > 0.1 in its final model, there was no need to assess gene-gene interaction. Adjustment for the effect of PPARλ SNP in the final model of the IL6 SNP was performed since the p-value of the PPARλ SNP in its final model was < 0.05.

5.5.2.2.7 Ln-Interleukin 6 (ln-IL6 pg/ml)

For ln-IL6 serum level, the following variables were entered in a stepwise fashion: ever smoking, menopause status, CRP serum level, SBP, DBP, LDL and physical activity(Kcal/Week); smoking was defined by smoking cigarettes or/&pipes or/&cigars.

5.5.2.2.7.1 IL6 G-174C SNP (N=72)

IL6 SNP interactions with race, PCOS status, BMI, fasting insulin and C-reactive protein level (CRP) were tested and found to be not significant. It is noteworthy to mention that the effect of the IL6 GG genotype on ln-IL6 serum level became borderline significant (p-value was 0.545, became 0.077) after adjusting for age, race, fasting insulin and PCOS status and then became not significant when BMI entered the regression model (p-value= 0.205). In the final model, race and CRP serum level were found to significantly associate with ln-IL6 serum level. IL6 GG genotype of the IL6 SNP was found to borderline significantly associate with IL6 serum level as well (p-value=0.093). Adjusting for IL6 SNP, age, race, fasting insulin, PCOS status, BMI and CRP serum level (when applicable), the increase in ln-IL6 serum level was found to be ~ 0.33 pg/ml, ~0.77 pg/ml and ~0.05 pg/ml for a female with the GG genotype of the IL6 SNP compared to a female with the wild genotype(CC), for an African American female compared to a Caucasian female and for a female whose CRP serum levels are 1 mg/L higher than another female's respectively (**Table 75**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, race and CRP serum level were found to significantly associate with ln-IL6 serum level and IL6 GG genotype of the IL6 SNP was found to borderline significantly associate with IL6 serum level (p-value=0.072). Adjusting for IL6 GG genotype of the IL6 SNP, race and CRP serum level (when applicable), the increase in ln-IL6 serum level was found to be ~ 0.3 pg/ml, ~0.87 pg/ml and ~0.07 pg/ml for a female with the GG genotype of the IL6 SNP compared to a female with the wild genotype(CC), for an African American female compared to a Caucasian female and for a female whose CRP serum level are 1 mg/L higher than another female's respectively. The residual heritability for ln-IL6 was found to be not significant, meaning that there is no genetic contribution to ln-IL6. Moreover the proportion of variance in ln-IL6 which is found to be explained by the IL6 GG genotype of the IL6 SNP, race and CRP serum level, was found to be ~ 56% **(Table 75).** Excluding the IL6 SNP and running the model including race and CRP serum level only and then comparing $\sigma^2_{\Sigma\beta}$ from this model ($\sigma^2_{\Sigma\beta}$ =0.54) and the $\sigma^2_{\Sigma\beta}$ from the model shown in **Table 75** ($\sigma^2_{\Sigma\beta}$ =0.56), showed that race and CRP serum level explain 54% of the variation in ln-IL6 and the IL6GG genotype of the IL6 SNP explains an additional 2%.

Table 75: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-IL6 and G-174C IL6 SNP in Females (N=72)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.07±0.28 p-value =0.40 $\sigma^2_{\Sigma\beta}$ = 0.56		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	0.112	0.141	0.430	-0.169,0.393	_____	_____	0.268
IL6 GG9	**0.332**	**0.195**	**0.093**	**-0.057,0.721**	**0.303**	**0.188**	**0.072**
Age	0.003	0.004	0.534	-0.006,0.011	_____	_____	_____
Race	**0.769**	**0.203**	**0.000**	**0.363,1.174**	**0.867**	**0.173**	**0.0001**
Fasting Insulin	-0.001	0.007	0.924	-0.014,0.013	_____	_____	_____
PCOS status	0.173	0.163	0.293	-0.153,0.499	_____	_____	_____
BMI	0.023	0.014	0.111	-0.005,0.052	_____	_____	_____
CRP(mg/L)	**0.049**	**0.015**	**0.002**	**0.019,0.079**	**0.074**	**0.010**	**$1x10^{-9}$**

5.5.2.2.7.2 PPARλ P12A SNP (N=72)

PPARλ SNP- interactions with fasting insulin, CRP serum levels and race were tested and found to be not significant. It is noteworthy to mention that the unadjusted effect of PPARλ SNP on ln-IL6 was significant (p-value=0.031), lost its significance after adjusting for age and race (p-value became 0.106), became significant again after adjusting for fasting insulin, PCOS and BMI(p-value =0.042) and last lost its significance when CRP entered the model(p-value=0.135). In the final model, race, BMI and CRP serum levels were found to significantly associate with ln-IL6 serum level. Adjusting for PPARλ SNP, age, race, fasting insulin, PCOS status, BMI and CRP serum levels (when applicable), the increase in ln-IL6 serum level was found to be ~ 0.63 pg/ml, ~0.03 pg/ml and ~0.04 pg/ml for an African American female compared to a Caucasian female, for a female whose BMI is 1 kg/m^2 higher and for a female whose CRP serum level are 1 mg/L higher than another female's respectively (**Table 76).**

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, race, BMI and CRP serum levels were found to significantly associate with ln-IL6 serum level. Adjusting for race, BMI and CRP serum levels (when applicable), the increase in ln-IL6 serum level was found to be ~ 0.63 pg/ml, ~0.03 pg/ml and ~0.05 pg/ml for an African American female compared to a Caucasian female, for a female whose BMI is 1 kg/m^2 higher and for a female whose CRP serum levels are 1 mg/L

higher than another female's respectively. The residual heritability for ln-IL6 was found to be not significant, meaning that there is no genetic contribution to ln-IL6. Moreover the proportion of variance in ln-IL6 which is found to be explained by race, BMI and CRP serum level, was found to be ~ 59% **(Table 76).**

Table 76: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-IL6 and Pro12Ala PPARλ SNP in Females (N=72)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.05±0.23 p-value =0.40 $\sigma^2_{\Sigma\beta}$ = 0.59		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.283	0.187	0.135	-0.657,0.090	_____	_____	0.112
Age	0.002	0.004	0.621	-0.006,0.010	_____	_____	_____
Race	**0.627**	**0.199**	**0.002**	**0.229,1.025**	**0.627**	**0.177**	**0.002**
Fasting Insulin	-0.001	0.007	0.935	-0.014,0.013	_____	_____	_____
PCOS status	0.139	0.161	0.390	-0.183,0.462	_____	_____	_____
BMI	**0.031**	**0.014**	**0.031**	**0.003,0.059**	**0.033**	**0.011**	**0.002**
CRP(mg/L)	**0.041**	**0.015**	**0.010**	**0.01,0.071**	**0.045**	**0.013**	**0.003**

Since the p-value for the PPARλ SNP was > 0.1 in its final model, there was no need to assess gene-gene interaction. Adjustment for the effect of either SNP was unnecessary since the p-value of each in its final model was > 0.05.

5.5.2.2.8 Ln-C Reactive Protein (ln-CRP mg/L)

For ln-CRP serum level, the following variables were entered in a stepwise fashion: current drinking, ever OC use, current OC use, ever HRT use, menopause status, IL6 serum level, SBP, DBP, LDL, TG, G0 and physical activity.

5.5.2.2.8.1 IL6 G-174C SNP (N=70)

No potential IL6 SNP-environment interactions were identified. In the final model, age, BMI, current OC use, DBP and IL6 serum levels were found to significantly associate with ln-CRP serum level. Adjusting for IL6 SNP, age, race, fasting insulin, PCOS status, BMI, current OC use, DBP and IL6 serum levels (when applicable), the increase in ln-CRP serum levels was

found to be ~ 0.03 mg/L,~ 0.13 mg/L, ~2.07 mg/L ~0.04 mg/L and ~ 0.18 mg/L for a female who is 1 year older, for a female whose BMI is 1 kg/m^2 higher, for a female who currently uses OC compared to a female non-current user, for a female whose DBP is 1mm higher and for a female whose IL6 serum levels are 1 pg/ml than another female's higher respectively (**Table 77**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. In the final model, age, BMI, current OC use and DBP were found to significantly associate with ln-CRP serum levels. However, IL6 serum levels were found to be not significantly associated with ln-CRP. Adjusting for IL6 age, BMI, current OC use and DBP (when applicable), the increase in ln-CRP serum level was found to be ~ 0.03 mg/L,~ 0.12 mg/L, ~2.05 mg/L and ~0.04 mg/L for a female who is 1 year older, for a female whose BMI is 1 kg/m^2 higher, for a female who currently uses OC compared to a female non-current OC user and for a female whose DBP is 1mm higher than another female's respectively. The residual heritability for ln-CRP was found to be not significant, meaning that there is no genetic contribution to ln-CRP. Moreover the proportion of variance in ln-CRP which is found to be explained by the age, BMI, current OC use and DBP, was found to be ~ 66% **(Table 77).**

Table 77: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-CRP and G-174C IL6 SNP in Females (N=70)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.02±0.37 p-value =0.48 $\sigma^2_{\Sigma\beta}$ = 0.66		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.175	0.325	0.592	-0.826,0.475	_____	_____	0.558
IL6 GG	-0.644	0.455	0.162	-1.554,0.266	_____	_____	0.188
Age	**0.025**	**0.010**	**0.012**	**0.006,0.044**	**0.033**	**0.008**	**0.0002**
Race	-0.653	0.480	0.179	-1.614,0.308	_____	_____	_____
Fasting Insulin	-0.024	0.017	0.161	-0.059,0.010	_____	_____	_____
PCOS status	-0.199	0.420	0.637.	-1.041,0.642	_____	_____	_____
BMI	**0.131**	**0.032**	**0.000**	**0.067,0.194**	**0.121**	**0.020**	**0.0001**
Current OC use	**2.068**	**0.477**	**0.000**	**1.113,3.023**	**2.052**	**0.401**	**$2x10^{-6}$**
DBP	**0.043**	**0.019**	**0.024**	**0.006,0.080**	**0.041**	**0.017**	**0.009**
IL6 serum level	**0.175**	**0.084**	**0.042**	**0.007,0.343**	_____	_____	0.129

5.5.2.2.8.2 PPARλ P12A SNP (N=70)

No potential PPARλ SNP interactions were identified. In the final model, age, BMI, current OC use and DBP were found to significantly associate with ln-CRP serum level. Adjusting for PPARλ SNP, age, race, fasting insulin, PCOS status, BMI, current OC use and DBP (when applicable), the increase in ln-CRP serum level was found to be ~ 0.03 mg/L,~ 0.13 mg/L, ~2.08 mg/L and ~0.04 mg/L for a female who is 1 year older, for a female whose BMI is 1 kg/m^2 higher, for a female who currently uses OC compared to a female non-current OC user and for a female whose DBP is 1mm higher than another female's (**Table 78**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, age, BMI, current OC use and DBP were found to significantly associate with ln-CRP serum levels. Adjusting for age, BMI, current OC use and DBP (when applicable), the increase in ln-CRP serum level was found to be ~ 0.03 mg/L, ~ 0.12 mg/L, ~2.05 mg/L and ~0.04 mg/L for a female who is 1 year older, for a female whose BMI is 1 kg/m^2 higher, for a female who currently uses OC compared to a female non-current OC user and for a female whose DBP is 1mm higher than another female's. The residual heritability for ln-CRP was found to be not significant, meaning that there is no genetic contribution to ln-CRP. Moreover the proportion of variance in ln-CRP which is found to be explained by the age, BMI, current OC use and DBP, was found to be ~ 66% **(Table 78).**

Table 78: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-CRP and Pro12 Ala PPARλ SNP (N=70)

Regression					**VARIANCE COMPONENTS TEST** H2r=0.02±0.37 p value=0.48 $\sigma^2_{\Sigma\beta}$ = 0.66		
Independent variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.406	0.431	0.351	-1.268,0.457	____	____	0.335
Age	**0.031**	**0.009**	**0.002**	**0.013,0.050**	**0.033**	**0.008**	**0.0001**
Race	-0.238	0.454	0.601	-1.146,0.669	____	____	____
Fasting Insulin	-0.001	0.014	0.939	-0.029,0.027	____	____	____
PCOS status	-0.166	0.427	0.698	-1.019,0.687	____	____	____
BMI	**0.131**	**0.032**	**0.000**	**0.067,0.195**	**0.121**	**0.020**	**$1x10^{-7}$**
Current OC use	**2.076**	**0.493**	**0.000**	**1.090,3.062**	**2.052**	**0.401**	**$3x10^{-6}$**
DBP	**0.039**	**0.019**	**0.042**	**0.001,0.077**	**0.041**	**0.017**	**0.030**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor to adjust for the effect of either SNP in the other SNP's final model.

5.5.2.2.9 Ln-HOMA-IR

For ln-HOMA-IR, the following variables were entered in a stepwise fashion: current smoking, menopause status, CRP/IL6 serum levels, LDL, HDL and TG; smoking was defined by smoking cigarettes or/&pipes or/&cigars. Current anti-diabetic medication use was forced into both the regression and variance components models.

5.5.2.2.9.1 IL6 G-174C SNP (N=72)

No potential IL6 SNP-environment interactions were identified. In the final model, BMI was found to significantly associate with ln-HOMA-IR. Adjusting for IL6 SNP, age, race, PCOS status and current anti-diabetic medication use , the increase in ln-HOMA-IR was found to be ~0.05 μU/ml* mmol/L for a female whose BMI is 1 kg/m^2 higher (**Table 79**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. In the final model, BMI and current anti-diabetic medication use were found to significantly/borderline significantly associate with ln-HOMA-IR respectively. Adjusting for current anti-diabetic medication use, the increase in ln-HOMA-IR was found to be ~0.04 μU/ml* mmol/L for a female whose BMI is 1 kg/m^2 higher. Adjusting for BMI, the increase in ln-HOMA-IR was found to be 0.27 μU/ml* mmol/L for a female who currently uses anti-diabetic medications compared to a female non-current user.The residual heritability for ln-HOMA-IR was found to be significant and equal to 50%, meaning that ~50% of the total variation in ln-HOMA-IR is due to other genetic factors, adjusting for BMI and current anti-diabetic medication use. Moreover the proportion of variance in ln-HOMA-IR which was found to be explained by BMI and current anti-diabetic medication use is ~ 39% **(Table 79).**

Table 79: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HOMA-IR and G-174C IL6 SNP (N=72)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.50±0.27 p-value =0.02 $\sigma^2_{\Sigma\beta}$ =0.39		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	-0.029	0.115	0.805	-0.259,0.202	____	____	0.530
IL6 GG	-0.066	0.158	0.679	-0.381,0.250	____	____	0.954
Age	-0.003	0.003	0.307	-0.010,0.003	____	____	____
Race	-0.244	0.159	0.131	-0.562,0.075	____	____	____
PCOS status	-0.119	0.145	0.414	-0.408,0.170	____	____	____
BMI	**0.049**	**0.009**	**0.000**	**0.031,0.066**	**0.038**	**0.007**	**$4x10^{-7}$**
Current Anti-diabetic Meds use	0.251	0.161	0.124	-0.07,0.572	**0.273**	**0.140**	**0.052**

5.5.2.2.9.2 PPARλ P12A SNP(N=72)

No potential PPARλ SNP interactions were identified. In the final model, BMI, current smoking and TG serum levels were found to significantly associate with ln-HOMA-IR. Adjusting for PPARλ SNP, age, race, PCOS status, BMI, current anti-diabetic medication use, current smoking and TG serum levels (when applicable), the increase in ln-HOMA-IR was found to be ~0.04 μU/ml* mmol/L and 0.001 μU/ml* mmol/L for a female whose BMI is 1 kg/m^2 higher and for a female whose TG serum levela are 1 mg/dl higher than another female's respectively. On the other hand, the decrease in ln-HOMA-IR was found to be ~0.36 μU/ml* mmol/L for a female who currently smokes compared to a female non-current smoker (**Table 80**).

The findings of the **VARIANCE COMPONENTS TEST** were consistent with the regression results. In the final model, BMI, current smoking and TG serum levels were found to significantly associate with ln-HOMA-IR. Adjusting for BMI, current smoking, TG serum levels and current anti-diabetic medication use (when applicable), the increase in ln-HOMA-IR was found to be ~0.04 μU/ml* mmol/L and 0.001 μU/ml* mmol/L for a female whose BMI is 1 kg/m^2 higher and for a female whose TG serum levels are 1 mg/dl higher than another female's respectively. On the other hand, the decrease in ln-HOMA-IR was found to be ~0.28 μU/ml* mmol/L for a female who currently smokes compared to a female non-current smoker. The residual heritability for ln-HOMAIR was found to be not significant, meaning that there is no genetic contribution to ln-HOMA-IR. Moreover, the proportion of variance in ln-HOMAIR

which is found to be explained by BMI, current smoking, TG serum levesl and current anti-diabetic medication use was found to be ~ 47% **(Table 80).**

Table 80: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-HOMA_IR and Pro12 Ala PPARλ SNP in Females (N=72)

Regression					**VARIANCE COMPONENTS TEST**		
					H2r=0.36±0.32 p-value =0.10 $\sigma^2_{\Sigma\beta}$ =0.47		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.227	0.142	0.114	-0.510,0.056	_____	_____	0.137
Age	-0.004	0.003	0.176	-0.010,0.002	_____	_____	_____
Race	-0.137	0.153	0.373	-0.443,0.168	_____	_____	_____
PCOS status	-0.084	0.134	0.532	-0.352,0.184	_____	_____	_____
BMI	**0.044**	**0.008**	**0.000**	**0.028,0.061**	**0.036**	**0.007**	**$2x10^{-6}$**
Current Anti-T2DM Meds use	0.161	0.153	0.299	-0.146,0.467	0.171	0.144	0.220
Current smoking	**-0.355**	**0.127**	**0.007**	**-0.608,-0.102**	**-0.283**	**0.128**	**0.018**
TG(mg/dl)	**0.001**	**0.001**	**0.046**	**0.000,0.003**	**0.001**	**0.0006**	**0.035**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor to adjust for the effect of either SNP in the other SNP's final model.

5.5.2.2.9.10 Ln-Testosterone

For ln-testosterone, the following variables were entered in a stepwise fashion: ever HRT use, and SBP. Current HRT and OC use were forced into both the regression and variance components models.

5.5.2.2.9.10.1 IL6 G-174C SNP (N=71)

IL6 SNP interactions with age and BMI were tested and found to be not significant. In the final model, age and current HRT use were found to significantly associate with ln-testosterone. Adjusting for IL6 SNP, race, fasting insulin, BMI, current HRT use and current OC use, the decrease in ln-testosterone was found to be ~0.01 ng/ml for a female who is one year older

than another female and. Adjusting for age, IL6 SNP, race, fasting insulin, BMI and current OC use, the decrease in ln-testosterone was found to be 0.56 ng/ml for a female who currently uses HRT compared to a female who does not (**Table 81**).

The findings of the **VARIANCE COMPONENTS TEST** were not consistent with the regression results. As opposed to the regression model, the IL6 CG genotype of the IL6 SNP and BMI were found to be significantly associated, rather than not significantly associated, with ln-testosterone. Moreover, current HRT use was found to be not significantly associated, rather than significantly associated, with ln-testosterone. In the final model, the IL6CG genotype of the IL6 SNP, age and BMI were found to significantly associate with ln-testosterone. Adjusting for IL6 SNP, age, BMI, current HRT use and current OC use(when applicable), the increase in ln-testosterone was found to be ~0.23 ng/ml and ~0.02 ng/ml, for a female with the IL6 CG genotype compared to a female with the wild genotype (CC) and for a female whose BMI is 1 kg/m^2 higher. On the other hand, the decrease in ln-testosterone was found to be ~0.01 ng/ml for a female who is one year older than another female, after adjusting for IL6 SNP, BMI, current HRT use and current OC use. The residual heritability for ln-testosterone was found to be not significant, meaning that there is no genetic contribution to ln-Testosterone. Moreover, the proportion of variance in ln-testosterone which is found to be explained by the IL6 CG genotype of the IL6 SNP, age, BMI, current HRT and current OC use was found to be ~ 28 %**(Table 81).** Excluding the IL6 SNP and running the model including age, BMI, current HRT and current OC use only and then comparing $\sigma^2_{\Sigma ß}$ from this model ($\sigma^2_{\Sigma ß}$ =0.26) and the $\sigma^2_{\Sigma ß}$ from the model shown in **Table 81** ($\sigma^2_{\Sigma ß}$ =0.28), showed that age, BMI, current HRT and current OC use explain 26% of the variation in ln-testosterone and the IL6GG genotype of the IL6 SNP explains an additional 2%.

Table 81: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-Testosterone and G-174C IL6 SNP in Females (N=71)

Regression					VARIANCE COMPONENTS TEST		
					H2r=0.26±0.39 p-value =0.24 $\sigma^2_{\Sigma B}$=0.28		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
IL6 CG	0.230	0.137	0.100	-0.045,0.504	**0.233**	**0.124**	**0.032**
IL6 GG	0.155	0.190	0.416	-0.224,0.534	____	____	0.290
Age	**-0.009**	**0.004**	**0.022**	**-0.017,-0.001**	**-0.009**	**0.004**	**0.011**
Race	-0.242	0.186	0.199	-0.614,0.130	____	____	____
Insulin	0.003	0.006	0.613	-0.009,0.015	____	____	____
BMI	0.019	0.012	0.135	-0.006,0.043	**0.018**	**0.008**	**0.034**
Current HRT use	**-0.562**	**0.274**	**0.044**	**-1.109,-0.014**	-0.498	0.301	0.104
Current OC use	-0.284	0.182	0.124	**-0.648,0.080**	-0.267	0.172	0.149

5.5.2.2.10.2 PPARλ P12A SNP (N=71)

No potential PPARλ SNP interactions were identified. In the final model, age and current HRT use were found to significantly associate with ln-testosterone. Moreover, race, BMI, and current OC use were found to be borderline significantly associated with ln-testosterone (p-value ~0.09, 0.099 and 0.08 respectively). Adjusting for PPARλ SNP, age, race, fasting insulin, current HRT and current OC use(when applicable), the increase in ln-testosterone was found to be 0.020 ng/ml for a female whose BMI is 1 kg/m^2 higher than another female. On the other hand, adjusting for PPARλ SNP, age, race, fasting insulin, BMI, current HRT and current OC use(when applicable), the decrease in ln-testosterone was found to be ~0.01ng/ml,0.32ng/ml,~ 0.62ng/ml and ~0.33ng/ml for a female who is 1 year older than another female, for an African American female compared to a Caucasian female, for a female who currently uses HRT compared to a female who does not and for a female who currently uses OC compared to a female who does not respectively(**Table 82**).

The findings of the **VARIANCE COMPONENTS TEST** were almost consistent with the regression results. Race and BMI were found to significantly, rather than borderline significantly, associate with ln-testosterone. In the final model, age, race, BMI and current HRT use were found to significantly associate with ln-testosterone. Adjusting for age, race, current HRT use and current OC use, the increase in ln-testosterone was found to be 0.025 ng/ml for a female whose BMI is 1 kg/m^2 higher than another female. On the other hand, adjusting for age, race, BMI, current HRT use and current OC use(when applicable), the decrease in ln-

testosterone was found to be ~0.01 ng/ml, 0.33 ng/ml and ~ 0.61 ng/ml and ~ 0.31 ng/ml for a female who is 1 year older than another female, for an African American female compared to a Caucasian female, for a female who currently uses HRT compared to a female who does not and for a female who currently uses OC compared to a female who does not respectively.The residual heritability for ln-testosterone was found to be not significant and equal to 0%, meaning that a sporadic model has a better likelihood than a genetic model in explaining the variation in ln-testosterone, adjusting for age, race, BMI, current HRT use and current OC use. Moreover the proportion of variance in ln-HOMAIR which is found to be explained by age, race, BMI, current HRT use and current OC use was found to be ~ 30% (**Table 82).**

Table 82: Results of Multiple Linear Regression and VARIANCE COMPONENTS TEST of ln-Testosterone and Pro12Ala PPARλ SNP in Females (N=71)

Regression					VARIANCE COMPONENTS TEST H2r=0 p-value =0.5 $\sigma^2_{\Sigma \beta}$ =0.30		
Independent Variable	**β**	**S.E.**	**p-value**	**95% CI**	**β**	**S.E.**	**p-value**
PPARλ P12A	-0.142	0.180	0.434	-0.501,0.218	_____	_____	0.409
Age	**-0.009**	**0.004**	**0.031**	**-0.017,-0.001**	**-0.009**	**0.004**	**0.011**
Race	**-0.324**	**0.186**	**0.086**	**-0.695,0.047**	**-0.327**	**0.166**	**0.040**
Insulin	0.003	0.006	0.586	-0.009,0.015	_____	_____	_____
BMI	**0.020**	**0.012**	**0.099**	**-0.004,0.045**	**0.025**	**0.009**	**0.004**
Current HRT use	**-0.619**	**0.276**	**0.028**	**-1.170,-0.067**	**-0.607**	**0.260**	**0.022**
Current OC use	**-0.325**	**0.183**	**0.080**	**-0.691,0.040**	**-0.314**	**0.173**	**0.061**

Since the p-value for each of the PPARλ and the IL6 SNPs was > 0.1 in its final model, there was no need to assess gene-gene interaction. Moreover, adjustment for either SNP was not needed in the final model of either SNP since the p-value of the each SNP in its final model was > 0.05.

5.5.2.3 Subgroup Analysis -Males (N=29)

5.5.2.3.1 Waist (cm)

For waist, the following variables were entered in a stepwise fashion: HDL, LDL and TG.

5.5.2.3.1.1 IL6 G-174C SNP (N=27)

IL6 SNP interactions with IR and BMI were tested and found to be not significant. It is noteworthy to mention that the effect of IL6 GG genotype on waist became borderline significant (p-value was 0.212 and became 0.09) after adjusting for age and IR and then became not significant when BMI entered the regression model (p-value=0.627). The effect of IR on waist was highly significant but lost significance when BMI entered the model. In the final model, age and BMI were found to significantly associate with waist. Adjusting for IL6 SNP, IR, and BMI, the increase in waist was found to be ~ 0.12 cm for a male who is one year older than another male. Adjusting for IL6 SNP, age and IR, the increase in waist was found to be ~2 for a male whose BMI is 1 kg/m^2 higher than another male (**Table 83**).

Table 83: Results of Multiple Linear Regression of Waist and G-174C IL6 SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	-4.232	2.654	0.126	-9.750,1.286
IL6 GG	-1.206	2.441	0.627	-6.283,3.872
Age	**0.118**	**0.054**	**0.040**	**0.006,0.230**
IR	2.272	2.642	0.400	-3.223,7.767
BMI	**2.030**	**0.197**	**0.000**	**1.621,2.440**

5.5.2.3.1.2 PPARλ P12A SNP (N=27)

PPARλ SNP interactions with IR and BMI were tested and found to be not significant. It is noteworthy to mention that the effect of PPARλ SNP on waist became borderline significant (p-value was 0.389 and became 0.05) when IR entered the regression model, after adjusting for age and IR and then became not significant when BMI entered the regression model (p-value=0.306). The effect of IR on waist was highly significant but lost significance when BMI entered the model. In the final model, age and BMI were found to significantly associate with

waist. Adjusting for PPARλ SNP, IR, and BMI, the increase in waist was found to be ~ 0.1 cm for a male who is one year older than another male. Adjusting for PPARλ SNP, age, and IR, the increase in waist was found to be ~2 for a male whose BMI is 1 kg/m^2 higher than another male (**Table 84**).

Table 84: Results of Multiple Linear Regression of Waist and Pro12Ala PPARλ SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
PPARλ P12A	-3.463	3.303	0.306	-10.314,3.388
Age	**0.111**	**0.053**	**0.049**	**0.001,0.222**
IR	4.354	2.671	0.117	-1.187,9.894
BMI	**1.912**	**0.191**	**0.000**	**1.517,2.308**

Since the p-value for the PPARλ SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor adjust for the effect of either SNP in the final model of the other SNP.

5.5.2.3.2 Ln-Triglycerides (ln-TG, mg/dl)

For ln-TG, no variables were entered in a stepwise fashion.

5.5.2.3.2.1 IL6 G-174C SNP (N=27)

No Potential IL6 SNP interactions were identified. In the final model, IL6 SNP, age and waist were found to be not significantly associated with ln-TG in males (**Table 85**).

Table 85: Results of Multiple Linear Regression of ln-TG and G-174C IL6 SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	0.142	0.355	0.693	-0.595,0.879
IL6 GG	0.149	0.336	0.661	-0.547,0.845
Age	0.003	0.008	0.709	-0.013,0.019
Waist	0.016	0.009	0.099	-0.003,0.035

5.5.2.3.2.2 PPARλ P12A SNP (N=27)

No Potential PPARλ SNP-environment interactions were identified. In the final model, PPARλ SNP, age and waist were found to be not significantly associated with ln-TG (**Table 86**).

Table 86: Results of Multiple Linear Regression of ln-TG and Pro12Ala PPARλ SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
PPARλ P12A	-0.422	0.410	0.314	-1.269,0.425
Age	0.004	0.007	0.590	-0.011,0.019
Waist	0.013	0.009	0.142	-0.005,0.031

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction or to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.3.3 Ln-HDL, mg/dl

For ln-HDL, no variables were entered in a stepwise fashion.

5.5.2.3.3.1 IL6 G-174C SNP (N=27)

No Potential IL6 SNP interactions were identified. In the final model, waist was found to borderline significantly associate with ln-HDL (p-value=0.065). After adjusting for IL6 SNP, age and IR, the decrease in ln-HDL was found to be ~ 0.01 mg/dl for a male whose waist is 1 cm higher than another male (**Table 87**).

Table 87: Results of Multiple Linear Regression of ln-HDL and G-174C IL6 SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	-0.132	0.118	0.276	-0.376,0.113
IL6 GG	0.074	0.111	0.512	-0.157,0.305
Age	0.000	0.003	0.853	-0.005,0.006
IR	0.026	0.121	0.834	-0.226,0.278
Waist	**-0.008**	**0.004**	**0.065**	**-0.016,0.001**

5.5.2.3.3.2 PPARλ P12A SNP(N=27)

No Potential PPARλ SNP interactions were identified. In the final model, waist was found to significantly associate with ln-HDL. After adjusting for PPARλ SNP, age and IR, the decrease in ln-HDL was found to be ~ 0.01 mg/dl for a male whose waist is 1 cm higher than another male (**Table 88**).

Table 88: Results of Multiple Linear Regression of ln-HDL and Pro12Ala PPARλ SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
PPARλ P12A	-0.114	0.154	0.465	-0.433,0.204
Age	0.001	0.002	0.652	-0.004,0.006
IR	0.116	0.128	0.378	-0.151,0.382
Waist	**-0.011**	**0.004**	**0.016**	**-0.019,-0.002**

Since the p-value for each of the PPARλ SNP and the IL6 SNP was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor to adjust for the effect of one SNP in the final model of the other SNP.

5.5.2.3.4 Ln-Systolic Blood Pressure (ln-SBP,mm)

For ln-SBP, current drinking variable was entered in a stepwise fashion.

5.5.2.3.4.1 IL6 G-174C SNP (N=27)

No potential IL6 SNP interactions identified. In the final model, PPARλ SNP was found to significantly associate with ln-SBP. After adjusting for IL6 SNP, age, race and waist, the decrease in ln-SBP was found to be ~0.15 mm for a male with at least one Ala12 allele of the PPARλ SNP compared to a male with the wild genotype (Pro12Pro) (**Table 89**).

Table 89: Results of Multiple Linear Regression of ln-SBP and G-174C IL6 SNP (N=27)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	0.003	0.048	0.952	-0.097,0.102
IL6 GG	0.021	0.046	0.658	-0.076,0.118
Age	0.001	0.001	0.310	-0.001,0.003
Race	0.074	0.046	0.120	-0.021,0.170
waist	0.002	0.001	0.225	-0.001,0.004
PPARλ SNP	**-0.151**	**0.058**	**0.017**	**-0.271,-0.03**

5.5.2.3.4.2 PPARλ P12A SNP (N=27)

PPARλ SNP interaction with current drinking was assessed and found to be not significant. It is noteworthy to mention that the unadjusted effect of the PPARλ SNP on ln-SBP was significant (p-value=0.022). In the final model, PPARλ SNP was found to significantly associate with ln-

SBP. After adjusting for age, race and waist, the decrease in ln-SBP was found to be ~0.15 mm for a male with at least one Ala12 allele of the PPARλ SNP compared to a male with the wild genotype (Pro12Pro) (**Table 90**).

Table 90: Results of Multiple Linear Regression of ln-SBP and Pro12Ala PPARλ SNP in Males (N=27)

Independent Variable	B	S.E.	p-value	95% CI
PPARλ P12A	**-0.152**	**0.054**	**0.010**	**-0.265,-0.039**
Age	0.001	0.001	0.205	-0.001,0.003
Race	0.067	0.041	0.113	-0.017,0.152
Waist	0.001	0.001	0.234	-0.001,0.004

Since the p-value for each of the IL6 SNP was > 0.1 in its final model, there was no need to assess gene-gene interaction. However, adjustment for the PPARλ SNP was performed in the IL6 final model since the p-value of the PPARλ SNP in its final model was < 0.05.

5.5.2.3.5 Diastolic Blood Pressure (DBP, mm)

For DBP, the following variables were entered in a stepwise fashion: ever smoking, current drinking, CRP serum level and IL6 serum level; smoking was defined by smoking cigarettes or/&pipes or/&cigars.

5.5.2.3.5.1 IL6 G-174C SNP (N=27)

IL6 SNP interaction with current drinking was tested and found to be not significant. It is noteworthy to mention that effect of the IL6 CG genotype on DBP became significant (p-value was 0.118 and became 0.036) when current drinking variable entered the regression model. One step further, it became borderline significant when we adjusted for PPARλ SNP (p-value~0.05). It is also worth mentioning that IL6 serum levels, current drinking and CRP serum levels entered the model after adjusting for both effects of the IL6 and PPARλ SNPs. In the final model, PPARλ SNP, IL6 serum levels, current drinking and CRP serum levels were found to significantly associate with DBP. Moreover, IL6 CG SNP, age and waist were found to borderline significantly associate with DBP (p-value ~0.05,~0.05 and~0.06 respectively). Adjusting for IL6 and PPARλ SNPs, age, race, waist, IL6 serum levels, current drinking and

CRP serum levels (when applicable), the increase in DBP was found to be ~ 5 mm,0.13 mm, 0.13 mm, ~ 5mm and ~0.8 mm for a male with the IL6 CG genotype compared to a male with the IL6 CC genotype, for a male who is 1 year older than another male, for a male whose waist is 1 cm higher than another male, for a male who currently drinks compared to another male who does not currently drink and for a male whose CRP serum levels are 1 mg/L higher than another male's. On the other hand, the decrease in DBP was found to be ~ 9.2 mm and ~3.2 mm for a male with at least one Ala12 allele of the PPARλ genotype of the PPARλ SNP compared to a male with the wild genotype (Pro12Pro) and for a male whose IL6 serum levels are 1 pg/ml higher than another male respectively (**Table 91**).

Table 91: Results of Multiple Linear Regression of DBP and G-174C IL6 SNP in Males (N=27)

Independent Variable	B	S.E.	p-value	95% CI
IL6 CG	**5.081**	**2.433**	**0.052**	**-0.052,10.214**
IL6 GG	-0.560	2.652	0.835	-6.156,5.036
Age	**0.130**	**0.062**	**0.051**	**0.000,0.260**
Race	3.855	2.389	0.125	-1.187,8.896
Waist	**0.133**	**0.067**	**0.062**	**-0.008,0.274**
PPARλ SNP	**-9.182**	**3.053**	**0.008**	**-15.624,-2.740**
IL6 serum level(pg/ml)	**-3.166**	**1.096**	**0.010**	**-5.744,-0.854**
Current drinking	**4.995**	**2.191**	**0.036**	**0.372,9.618**
CRP(mg/L)	**0.801**	**0.378**	**0.049**	**0.004,1.598**

5.5.2.3.5.2 PPARλ P12A SNP(N=27)

No potential PPARλ SNP interactions were identified. It is noteworthy to mention that the unadjusted effect of the PPARλ SNP on DBP was highly significant (p-value = 0.001); the decrease in DBP was ~ 15 mm for a male with at least 1 Ala12 allele compared to a male with the wild genotype (Pro12Pro). In the final model, PPARλ SNP, IL6 serum levels, current drinking and CRP serum levels were found to significantly associate with DBP. Moreover, age, waist and IL6 CG genotype of the IL6 SNP, were found to borderline significantly associate with DBP (p-value ~0.05,~0.06 and~0.05 respectively). Adjusting for IL6 and PPARλ SNPs, age, race, waist, IL6 serum levels, current drinking and CRP serum levels (when applicable), the decrease in DBP was found to be ~ 9.2 mm and ~3.2 mm for a male with at least one Ala12 allele of the PPARλ genotype of the PPARλ SNP compared to a male with the wild genotype

(Pro12Pro) and for a male whose IL6 serum levels are 1 pg/ml highe than another male respectively. On the other hand, the increase in DBP was found to be 0.13 mm, 0.13 mm, ~ 5 mm , ~ 5 mm and 0.8 mm, for a male who is 1 year older than another male, for a male whose waist is 1 cm higher than another male's, for a male with the IL6 CG genotype compared to a male with the IL6CC genotype, for a male who currently drinks compared to another male who does not currently drink and for a male whose CRP levels are 1 mg/L higher than another male's respectively (**Table 92**).

Table 92: Results of Multiple Linear Regression of DBP and Pro12Ala PPARλ SNP in Males (N=27)

Independent Variable	B	S.E.	p-value	95% CI
PPARλ P12A	**-9.182**	**3.053**	**0.008**	**-15.264,-2.740**
Age	**0.130**	**0.062**	**0.051**	**0.000,0.260**
Race	3.855	2.389	0.125	-1.187,8.896
Waist	**0.133**	**0.067**	**0.062**	**-0.008,0.274**
IL6 CG	**5.081**	**2.433**	**0.052**	**-0.052,10.214**
IL6 GG	-0.560	2.652	0.835	-6.156,5.036
IL6 serum level (pg/ml)	**-3.166**	**1.096**	**0.010**	**-5.744,-0.854**
Current drinking	**4.995**	**2.191**	**0.036**	**0.372,9.618**
CRP(mg/L)	**0.801**	**0.378**	**0.049**	**0.004,1.598**

The p-value for each of the IL6 and PPARλ SNPs was < 0.05 in its final model; p-value=0.036 and 0.007 respectively, before adjustment for either SNP in the final model of the other SNP. Therefore, gene-gene interaction was assessed and found to be not significant. Adjustment for one SNP in the final model of the other SNP was performed as well.

5.5.2.3.6 Fasting Glucose (G0, mg/dl)

For G0, the following variables were entered in a stepwise fashion: current drinking and HDL.

5.5.2.3.6.1 IL6 G-174C SNP (N=25)

No potential IL6 SNP interactions were identified. In the final model, age was found to significantly associate with G0. Adjusting for IL6 SNP and waist, the increase in G0 was found to be ~ 0.3 mg/dl for a male who is one year older than another male (**Table 93**).

Table 93: Results of Multiple Linear Regression of Fasting Glucose and G-174C IL6 SNP in Males (N=25)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	-1.382	7.296	0.852	-16.601,13.837
IL6 GG	-1.989	6.252	0.754	-15.031,11.053
Age	**0.327**	**0.142**	**0.032**	**0.031,0.622**
Waist	0.310	0.177	0.095	-0.059,0.680

5.5.2.3.6.2 PPARλ P12A SNP(N=25)

No potential PPARλ SNP interactions were identified. In the final model, age and waist were found to significantly associate with G0. Adjusting for PPARλ SNP and waist, the increase in G0 was found to be ~ 0.3 mg/dl for a male who is one year older than another male. Adjusting for PPARλ SNP and age, the increase in G0 was found to be ~ 0.4 mg/dl for a male whose waist is 1 cm higher than another male's (**Table 94**).

Table 94: Results of Multiple Linear Regression of Fasting Glucose and Pro12Ala PPARλ SNP in Males (N=25)

Independent Variable	B	S.E.	p-value	95% CI
PPARλ P12A	6.899	7.506	0.368	-8.711,22.509
Age	**0.311**	**0.130**	**0.026**	**0.04,0.582**
Waist	**0.355**	**0.162**	**0.040**	**0.018,0.692**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor adjust for the effect of either SNP in the final model of the other SNP.

5.5.2.3.7 Ln-Interleukin 6 (ln-IL6 pg/ml)

For ln-IL6 serum level, the following variables were entered in a stepwise fashion: CRP serum levels and DBP.

5.5.2.3.7.1 IL6 G-174C SNP (N=27)

No IL6 SNP interactions were identified. In the final model, waist, CRP serum levels and DBP were found to significantly associate with ln-IL6 serum levels. Adjusting for IL6 SNP, age, IR, waist, CRP serum level and DBP (when applicable), the increase in ln-IL6 serum level was

found to be ~ 0.02 pg/ml and 0.05 pg/ml for a male whose waist is 1 cm higher than another male's and for a male whose CRP levels are 1 mg/L higher than another male's respectively. On the other hand, the decrease in ln-IL6 was found to be ~0.03 pg/ml for a male whose DBP is 1 mm higher than another male's (**Table 95**).

Table 95: Results of Multiple Linear Regression of ln-IL6 serum levels and G-174C IL6 SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	0.227	0.204	0.280	-0.2,0.655
IL6 GG	0.033	0.185	0.861	-0.354,0.420
Age	0.007	0.004	0.146	-0.003,0.016
IR	0.007	0.232	0.975	-0.478,0.492
Waist	**0.019**	**0.008**	**0.037**	**0.001,0.036**
CRP(mg/L)	**0.054**	**0.011**	**0.000**	**0.031,0.077**
DBP	**-0.028**	**0.013**	**0.043**	**-0.056,-0.001**

5.5.2.3.7.2 PPARλ P12A SNP (N=27)

PPARλ SNP- interactions with waist, CRP serum levels and age were tested and found to be not significant. It is noteworthy to mention that the effect of PPARλ SNP became significant at the time waist entered the regression model (p-value was 0.216; became 0.049) and then became not significant when CRP serum levels entered the model (p-value was 0.049, became 0.905). In the final model, CRP serum levels were found to significantly associate with ln-IL6 serum levels. Adjusting for PPARλ SNP, age, IR and waist, the increase in ln-IL6 serum levels were found to be ~ 0.07 pg/ml for a male whose CRP levels are 1 mg/L higher than another male's (**Table 96**).

Table 96: Results of Multiple Linear Regression of ln-IL6 serum levels and Pro12Ala PPARλ SNP in Males (N=27)

Independent Variable	B	S.E.	p-value	95% CI
PPARλ P12A	0.036	0.3	0.905	-0.588,0.661
Age	0.005	0.005	0.292	-0.005,0.015
IR	0.169	0.250	0.505	-0.350,0.689
Waist	0.009	0.008	0.236	-0.007,0.025
CRP(mg/L)	**0.066**	**0.011**	**0.000**	**0.044,0.089**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor adjust for the effect of either SNP in the final model of the other SNP.

5.5.2.3.8 Ln-C Reactive Protein (ln-CRP mg/L)

For ln-CRP serum level, only IL6 serum levels were entered in a stepwise fashion.

5.5.2.3.8.1 IL6 G-174C SNP (N=26)

No potential IL6 SNP -environment interactions were identified. In the final model, IL6 serum levels were found to significantly associate with ln-CRP serum levels. After adjusting for IL6 SNP, age and waist, the increase in ln-CRP serum levels were found to be ~ 0.3 mg/L for a male whose IL6 serum levels are 1 pg/ml higher than another male's (**Table 97**).

Table 97: Results of Multiple Linear Regression of ln –CRP serum levels and G-174C IL6 SNP in Males (N=26)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	0.579	0.618	0.360	-0.710,1.868
IL6 GG	-0.001	0.592	0.999	-1.235,1.233
Age	0.002	0.015	0.897	-0.029,0.033
Waist	0.025	0.017	0.143	-0.009,0.06
IL6 serum level (pg/ml)	**0.314**	**0.081**	**0.001**	**0.145,0.484**

5.5.2.3.8.2 PPARλ P12A SNP(N=26)

No potential PPARλ SNP interactions were identified. In the final model, IL6 serum levels were found to significantly associate with ln-CRP serum levels. Adjusting for PPARλ SNP, age and waist, the increase in ln-CRP serum levels were found to be ~ 0.3 mg/L for a male whose IL6 serum levels are 1 pg/ml higher than another male's (**Table 98**).

Table 98: Results of Multiple Linear Regression of ln-CRP serum levels and Pro12Ala PPARλ SNP in Males (N=26)

Independent Variable	B	S.E.	p-value	95% CI
PPARλ P12A	-0.210	0.758	0.785	-1.786,1.366
Age	0.001	0.015	0.956	-0.03,0.031
Waist	0.024	0.016	0.144	-0.009,0.057
IL6 serum level (pg/ml)	**0.311**	**0.082**	**0.001**	**0.140,0.482**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need to neither assess gene-gene interaction nor to adjust for the effect of either SNP in the other SNP's final model.

5.5.2.3.9 Ln-HOMA-IR

For ln-HOMA-IR, the following variables were entered in a stepwise fashion: current drinking, HDL and TG. Current anti-diabetic medications use was forced into the model.

5.5.2.3.9.1 IL6 G-174C SNP (N=27)

No potential IL6 SNP interactions were identified. In the final model, waist and current anti-diabetic meds use were found to significantly associate with ln-HOMA-IR. Adjusting for IL6 SNP, age and current anti-diabetic meds use, the increase in ln-HOMA-IR was found to be ~0.02 μU/ml* mmol/L for a male whose waist is 1 cm higher than another male's. Adjusting for IL6 SNP, age and waist the increase in ln-HOMA-IR was found to be ~0.80 μU/ml* mmol/L for a male who currently uses anti-diabetic medications compared to a male who does not (**Table 99**).

Table 99: Results of Multiple Linear Regression of ln-HOMA-IR and G-174C IL6 SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
IL6 CG	-0.011	0.215	0.960	-0.457,0.435
IL6 GG	0.086	0.203	0.674	-0.335,0.508
Age	0.000	0.005	0.952	-0.010,0.009
Waist	**0.020**	**0.006**	**0.003**	**0.008,0.032**
Current Anti-diabetic meds use	**0.796**	**0.279**	**0.009**	**0.216,1.376**

5.5.2.3.9.2 PPARλ P12A SNP(N=27)

No potential PPARλ SNP interactions were identified. In the final model, waist and current anti-diabetic meds use were found to significantly associate with ln-HOMA-IR. Adjusting for PPARλ SNP, age and current anti-diabetic meds use, the increase in ln-HOMA-IR was found to be ~0.02 μU/ml* mmol/L for a male whose waist is 1 cm higher than another male's. Adjusting for PPARλ SNP, age and waist,the increase in ln-HOMA-IR was found to be ~0.78 μU/ml*

mmol/L for a male who currently uses anti-diabetic medications compared to a male who does not (**Table 100**).

Table 100: Results of Multiple Linear Regression of ln-HOMA-IR and Pro12Ala PPARλ SNP in Males (N=27)

Independent Variable	β	S.E.	p-value	95% CI
PPARλ P12A	0.015	0.251	0.954	-0.506,0.535
Age	0.000	0.004	0.941	-0.009,0.009
Waist	**0.019**	**0.006**	**0.002**	**0.008,0.031**
Current Anti-diabetic Meds use	**0.775**	**0.269**	**0.009**	**0.218,1.332**

Since the p-value for each of the IL6 and PPARλ SNPs was > 0.1 in its final model, there was a need neither to assess gene-gene interaction nor to adjust for the effect of either SNP in the other SNP's final model.

5.6 RESULTS OF COMPARISON OF THE ALLELE FREQUENCIES BETWEEN PCOS FAMILIES AND THE GENERAL POPULATION

To accomplish specific aim 5, we compared the genotype frequencies obtained in our PCOS families to those reported in dbSNP build 125 database. According to the information submitted by **PERLEGEN** for **24 European Americans**, the minor allele frequency G (Ala12) for PPARλ P12A variant is 0.062 (6.2%); the frequencies of the GG, CG and CC genotypes are 0(0%), 0.125(12.5%) and 0.875(87.5%) respectively. As for the IL-6 G-174C variant, the reported minor allele G is 0.479 (47.9%); the frequencies of the GG, CG and CC genotypes are 0.208(20.8%), 0.542(54.2%) and 0.250(25.0%) respectively. According to the information also submitted by **PERLEGEN** for **23 African Americans**, the minor allele frequency G(Ala12) for PPARλ P12A variant is 0.043 (4.3%); the frequencies of the GG, CG and CC genotypes are 0(0%), 0.087(8.7%) and 0.913(91.3%) respectively. As for the IL-6 G-174C variant, the reported minor allele C is 0.022 (2.2%); the frequencies of the CC, CG and GG genotypes are 0(0%), 0.043(4.3%) and 0.957(95.7%) respectively. In 82 **White Americans in our study population,** the minor allele frequency G (Ala12) for PPARλ P12A variant is 0.085 (8.5%); the frequencies of the GG, CG and CC genotypes are 0.012(1.2%), 0.146(14.6%) and 0.841(84.1%) respectively. As for the IL-6 G-174C variant, the minor allele G is 0.41 (41%);

the frequencies of the GG, CG and CC genotypes are 0.207(20.7%), 0.402(40.2%) and 0.390(39.0%) respectively. On the other hand, in 19 **African Americans in our study population**, the minor allele frequency G(Ala12) for PPARλ P12A variant is 0 (0%); the frequencies of the GG, CG and CC genotypes are 0(0%), 0(0%) and 0.1(100 %) respectively. As for the IL-6 G-174C variant, the minor allele was found to be the G allele as opposed to the C allele that was reported by PERLEGEN. The minor allele frequency G, in our African American population, is 0.24(24%). The frequencies of the CC, CG and GG genotypes are 0.579 (57.9%), 0.368 (36.8%) and 0.053 (5.3%) respectively.

6.0 DISCUSSION

This investigation was successful in demonstrating the feasibility of recruiting multigenerational, multiplex family members of women with PCOS, to study insulin resistance and inflammation markers in families with PCOS; this was despite the association of PCOS with infertility and low fecundity, and therefore the inherent difficulty in identifying large extended families with multiple PCOS-affected individuals. In spite of the small sample size, we noted several interesting significant SNP-phenotype associations. On the other hand, several of the resulting not-significant associations in our study may be attributed to the sample size limitation; this is especially valid for associations tested in subgroup analyses. These facts strongly encourage the conduction of similar studies on a larger scale, which have enough power to test different hypotheses in such an exquisite population. Imminent studies should aim as well at recruiting an adequate number of each of the different racial and gender groups, so that enough power is attained to disentangle potential important genotype-phenotype racial and gender differences.

The major challenge to future similar studies is the conduction of a successful recruitment process. Therefore, it would be worthy to present some of the major obstacles which we encountered during recruitment to provide useful guidance tips for imminent studies. An attempt was made to recruit our families from a total of 61 PCOS probands. In total, 38 (62%) indicated interest in participation; 10 (16%) PCOS families were eligible-9 (15%) PCOS probands and their families were recruited and the remaining 1(1%) was not because recruitment goal was achieved- and the remaining 28(46%) were not eligible. Of the remaining 23(38%), 5(8%) indicated no interest to participate and 18(30%) did not return the postcard indicating interest to participate. These figures indicate a high positive response rate and relatively high recruitment success rate; almost two-thirds of the probands whom we contacted indicated interest in participation (38/61) and one-fourth of those were eligible to participate

(10 eligible out of the 38 who indicated interest). Therefore, the results of this pilot study strongly support the feasibility of conducting larger similar studies in the future. It is notable that the size of some of our families was smaller than others; this is because of a latency period in the recruitment process, which was ensued due to a major personnel change(study's coordinator). Therefore, by the time we were able to resume recruitment and re-contacted the probands, we faced several problems in recruiting the family members who originally indicated interest but did not actually participate. These obstacles include: unwillingness of the proband to re-contact those family members to ascertain their sustained interest in participation or lack of continued interest in participation by those family members. The underlying reasons for the family member's discontinued interest were emerging health problems during the period of no active recruitment, lack of time, lack of enthusiasm mostly due to interrupted recruitment and the resulting absence of consistent follow-up. The lessons learned from carrying out the recruitment process for this study could be used as "potential recruitment success tips" in future similar studies. There is no doubt that the recruiter is the most critical element in a successful recruitment campaign. Important qualities that a successful recruiter should have include persistence, enthusiasm, intense follow-up and good listening skills. The recruiter should be aware of the inherent difficulty in recruiting PCOS families and therefore be persistent, no matter how frustrating and never-ending mission the recruitment process would seem. He/she should be a true believer in the importance of the study and can communicate this clearly to a proband. Follow-up phone calls with the proband are crucial for successful recruitment; prior arrangements for these calls should be made with the proband, so that she puts the effort in working towards a deadline date (Examples include contacting her family members, her physician for a clinical documentation of her PCOS diagnosis etc..). Monetary compensation and flexible clinic schedule (weekends and early morning clinic hours) were big incentives in a successful recruitment process; this is especially true for studies that involve adolescents.

Phenotypically, the adult blood relatives of the PCOS probands were on average obese, pre-hypertensive, insulin resistant, and had borderline-high TC and LDL levels. Furthermore, 37(48%) of the adults had one or more of the glucose abnormalities (6(~8%) had both IFG and IR, 30(39%) had IR only and 1(1%) had IFG only) and 26(34%) had MS with 54(~70%) satisfying at least 1 component of the MS ATP III diagnostic criteria. Interestingly, blood-

related adolescents to the PCOS probands were on average at risk of overweight, insulin resistant and had borderline low HDL levels. Furthermore, 11(~69%) of the adolescents had one or more of the glucose abnormalities (1(~6%) had both IFG and IR and 10(63%) had IR only) and 1(6%) had MS, with 12 adolescents (75%) satisfying at least 1 component of the MS ATP III diagnostic criteria, modified for age**.** Moreover, high hormonal and inflammation levels were indicated among both the adult and adolescent blood-relatives to the PCOS probands. These phenotypic characteristics strongly support the increased risk of blood-relatives, including adolescents, of 2 PCOS cases or more to PCOS-associated serious disorders (Metabolic Syndrome, Insulin Resistance, T2DM, dyslipedimia and heart disease).

Trying to validate the above phenotypic observations, we determined the prevalence rates of IFG, T2DM and MS in our study population and then compared them with those reported in the general population. For this purpose, IFG was defined as 100mg/dl≤G0≤125mg/dl, T2DM as G0 ≥ 126 mg/dl, adolescents as 12-19 years old and adults as ≥ 20 years of age. This is because these were the definitions used in the literature in reporting prevalence figures of such phenotypes in the general population. Metabolic Syndrome (MS) was defined according to the ATP III diagnostic criteria in adults and ATP III diagnostic criteria, modified for age in adolescents. The sample size of our sample of blood-related adolescents 12-19 yrs old and adults ≥ 20 years of age was equal to 24 and 69, respectively.

For comparison of prevalence figures ***in the adult sub-population***, the prevalence rate for **IFG and the mean HOMA-IR were obtained from NHANES$_{1988-1994}$** sample (**N=7347**)(728), whereas the prevalence figures **for T2DM and MS** in the general population were **obtained from NHANES$_{1999-2000}$ sample where N=4387 for T2DM analyses** (729) and **N=1677 for MS analyses**(730). For comparison of prevalence figures ***in the adolescent sub-population***, the **prevalence figures in the general population were obtained from NHANES$_{1999-2000}$ sample** where **N=1496 for IFG subsample analyses, N=4370 for T2DM analyses** (731) and **N=991, for MS analyses** (732).

The ***prevalence rates*** **of IFG, T2DM and MS in our sample of** ***adult blood-relatives vs. those reported in the general population*** were **35 %(n=24) vs. 27.3 % (n=2008), 20% (n=14) vs. 9.8% (n=430)** and **37.7 %(n=26) vs. 26.7 % (n=448).** As for insulin resistance severity, the **mean HOMA IR** in our study sample of blood-relatives vs. that in the general

population was **5.07 vs. 3.45**. ***As for adolescents***, the ***prevalence rates*** **of IFG, T2DM and MS in our sample of** ***blood- related adolescents vs. those reported in the general population*** were **25 %(n=6) vs. 12 % (n=178), 4.2% (n=1) vs. 0.2% (n=8)** and **8.3 %(n=2) vs. 6.4% (n=63).**

The above comparisons clearly show that the adult and adolescent sub-populations in our PCOS families had higher prevalence rates of IFG, T2DM and MS, compared to those reported in the general population. Moreover, the adult sub-population in this study had higher mean HOMA-IR than that reported in the general population, indicating higher insulin resistance severity as well; according to our criteria for identifying IR adults (HOMA-IR>3.9), the adult sub-population in this study is on average IR whereas that selected from the general population is not. These findings strongly support the importance of conducting research studies, which examine closely the genetic contributing factors to these higher rates of critical metabolic abnormalities/disorders in populations of PCOS families compared to those found in the general population. There are some limitations inherent in these comparisons, which are worth mentioning though. Our sample and NHANES sample are incomparable in size and female: male ratio. To illustrate this limitation, we used descriptive data of the study adult population of $NHANES_{1999\text{-}2000.}$ The adult sub-sample in this study was much smaller in size ($N=69_{>=20yrs,\ probands+\ blood\ relatives}$ vs. $N=4387_{>=20yrs}$ for NHANES), and weighted more towards women ($Males_{our\ sample} = 31.9\%$ vs. $Males_{NHANES} = 47.7\%$) than NHANES sample. However, both samples were comparable on other study population descriptive parameters ($Mean\ age_{adult\ sub\text{-}\ sample,\ this\ study} = 46.7$ yrs vs. $Mean\ age_{NHANES} = 45$ yrs; $Race_{adult\ sub\text{-}\ sample,\ this\ study}$: 81.2% Caucasians, 18.8% African Americans vs. $Race_{NHANES}$: 73.6% Caucasians, 11.4% African Americans

No evidence for linkage of each of the IL6 and the PPARγ markers to any of the examined phenotypes was found in this population of PCOS families. Both the IL6 and the PPARγ SNPs resulted in very low LOD scores in relation to all studied phenotypes, which was an expected finding given the extremely small sample size and the resulting low power. In numbers, we had approximately one-fourth the power needed to detect significant linkages and about 36-45 whole families(4-5 times our number of families) will be required for sufficient power in future similar studies. Moreover, all the obtained LOD scores were within the range of -2 and +2, indicating undetermined conclusions about linkage status. Therefore, given the

sample size limitation and considering the potential roles of IL6 and PPARγ in relation to the examined phenotypes, these loci may deserve further exploration. The possibility remains that the loci of the IL6 or/ and the PPARγ genes could play a major role in any of the examined phenotypes in PCOS families and with a larger sample size, the result may show to be positive.

The findings from the variance components association test and FBAT related to quantitative and dichotomous outcomes respectively, represent among the most important findings; this is because both tests account for the pedigree structure, and therefore family relationships. The FBAT results supported no association of either the PPARγ P12A SNP or the IL6 G-174C SNP with either metabolic syndrome (MS) or insulin resistance (IR). In conceptual disagreement to our finding of no association between the PPARγ P12A variant and MS, a study by Hasstedt et al which adopted a similar analytic strategy (family-based association strategy) in members of Caucasian familial T2DM kindreds found a significant positive association of the Ala12 allele with several traits commonly attributed to MS (BMI, systolic and diastolic blood pressures, triglyceride levels, and glucose concentration.)(561). This same study, however, supported our finding of no association between the P12A SNP and IR (561). In conceptual disagreement to our finding of no association between the P12A SNP and IR, however, another study (454) involving Caucasian parent-offspring trios, with T2DM or abnormal glucose homeostasis, and replication samples found a significant positive association of the Pro12 allele with T2DM risk. As for our finding regarding no association of IL6 G-174C SNP with MS and IR, no studies in the literature used family based association tests to study this IL6 SNP in relation to either phenotype.

The regression analyses related to dichotomous traits (IR and MS), on the other hand, showed a borderline significant negative association of the Ala12 allele of the PPARγ SNP with MS in both the total sample and females. Moreover, this kind of analyses supported a borderline significant positive association of the Ala12 allele of the PPARγ SNP with IR in males. In conceptual agreement to our former finding, several studies supported a protective effect of this variant on traits attributed to MS in different populations. In T2diabetic populations, the Ala12 allele has been associated with protective effect against T2DM (586;454;358;451;460;620; 624;627-635), increased insulin sensitivity (358; 626; 634; 636), lower BMI (358; 634; 638) and better lipid profile as well (358; 460; 637,458,460*)*. On the

other hand, other studies either did not support an association of this variant with T2DM (600; 615; 616-618; 622; 625-626; 639-641;457;642-644), insulin sensitivity (451;616; 621;624; 638; 647-648), insulin secretion (451), BMI (454; 451;616; 618; 624; 643; 645; 647-649; 633] dyslipidemia (451;616; 618 643; 645; 647-648) and blood pressure (615-616; 624; 643; 645-646; 648) in T2 diabetic populations, or reported opposite effects of this genetic variant on T2DM (626; 652), insulin sensitivity (624), BMI (561,460; 642; 650-651), lipid profile (561; 624; 643; 653) glucose concentration and blood pressure (561, 458) in T2 diabetic populations. There was a support for a protective effect of the Ala12 allele on MS traits in non-diabetic populations as well**.** Ala12 alleles of PPARλ have been shown to improve insulin sensitivity among Swedish Caucasian men (252), Caucasian women (461) and Caucasian women presenting with PCOS defined by NICHD (253), Caucasian young adults and children independent of sex, age, and BMI (455) and among siblings in Chinese and Japanese populations independent of obesity (453).

Ala12 carriers were also shown to have a better lipid profile among Asians (458) and Caucasian adults (461-462). However, in a population-based sample of Caucasian non-diabetic severely obese and normal weight , age- and sex-matched controls, no association of the P12Ala variant with any of the components of the metabolic syndrome (fasting glucose, triglycerides, high density lipoprotein cholesterol, insulin, waist circumference and blood pressure) was observed (733). As for the borderline significant positive association found between the Ala12 allele and IR in males, this finding was not supported in 2 previous studies by Hegele et al. and Ek et al. One case-control, in conceptual disagreement to our finding of a positive association of the Ala12 allele with IR in males, found no association of this allele and T2DM among Caucasian men (621).The other study was case series and found the Ala12 allele to be associated with improved insulin sensitivity in Caucasian men (252). Although the association of the Ala12 allele with IR in males was accompanied by a huge confidence interval due to the extremely small number of male subjects carrying the Ala12 allele of the PPARγ SNP and may represent a spurious finding, we believe that potential differential effect of the Ala12 allele on IR or T2DM across gender is worth exploring in future research. This is further supported by the following other findings in our study and other studies. In our study, the direction of the association between the Ala12 allele of the PPARγ SNP and IR was found

to be negative among women (association was not significant, p-value=0.1) and positive in men (association was significant). Furthermore, the Ala12 allele was found to positively associate with fasting glucose (G0) and ln-HOMAIR in males but, negatively associate with G0 and ln-HOMAIR in females (associations were not significant). One case-control study by Hegele et al. of Caucasian subjects reported a differential effect of the Ala12 allele on risk of T2DM across gender; this study found Ala12 allele to be significantly strongly positively associated with T2DM in women, but not associated with T2DM in men (621). As for our finding regarding no association of the G-174C IL6 SNP with either MS or IR, one population-based study supported these findings (604).

The **variance components association test** showed **significant /borderline significant negative association of the Ala12 allele of the PPARγ SNP with diastolic Blood Pressure** (DBP) in the total sample (β=-5.3, p-value ~ 0.016) **and with fasting glucose** (G0) in both the total sample and females ($\beta_{total,\ females}$ ~ -7, p-value$_{total,\ females}$~ 0.07) respectively.These associations are especially important because they were adjusted for covariates, which significantly associated with the outcome in the multivariate regression analyses (gender, BMI and current drinkinging for DBP; age, race and BMI for G0). The finding of a protective effect of the Ala 12 allele on DBP was inconsistently supported in previous literature in different populations. Two studies supported this finding in T2 diabetic populations. The first study by Altshuler et al involving Caucasian parent-offspring trios with T2DM or abnormal glucose homeostasis, and replication samples, found a trend towards lower DBP in subjects that were homozygous for the Ala12 allele (454). The other study by Horiki et al, involving Japanese normotensive diabetic and non- diabetic subjects and hypertensive diabetic and non-diabetic subjects found that the Ala phenotype was negatively associated with hypertension, when stratified by diabetic status (663). On the other hand, other studies did not support an association of the Ala12 allele with DBP (451; 616;645-646;648) or reported a positive association of this allele with DBP (460,561) in T2 diabetic populations. This finding has been also supported in two studies involving non-diabetic populations. The first study involved Caucasians, the Danish MONICA cohort study, found that Ala12Ala homozygotes of the variant have lower DBP compared with wild-type carriers, even after adjusting for BMI, age, and gender (462). The other study was population-based sample of middle-aged African-

American and found nonobese Ala12 carriers to have a significantly lower DBP, adjusting for age, sex and BMI (457). On the other hand , other studies did not support an association of the Ala12 allele with DBP (453,664,451,733) or reported a positive association of this allele with DBP (734, 460) in non-diabetic populations. **The finding of a protective effect of the Ala12 allele PPARγ SNP on fasting glucose** was inconsistently supported by previous literature in various populations a well. In T2 diabetic populations, three studies in Caucasian and Korean T2diabetic populations, found that the Ala12 carriers had significant lower fasting plasma glucose as compared to Pro12Pro carriers. In two of these studies, this was even after adjusting for age, changes in insulin and BMI (654-655;735). On the other hand, two studies in Caucasian and Asian T2 diabetics reported a positive association of the Ala12 allele and fasting glucose (561, 458); the latter study's finding was after multivariate adjustment (age, gender, ethnicity, BMI, alcohol, tobacco, and physical activity). In non-diabetic populations, findings were inconsistent as well. A sibling-controlled association study found that siblings with the Ala12 allele tended to have lower levels of fasting plasma glucose, after adjusting for age, gender, and body mass index in Chinese and Japanese populations (453). On the other hand, in a population-based sample of Caucasians , non-diabetic severely obese and normal weight ,age- and sex-matched controls, no association of the Pro12Ala variant with any of the component of the metabolic syndrome , including fasting glucose, was observed in either obese, juvenile obese or normal weight participants (733).

As for the IL6 G-174C SNP, the variance components association test showed a **borderline significant/ significant positive association of the CG genotype with DBP** in the total sample (β=2.2, p-value ~ 0.09) **and with ln-Testosterone in females** respectively (β=0.23 p-value ~ 0.032). **Moreover,** this test **showed significant /borderline significant positive association of the GG genotype of the IL6 G-174C SNP with ln-IL6 serum level** in the total sample (β=0.25, p-value ~ 0.045) and females (β=0.30, p-value ~ 0.07) respectively. These associations are particularly important because they were adjusted for covariates, which are known to associate or were significantly associated with the outcome in the multivariate regression analysis (gender, race, BMI, current drinking and PPARγ SNP for DBP; age, BMI, current HRT and current OC use for ln-Testosterone and race, BMI and CRP serum level for IL6 serum levels). The finding of an association of the G allele of the IL6 SNP with DBP was

mostly not supported in previous literature. Only one study involving Caucasian subjects found that carriers for the G allele of the IL-6 SNP have higher DBP, than CC homozygotes, despite similar age and body composition. The association was of borderline significance (p-value~0.05), probably due to small sample size (n=32)(516). All other studies either found no association of the IL-6 G-174C SNP (736, 604) or a positive association of the C allele (737-738,529) with DBP. This latter study, the Cardiovascular Health Study, involved elderly subjects (>=65 years) and therefore the conflicting results may be due to chance or that the IL6 G-174C SNP exhibits different effects in older individuals compared with younger people (mean age in our study = 40 years). However, **the finding of a positive association of the G allele of the IL6 SNP with testosterone levels** was mostly supported in previous literature. Two case control studies, which have considered IL-6 G–174C SNP in relation to functional hyperandrogenism and PCOS in Caucasian populations, found that the G allele is associated with hyperandrogenism, including higher total testosterone concentrations, or androgen-related phenotypic trait higher total testosterone concentrations (289,300). On the other hand, another case control study of Caucasian patients with PCOS and healthy controls found that PCOS women carrying at least one C allele of the IL6 G-174C SNP were more likely to present with elevated total T serum levels (515). **As for the positive association found between the GG genotype of the IL6 G-174C SNP and IL6 serum levels,** this finding was mostly supported in different populations (Caucasian hyperandrogenic/ with PCOS/ with CHD/with inflammatory disease or healthy subjects) in previous literature (300, 516, 739-741, 514). On the other hand, other studies either supported a positive association between the C allele of this SNP and IL6 plasma concentrations (529,742) or did not support an association of the IL6 G-174C SNP and IL6 serum levels (604, 289,736;743-744).

The regression results, but not the variance components test, **related to quantitative traits** showed significant negative associations between the **Ala12 allele of the PPARγ P12A SNP** and both ln-SBP and DBP in males (β ~ -0.15 and -9 respectively). This finding was supported in one study only involving T2diabetics where the Pro12Ala variant was found to be associated with lower DBP in Caucasian T2diabetic male subjects, even after adjusting for age, body mass index (BMI), fasting serum triglycerides, fasting serum insulin and hemoglobin A_{1c} (HbA_{1c}) (647).

As for the IL6 G-174C SNP, linear regression analyses showed significant /borderline significant negative association of the G allele (GG genotype) of the IL6 SNP with waist in the total sample and females respectively ($\beta_{total,females}$ ~ -4). Moreover, a borderline significant positive association between the G allele of this IL6 SNP (CG genotype) and DBP (β~5) was found in males. The former finding of a negative association between the G allele of the IL6 SNP and waist has been conceptually supported in one study involving Caucasian subjects; this study found the −174C allele to be associated with higher BMI in T2DM (608). On the other hand, one population-based sample involving Caucasian T2Diabetics, IGT subjects and normoglycemic controls frequency-matched for age and sex, found no association of the G-174C IL6 variant and waist circumference (604). This latter study did not support our finding of a positive association between the G allele of this IL6 SNP and DBP in males as well (604).

PPAR γ plays a pivotal role in the regulation of energy storage, adipocyte differentiation, insulin sensitivity, and lipoprotein metabolism (355-356); hence, variation in the PPARγ gene may be an important factor for the development of T2DM and the metabolic syndrome (357-359). Our variance components test showed negative associations of the Ala12 allele of the PPARγ SNP to both DBP and fasting glucose in our PCOS families. The association between the Pro12Ala variant and blood pressure is of particular interest. PPARγ is a nuclear hormone receptor that functions as a transcriptional regulator in a variety of tissues; it stimulates transcription of multiple genes necessary for adipogenesis, lipid metabolism, and insulin signaling (355-356). It is possible that the Pro12Ala variant of this protein affects several of the components of the MS by other mechanisms than altered insulin sensitivity (Blood pressure, for example). The Ala12 allele has been consistently associated with improved insulin sensitivity and decreased risk of T2DM in many studies (252,451,453) including a meta-analysis (454) and a protective effect on CVD, after adjusting for various conventional cardiovascular risk factors (658-660). One case control study of first-degree relatives of subjects with PCOS and healthy control subjects without a family history of diabetes or PCOS reported a significantly reduced frequency of the Ala12 allele in the first-degree relatives of PCOS subjects (4%) compared with the control group (11%). This study also found that fasting insulin, HOMA-IR and AUC insulin were significantly higher in first-degree relatives of PCOS subjects than in controls. Furthermore, it was found that among first-degree relatives of PCOS

subjects, carriers of the Pro12Ala variant had lower HOMA-IR compared to carriers of the wild genotype (Pro12Pro genotype). These findings have lead the authors to conclude that the decreased frequency of the Ala12 allele in 1st degree relatives of PCOS subjects compared to controls, may be the underlying reason for these findings(745). Trying to evaluate the findings of this case-control study, we determined the Ala12 allele frequency ("good allele", according to our findings) in our study population by degree of blood-relationship to proband. As expected, we found an almost consistent increasing trend of the Ala12 allele frequency as the blood-relationship to the proband gets farther; among the 1st & 2nd degree relatives, the Ala12 allele frequency was distributed as follows: 7% & 17% respectively. The bulk of the family members were in the 1st & 2nd degree relative categories; therefore, we were not able to accurately examine the trend in the Ala12 frequency across all "degree of relationship" categories and the group of spouses of probands and spouses of a blood relative to a proband ("control group"). In conceptual agreement to the findings of the above-mentioned study, the mean HOMA-IR and the prevalence of one or more glucose abnormalities (IFG or/and IR or T2DM) and metabolic syndrome, in our study population, decreases as the blood-relationship to the proband gets farther (as the Ala12 frequency increases). This suggests an increased risk of immediate blood relatives to the proband compared to other blood relatives as well as a protective effect of the Ala12 allele against IR, T2DM and other associated disorders in PCOS families.

Currently, treatments for PCOS women are specific to help relieve distinct associated symptoms. This is accomplished by using methods/treatment regimens, originally designed for other purposes/medical conditions. Oral contraceptive pills (OC), for example, are prescribed to help treat the problem of irregular menstrual cycles. OC are also, together with androgen-lowering drugs, the drugs of choice to lower levels of androgens to improve hyperandrogenic related symptoms such as acne and hair growth in PCOS women. Insulin-sensitizing medications (Metformin, most commonly used drug in PCOS women), used to treat adult-onset diabetes, are prescribed to help improve insulin sensitivity and may help regulating menstrual cycles.These treatment regimens tend not to work for every PCOS woman and this could probably be attributed to the fact that the currently adopted treatment regimens are targeted

towards interrupting a particular biological mechanism, which may not be true in some PCOS cases.

The findings of this study of positive associations between the G allele of the IL6 SNP (an inflammatory marker) and DBP, serum IL6 and testosterone levels, adjusted for covariates known to associate or were found to significantly associate with the outcome, could be an evidence of a novel biological mechanism, inflammatory pathway, which directly or indirectly lead to various metabolic disorders, such as IR and MS, and other hyperandrongenic symptoms associated with PCOS in PCOS families. Support for positive associations between increased plasma IL6 concentrations and various metabolic disorders or conditions associated with PCOS had been widely indicated in previous literature. Several lines of evidence support positive associations of increased transcription rates of IL6 with metabolic derangement and simultaneously with coronary heart disease (272). Increased IL-6 plasma levels have been found to be associated with lipid abnormalities (467,489,746) and to be positively associated with MS (467,489,747). Both increased IL6 levels and IL6 gene transcripts have been localized within atherosclerotic plaques (491-492) supporting a possible local role of inflammation in the initiation and progression of atherosclerosis (493). Furthermore, prospective clinical studies have shown that elevated serum levels of IL6 are predictive of the risk of future coronary events in apparently healthy men and women (501-503,6, 494]. Specifically, the G allele of the G-174C IL6 SNP has been positively associated with dyslipidemia(490), thicker carotid IMT (524-525), peripheral artery occlusive disease (534) and stroke (748). Positive associations have been also reported between increased serum levels of IL-6 and indices of insulin resistance (275,490,746,478,500), insulin resistance and associated syndromes (512-513, 272, 297-299,747) and T2DM (467,489,747). Specifically, the G allele of the G-174C IL6 SNP has been positively associated with indices of insulin resistance (490, 516; 743) IR (517; 743) and T2DM (517). Moreover, increased IL6 serum levels have been positively associated with altered intrafollicular steroid milieu, leading to infertility in some women (749).

Increased plasma IL6 concentrations may exert the above biological effects in one of two mechanisms; direct or indirect. As far as the relationship between elevated IL6 levels and DBP, several studies, including our study, supported significant positive correlations between

DBP and IL6 serum levels (478,500,299,746,750-753). However, most of these studies were cross sectional and therefore the direction of the association is not clear (478,299,752-753,746). The literature supports one of two mechanisms in explaining the relationship between DBP, IL6 serum levels and the resulting biological effect. The first potential mechanism is a direct effect of increased IL6 levels, in which hypertension may increase the risk of atherosclerosis via proinflammatory effects. The second one would be opposite in concept, an indirect effect of elevated IL6 concentrations; circulating IL-6 levels, by inducing hypertension constitute a significant proatherogenic cytokine (299; 495-498). On the other hand, most available literature either supports no correlation between testosterone and IL6 serum levels (289;754-755) or supports suppression of endogenous IL6 gene expression and secretion by testosterone and vice versa (756-757).Therefore, it seems from the existing literature that PCOS-related endocrine abnormalities do not activate inflammatory parameters thereby enhancing the risk of metabolic disoders associated with high levels of IL6; the opposite scenario seems unlikely as well. It is noteworthy, however, that positive weak correlation was found between testosterone and IL6 serum levels in our study population.

It is possible that in PCOS families, the inflammatory pathway is one explanation for PCOS metabolic disorders and well-defined associated symptoms. Some potential inflammatory pathways could be depicted in the following diagrams. IL6 SNP→ ↑ Plasma IL6 levels →↑ DBP→ PCOS metabolic disorders; IL6 SNP→↑ DBP→ ↑ Plasma IL6 levels → PCOS metabolic disorders. IL6 SNP→ ↑ Plasma IL6 levels→ ↑ Testosterone levels → hyperandrogenic symptoms + PCOS metabolic disorders. The last mechanism shown in the last diagram is not widely supported in the literature however, based on the positive correlation found in our study between IL6 and testosterone levels, it is worth exploring in similar bigger future studies. The current applied treatment regimens for PCOS are directed towards targeting the endpoint result (treating the symptoms) rather than examining the underlying mechanism (which could be inflammatory) behind these apparent symptoms and disorders. In the first and second diagrams and assuming that, the biological outcome is T2DM or IR, clinicians aim at preventing the occurrence of the outcome by prescribing insulin-sensitizing drugs; our argument is that the appropriate treatment regimen to follow in some PCOS cases or family members of PCOS women could be anti-inflammatory drugs instead. Future research should be

targeted on approaches to managing PCOS as an overall condition rather than as a constellation of discrete symptoms; this is achieved by understanding the various potential underlying mechanisms to PCOS associated disorders and symptoms. Efforts should be made to identify biogenetic markers of specific pathophysiology to enable more individualized therapeutic regimens. These arguments are supported by an excellent review by Wellen KE and Hotamisligil GS (747).

To reach meaningful conclusions from the findings on heritability estimates for several phenotypic traits in our PCOS families, a comparison was made between the statistically significant estimates (p-value <0.05) obtained in this study with those reported in the general population. Interestingly, the heritability estimates for waist, SBP and CRP serum level were found to be higher in our PCOS families (64%, 16% and 31% respectively) compared to those found in the general population (39%, 11% and 27% respectively) (758-760)**(Table 101).** These findings support future research studies, which involve PCOS families, to direct its efforts towards exploring the genes that would contribute towards expression of these highly heritable traits in such families.

Table 101: Heritability Estimates for Waist, HDL, SBP, CRP and HOMA-IR in our PCOS families compared with those in the General Population

Phenotypic Trait	**h2*(%)**	
	PCOS Families	**General population****
Waist**	**64**	**39**
Ln-HDL	28	50
Ln-SBP	**16**	**11**
Ln-CRP	**31**	**27**
Ln-HOMA IR	25	31

*Heritability estimates for all traits, except waist, are the proportion of the total phenotypic variability of the trait

** Heritability estimate for waist is residual heritability estimate, adjusted for age and sex

***Heritability estimates obtained from general population were based on un-transformed traits.

Other interesting observations in our study population of PCOS families were the following: the Ala12 allele of the PPARγ SNP was found to explain additional 5 % of the variation in DBP and 4% of the variation in G0. As for the IL6 C-174G SNP, the IL6 CG genotype was found to explain an additional 3% of the variation in DBP and the IL6 GG genotype of this SNP was found to explain an additional 2% of the variation in ln-IL6 serum

levels. These variation estimates seem to imply fair-to-good amount of genetic contribution of these SNPs to some important phenotypes, which have major roles in serious disorders in PCOS families (IR, T2DM, metabolic syndrome). The other interesting observation could be summarized in the magnitudes of the effect of the significant SNP-phenotype associations we obtained in this population of PCOS families. These were as follows: Ala12 allele of the PPARγ SNP with G0 ($\beta \sim -7$) and DBP (β=-5.3) ; the G allele of the IL6 G-174C SNP with DBP ($\beta \sim 2$), testosterone ($\beta \sim 0.23$) and IL6 serum level ($\beta \sim 0.25$). An attempt was made to compare these observed variation and coefficient estimates in this study population of PCOS families with similar SNP-phenotype estimates in the general population however, none were reported. This is a major limitation in the existing literature, and should be addressed in prospective studies. This is important, so that future similar studies to ours could have the opportunity to interpret such findings in an educational context for this special population of PCOS families and the medical community (examples include, is there any differential magnitude of effect of the SNP on a specific trait between PCOS families and the general population? Does the SNP explain more of the variation of a specific trait in PCOS families, compared to the general population?)

A significant difference in the allele frequencies of each of Pro12Ala SNP at PPARγ, and G-174C SNP at IL-6 between our PCOS families and the general population was expected. In particular, we hypothesized decreased frequencies of Ala12 allele and C-174 allele in our PCOS families relative to the frequencies of these alleles in the general population. The frequencies of the Ala12 and the C-174 allele in our Caucasian population vs. those from a selected sample of Caucasians from the general population were found to be 8.5% vs. 6.2% for Ala12 allele and 59% vs. 52.1% for C-174 allele. In conceptual agreement to this finding about the Ala 12 allele frequency in Caucasians, one study reported Ala12 allele frequencies of 8% in PCOS Caucasian women (253). Moreover, another case control study reported a similar increase in the IL6 -174C allele frequency among Caucasian PCOS cases as compared to controls (37.1% and 35.6% respectively)(761). On the other hand, the frequencies of the Ala12 and the C-174 allele in our African American population vs. those from a selected sample of African Americans from the general population were found 0% vs. 4.3% for Ala12 allele and 76% vs. 2.2% for C-174 allele. In conceptual agreement to our finding about the Ala 12 allele

frequency in African Americans, one study reported Ala12 allele frequencies of 1% in PCOS African-American women (253). In conclusion, our hypothesis did not hold true for Caucasians and for the C-174 allele frequency in African Americans, but held true for the Ala12 allele in African Americans in our study population. Both the Ala12 and the C-174 allele frequencies in our Caucasian population were a little bit higher that those reported in the general population. On the other hand the Ala 12 allele frequency in our African American population, as we hypothesized, was lower than that in the general population; however the difference was minimal. One remarkable observation, though, is that the minor allele for the G-174C IL6 SNP was reported to be the C-174 allele in the selected sample of African Americans from the general population as opposed to the G-174 allele for that SNP in our African American population. In our study, our African American subpopulation was made up from one African family only (N=19). Therefore, this huge discrepancy in the C allele frequency between our African American population (76%) and that from the general population (~2%) could be attributed to a sampling issue; although the C allele occurs at a low frequency in the general population (2 %), but once it occurs in a family setting it will manifest at a much higher frequency. Another potential explanation for this observation could be attributed to an admixture issue; if our selected African Americans are closer to Caucasians in their genetic constitution than to African Americans, this selected "African American" family will have a -174C allele frequency closer to that in Caucasians (59%). Since the allele frequencies did not differ much between our study population of PCOS families and the general population, future research should be directed towards examining other SNPs in the IL6 and PPARγ genes in relation to different characterizing phenotypes in these families.

In this study, the direction of several associations (significant and not-significant) between a SNP and a trait was found to be differential across gender. All these different SNP-gender interactions were tested and found to be not significant, most probably due to small sample size and the resulting instability of the model when too many parameters are included in it. It is worth mentioning, however, that some of these interactions had a $0.08 \leq$p-value≤ 0.2, which made it worth to present these potential SNP-gender interactions in relation to relevant traits, so that future studies with large enough sample size and power can better examine these interactions. The Ala12 allele of the PPARγ SNP was found to negatively associate with waist

and ln-TG and positively associate with G0, ln-IL6 serum level, IR and ln-HOMAIR in males. Conversely, this allele was found to positively associate with waist and ln-TG and negatively associate with G0, ln-IL6 serum level, IR and ln-HOMAIR in females. As for the IL6 SNP-gender interactions, the G allele of the IL6 G-174C SNP was found to negatively associate with ln-HDL and DBP and positively associate with MS, ln-CRP serum level, ln-HOMAIR, IR and ln-TG in males. In contrast, this genotype was found to positively associate with ln-HDL and DBP and negatively associate with MS, ln-CRP serum level, ln-HOMAIR, IR and ln-TG in females. Another observation, which we think is worth examining in prospective studies, is the following: significant associations were found between the Ala12 allele of the PPARγ SNP and DBP in total sample and males but not in females; since the sample size of females was greater than that of males in our study ($N_{females} \sim 2.5\ N_{males}$), this finding could be a random or a real finding and it may be worth future investigation.

Due to the lack of power to test for racial differences, an effort was made to look at SNP-phenotype associations in the raw data. **Among Caucasians,** the Ala12 allele of the PPARγ SNP seems to be protective against both IR and MS; this is because the proportion positive for each of these outcome decreases with the number of the Ala12 allele. On the other hand, the G allele of the G-174C IL6 SNP seems to be a risk factor for both IR and MS; this is because the proportion positive for each of these outcome increases with the number of the G allele. **Among African Americans,** the Ala12 allele of the PPARγ SNP did not exist and therefore conclusions regarding its association with MS or IR cannot be made. As for the G-174C IL6 SNP, the G allele seems to be protective against both IR and MS; this is because the proportion positive for each of these outcome decreases with the number of the G allele. Based on these findings, we attempted to test interactions between the IL6 G-174C SNP with race on each of IR and MS. When testing for these interactions, the standard error for the IL6 GG genotype was enormously big due the extremely small number of African Americans carrying the G allele of this SNP (for CG 7; for GG 1). Therefore, the test of interaction between the IL6 SNP and race on MS was inconclusive. In spite of this though, the interaction term between the IL6 SNP and race on IR turned out to be borderline significant and actually significant between the IL6 CG genotype and race on IR. This is definitely worth exploring in future studies.

The findings of this study are valuable for several reasons: 1) The findings of this study are the first to be reported in PCOS extended families 2) The significant (borderline significant) SNP-phenotype associations seem robust enough to show, despite the small sample size limitation in this study and the adjustment for the significant effect of many covariates on the examined trait; this is especially true for associations which showed to be significant/ borderline significant in total as well as in subgroup analyses. 3) The magnitude of some of these associations was potentially big. This illustrates the likely significant contribution of the studied SNP to the trait of interest in PCOS families. 4) The levels of each of the 4 traits (DBP, G0, testosterone, IL6 serum level), which showed a significant association with any of our studied SNPs, are well known to be associated with serious PCOS-associated disorders (762-769).

This study had its limitations and strengths. The major limitation was the small sample size, which "most probably" deterred us from detecting many potential important associations and linkages in this special population of PCOS families. Another limitation is the inability to examine potential ethnic/gender disparities in relation to SNP-phenotype associations due to the limited number of participating African Americans and males respectively. A third potential limitation lies in our definition of an adult; in our study, adult status was defined by subjects ≥ 18 years of age. This was based on the conventional definition of an adult by the US government. This could be a limitation to studies, which wish to compare our findings with similar findings reported in different populations where adult/adolescence status was defined differently. There is also the potential for selection bias as we advertised for PCOS women with one or more PCOS cases in their family and announced the major aim as the availability of screening for CVD risk factors (glucose, insulin, lipid levels). However, the higher prevalence rates of the glucose abnormalities found in the adolescent sub-population of these PCOS families, whose participation is probably not out of health concerns, compared to those reported in the general population argue against this kind of bias. The possibility still remains, though, that our population of PCOS familes is not representative of all PCOS families. This study had several strengths as well. These include 1. This study was the first to attempt recruiting multigenerational, multiplex family members of women with PCOS, despite the association of PCOS with infertility and low fecundity, and therefore the inherent difficulty in finding large

extended families with multiply PCOS-affected individuals. 2. By nature of the study design, we were pioneers in examining phenotypic characteristics of a special subpopulation-adolescents-within a unique population, PCOS families 3. This study was the first to test for linkage and association of IL6 gene locus / IL6 gene SNPs with phenotypic features in PCOS families. 4. There has been one linkage and association study, which studied IRS1 and PPARγ genes in relation to PCOS/hyperandrogenemia in nuclear families of European and Caribbean origins. This study was the first family-based study, which recruited extended, rather than nuclear families to assess linkage and association of PPARγ and IRS1 gene locus / PPARγ and IRS1 gene SNPs to phenotypic features in PCOS families. 5. This study was the first to assess linkage and association of PPARγ and IRS1 gene locus / PPARγ and IRS1 gene SNPs to different phenotypes (PCOS/IR phenotype, Metabolic Syndrome and its components and Insulin Resistance, inflammatory markers' and testosterone serum levels) than those previously reported (PCOS or hyperandrogenemia) 6. This study was the first to assess linkage and association of PPARγ or IRS1 gene locus / PPARγ and IRS1 gene SNPs in different populations (African Americans and Caucasians) than those previously studied (Families of European and Caribbean origins) 7. This study was the first to report on important SNP-phenotype associations and prevalence rates of important phenotypes in adults and adolescents of PCOS extended families.

APPENDIX A

QUESTIONNAIRES

A.1 CLINIC QUESTIONNAIRE

GeneIRP

Health Sciences IRB
Protocol #
Approval Date
University of Pittsburgh

ID# |___|___|___|___|___|___| - |___|

Genetics of Insulin Resistance

The PPAR Pathway

Clinic Questionnaire

Respondent Name

Last_______________________________ First ____________________________ MI _________

Gender: Male □ Female□

Address: Street ____

City ______________________________ State ________ ZIP ____________

Phone # Home: (___)_____________ Work: (___)_____________

Area **Area**

Social Security # ____________ - _________ - ______________

Date of Birth __________/_________/________________

Month Day Year

Current Age _________

Ethnicity ____________________________

Interviewer Initials ____________

Date of Visit __________/_________/_______________

Month Day Year

Visit Setting: Clinic Home

SECTION A. DEMOGRAPHIC INFORMATION

A1. What is your current marital status?

1. Married (Spouse's First Name and MI ____)
2. Separated
3. Widowed
4. Divorced
5. Never married
6. Other (Specify) ____________________________

A2. What is your highest grade or level of schooling completed? (Circle number)

Elementary	0	1	2	3	4	5	6	7	8
Secondary	9	10	11	12					
Post Secondary		13	14	15	16	17+			

A2.1 Degree earned ____

A3. What is your current occupation?

____

A3.1 What category best describes your current job area?

1. Professional
2. Managerial
3. Sales
4. Technical (i.e. Electronics, computers)
5. Service (i.e. Cook, waitress)
6. Clerical
7. Laborer (i.e. Construction, home repair)
8. Homemaker
9. Retired
10. Unemployed (Disabled)
11. Unemployed (Not disabled)
12. Other (Specify) _________________________________

A3.2 How many hours do you work per week? _________________

SECTION B. MEDICAL HISTORY

B1. Have you ever been <u>told by a physician</u> that you have any of the following conditions?

	(a)		(b)	(c)		(d)
	Doctor Diagnosed		Onset Date	Currently Treated?		Medication
	Yes	No	Year	Yes	No	
Diabetes – Insulin Dependent Childhood Onset						
Diabetes – Non-insulin dependent Mature Onset (After age 25 years)						
Kidney Disease						
Thyroid – Hypoactive						
Thyroid – Hyperactive						
Thyroid – Other						
(Specify ________________)						
Ulcer – Peptic						
Ulcer – Duodenal						
Nervous or Emotional Problem						
Cancer						
(Specify ________________)						
High Blood Pressure						
Angina						
Heart Attack/ MI						
Bypass Surgery/Angioplasty						
Circulation Problems						
Stroke						

SECTION C. CURRENT MEDICATIONS

C1. Are you currently taking any of the following prescription medications?

	(a)		(b)		(c)
	Currently Taking Medication?		Duration of Use		Medication Name and Dosage
	Yes 1	No 2	Months	Years	
Antiandrogens (Flutamide)					
GnRH agonists (Leuprolide)					
Cholesterol-lowering					
Other					
Other					
Other					
Other					
Digitalis					
Diuretics 1. Thiazide 2. Non-thiazide					
Coronary Vasodilators					
Antiarrhythmics					
Sedatives					
Barbiturates					
Major tranquilizers (Thorazine)					
Minor tranquilizers (Librium, Valium)					
Antidepressants (Prozac, Zoloft)					
Antihypertensives					
Antidiabetics (Glipizide, Metformin)					
Insulin					
Anticonvulsants (Dilantin)					
Thyroid Medication (Synthroid)					
Corticosteroids (Prednisone)					
Anticoagulants (Coumadin)					
Chemotherapy (Methotrexate)					

C2. Are you taking any over-the-counter medications? Yes No

If yes, please specify.

1. ______________________________
2. ______________________________
3. ______________________________
4. ______________________________
5. ______________________________

SECTION D. FAMILY HISTORY

D1. Do you or any of your family members have Polycystic Ovary Syndrome* (PCOS)?

*This is a condition marked by menstrual irregularity and/or infertility, central body weight gain ("apple shape") and sometimes increased body hair and severe acne.

1. Yes
2. No
3. Possible
4. Unknown

D2. Which of the following have been diagnosed with PCOS or are suspected to have PCOS?

Relative	Diagnosed	Number	Suspected	**Number**
Yourself				
Maternal grandmother		N/A		N/A
Paternal grandmother		N/A		N/A
Mother		N/A		N/A
Sister(s)				
Daughter(s)				
Other1				
Other2				

D3. Have you or any of the following first- and second-degree relatives developed early baldness (i.e., before the age of 30 years)?

1. Yes
2. No
3. Possible
4. Unknown

D4. Which of the following developed early baldness?

Relative	Diagnosed	Number	Suspected	**Number**
Yourself				
Maternal grandfather		N/A		N/A
Paternal grandfather		N/A		N/A
Father		N/A		N/A
Brother(s)				
Son(s)				
Other				

D5. GeneIRP FAMILY HISTORY TABLE ID# |___|___|___|___| - |___|

	Zip Code	Date of Birth	Alive		If No, Cause of Death	Age(current or at death)	Date of Death	PCOS		NIDDM		CVD		Baldness	
			Yes	No				Yes	No	Yes	No	Yes	No	Yes	No
Mother															
Father															
Sibling															
1.															
2.															
3.															
4.															
Children															
1.															
2.															
3.															
4.															
Other _______															
Other _______															
Other _______															

<u>Definitions:</u> PCOS → Polycystic Ovary Syndrome CVD → Coronary vascular disease (i.e. stroke, heart attack, etc.)
NIDDM → Adult-onset or Type 2 diabetes BALDNESS → In men or women

SECTION E. LIFESTYLE DESCRIPTION AND HABITS

E1. Have you ever smoking cigarettes?

1. Yes
2. No

If yes:
E1.A. At what age did you start smoking cigarettes? __________ years old

E2. Are you currently smoking?

1. Yes (Go to E2.A)
2. No (Go to E2.D)

IF YES:

E2.A How many cigarettes, <u>on average</u>, do you smoke daily?

1. Once in a while, not daily
2. Less than half a pack per day
3. Half a pack up to one pack per day
4. One pack per day
5. One pack up to two packs per day
6. Two packs per day or more

E2.B For how many years have you smoked? ____________years

E2.C Has the smoking been continuous?

1. Yes
2. No

IF NO:

E2.D If not currently smoking, for how many years did you smoke before you stopped?

Total number of years ____________

Age when stopped _____________

E2.E How many cigarettes, <u>on average</u>, were you smoking at the time you stopped?

1. Once in a while, not daily
2. Less than half a pack per day
3. Half a pack to one pack per day
4. One pack per day
5. One pack up to two packs per day
6. Two packs per day or more

E2.F Had the smoking been continuous at the time you stopped?

1. Yes
2. No

E3. Have you ever smoking cigars?

1. Yes
2. No

If yes:
E3.A. At what age did you start smoking cigars? __________ years old

E4. Are you currently smoking cigars?

1. Yes (Go to E4.A)
2. No (Go to E4.D)

IF YES:

E4.A How many cigars, on average, do you smoke daily?

1. Once in a while, not daily
2. 1-2 cigars
3. 3-4 cigars
4. Four cigars per day or more

E4.B For how many years have you smoked cigars? ____________years

E4.C Has the smoking been continuous?

1. Yes
2. No

IF NO:

E4.D If not currently smoking, for how many years did you smoke cigars before you stopped?

Total number of years ____________

Age when stopped _____________

E4.E How many cigars, on average, were you smoking at the time you stopped?

1. Once in a while, not daily
2. 1-2 cigars
3. 3-4 cigars
4. Four cigars per day or more

E4.F Had the smoking been continuous at the time you stopped?

5. Yes
6. No

E5. Have you ever smoking pipes?

2. Yes
2. No

If yes:
E5.A. At what age did you start smoking **pipes**? __________ years old

E6. Are you currently smoking pipes?

3. Yes (Go to E4.A)
4. No (Go to E4.D)

E7. Do you current drinking alcoholic beverages when out socially or when relaxing?

1. Yes
2. No

IF YES:

E7.A On the days that you drink, are you more likely to drink:

1. Beer

2. Wine
3. Mixed drinks
4. Hard liquor

E7.B One the days that you drink, how many 8 oz. glasses do you drink <u>on average</u>? _________

E7.C How often do you drink this amount?

Beer __________	Wine __________	Mixed _________	Liquor ______
1. Every day	1. Every day	1. Every day	1. Every day
2. Almost every day	2. Almost every day	2. Almost every day	2. Almost every day
3. 3-4 times/week	3. 3-4 times/week	3. 3-4 times/week	3. 3-4 times/week
4. 1-2 times/week	4. 1-2 times/week	4. 1-2 times/week	4. 1-2 times/week
5. 2-3 times/month	5. 2-3 times/month	5. 2-3 times/month	5. 2-3 times/month
6. Once a month	6. Once a month	6. Once a month	6. Once a month
7. 6-11 times/year	7. 6-11 times/year	7. 6-11 times/year	7. 6-11 times/year
8. 1-5 times/year	8. 1-5 times/year	8. 1-5 times/year	8. 1-5 times/year

SECTION F. ANTHROPOMETRICS

F1. Preliminary Measurements:

Clinic	**Home**
Weight ______________ pounds (without shoes)	Weight _____________ pounds (without shoes)
Height ______________ inches (without shoes)	Height _____________ inches (without shoes)

F2.A Have you lost 10 pounds or more in the last year?

1. Yes 2. No 9. Unknown

F2.B Have you gained 10 pounds or more in the last year?

1. Yes 2. No 9. Unknown

F3. Approximately how much did you weigh (in pounds):

F3.A When you were 18 years old? __________________

F3.B When you were 25 years old? __________________

F3.C When you were 35 years old? __________________

F3.D When you were 45 years old? __________________

F3.E When you were 55 years old? __________________

F4. Circumference Measurements (to nearest 0.1 cm)

Waist (abdominal) Girth 1) __________________________________ cm

2) __________________________________ cm

Hip Girth 1) _________________________________ cm

2) _________________________________ cm

SECTION G. STANDARD PULSE AND BLOOD PRESSURE ASSESSMENT

Participants must avoid caffeine for at least 30 minutes prior to assessment. Subject must be quiet and remain in a seated position continuously for 5 minutes prior to and during the 2 blood pressure measurements. During the measurements of blood pressure, there should be no change in the participant's position.

Observers Initials: ___________

G1. Radial Pulse

Beats in 30 seconds ______________ X 2 = _________________ Beats/minute

Is pulse regular? 1. Yes 2. No

G2. Cuff Size and Peak Inflation Level

______ 1. Child
______ 2. Regular Adult (16.0 – 22.5 cm) Pulse obliteration pressure (POP) ____________
______ 3. Large Adult (30.1 – 37.5 cm) Peak Inflation Level Std. Man. (PIL) _____30____
______ 4. Thigh (37.6 – 43.7 cm) Peak Inflation Level (POP + PIL) ____________

G3. Systolic/Diastolic Blood Pressure

	Systolic Reading			**Disappearance 5th Phase Diastolic**		
Reading 1 (Std)	______	______	______	______	______	______
Reading 2 (Std)	______	______	______	______	______	______
Sum of Readings						
Average						

SECTION H. REPRODUCTIVE HISTORY <u>(WOMEN ONLY Except H5)</u>

H1. Have you ever used oral contraceptives?

1. Yes
2. No
3. Unknown

If yes:

H1.A At what age did you start using oral contraceptives? ___________ years

H1.B For how long have you used/did you use oral contraceptives? _________ years

_________ months

H2. Are you currently using oral contraceptives?

1. Yes
2. No
3. Unknown

If no:

H2.A At what age did you stop using oral contraceptives? ___________ years

H3. Have you ever used hormone replacement therapy?

1. Yes
2. No
3. Unknown

If yes:

H3.A At what age did you start using hormone replacements? ___________ years

H3.B For how long have you used/did you use hormone replacements? __________ years

__________ months

H4. Are you currently using hormone replacement therapy?

1. Yes
2. No
3. Unknown

If no:

H4.A At what age did you stop using hormone replacements? ____________ years

H5. Reproductive Tables (Apply to both MEN and WOMEN)

H5.A Have you ever conceived a child?

1. Yes
2. No (Go to H5.D)

H5.B What are the total number of conceptions you have had? __________

H5.C What are the total number of live births you have had? __________

	Gender	Date of Birth	Current Age
Child 1			
Child 2			
Child 3			
Child 4			
Child 5			
Child 6			
Child 7			
Child 8			
Child 9			
Child 10			

H5.D Have you (**OR <u>FOR MEN:</u>** your female partner(s) with whom you have had children) ever taken any medication to induce ovulation (fertility drugs*)?
* Clomid, Serophene, Pergonal, Metrodin, hcG.

1. Yes
2. No

Name of Fertility Drug	Approximate Age When Taken	Number of Cycles Taken

H6. Menstrual History(Applies to <u>WOMEN ONLY)</u>

The following questions are related to your menstrual periods. We are interested in how often and how predictable your cycles have been throughout your life.

H6.A How old were you when you had your menstrual period? _____________ years

I will be asking you a series of questions related to your menstrual cycle and hormone use throughout several stages of your life (i.e., during your teen years, twenties, thirties, etc.). We are interested in continuous exposure to lifetime hormones in this section.

H6.B Lifetime Menstrual/Exogenous Hormone History Table

	Teens	20s	30s	40s	50s	60s	70s
Hormone Use and Duration							
1. OC							
Duration							
2. Provera							
Duration							
3. HRT							
Duration							
4. None							
Total Duration (months)							

When NOT pregnant or using hormones:							
Avg # Periods/Year							
Avg Cycle Length (days)							
1. <21							
2. 22-26							
3. 27-32							
4. 33-40							
5. >40							
6. Irregular							
7. No periods							

H6.C Have you had at least one period in the last 12 months?

1. Yes
2. No

H6.C.1 How old were you when you stopped having you menstrual period? ________ yrs

H6.C.2 For what reason did your periods stop?

1. Surgery (Uterus and/or ovaries removed)
2. Natural Menopause
3. Radiation therapy
4. Drug therapy
5. Other __________________

H6.D What was the first day of your last menstrual period?

Date ______/________/_______

H6.E Thinking back over the past 12 months, in how many of those months did you have a period?

____

H6.F What would you estimate was the length of your cycle over the past 12 months?

1. <21 days
2. 22-26 days
3. 27-32 days
4. 33-40 days
5. >40 days

SECTION I. ENDOGENOUS HORMONE HISTORY

I1. Were you troubled by acne ? **(Applies to both MEN AND WOMEN)**

1. Yes
2. No

If yes:

I1.A When were you troubled by acne?

1. During your teen years
2. After your teen years
3. Both 1 and 2

I1.B For how long (years)? __________ yrs

I1.C Where was (is) the acne located?

1. Face and head
2. Shoulder, back, or chest
3. Both

I1.D Do you currently have acne?

1. Yes
2. No

I2. Have you ever been troubled by unwanted body hair?(**WOMEN ONLY)**

1. Yes
2. No

If yes:

I2.A Where was the unwanted body hair? (Check all that apply)

1.	Upper lip	___________
2.	Chin	___________
3.	Neck	___________
4.	Chest	___________
5	Lower stomach	___________
6.	Inner upper thighs	___________
7.	Sideburns	___________

A.2 SF36 QUESTIONNAIRE

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. ***Thank you for completing this survey!***

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
▼	▼	▼	▼	▼
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
▼	▼	▼	▼	▼
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d Climbing several flights of stairs	☐ 1	☐ 2	☐ 3
e Climbing one flight of stairs	☐ 1	☐ 2	☐ 3
f Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g Walking more than a mile	☐ 1	☐ 2	☐ 3
h Walking several hundred yards	☐ 1	☐ 2	☐ 3
i Walking one hundred yards	☐ 1	☐ 2	☐ 3
j Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Had difficulty performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very Severe
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

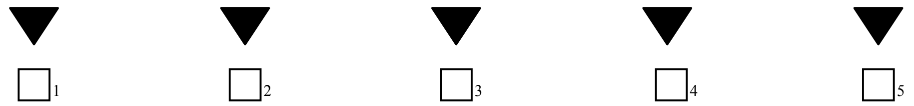
9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Have you been very nervous?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f Have you felt downhearted and depressed?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h Have you been happy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
i Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time



All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a I seem to get sick a little easier than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

THANK YOU FOR COMPLETING THESE QUESTIONS!

A.3 PAFFENBARGER PHYSICAL ACTIVITY QUESTIONNAIRE

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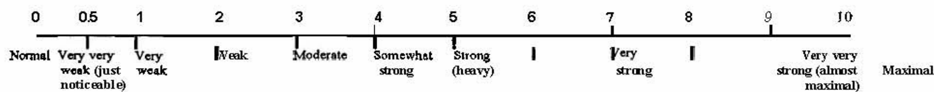
Paffenbarger Physical Activity Questionnaire

1. How many city blocks or their equivalent do you normally walk each **day?** ______ **blocks/day** (Let 12 blocks = 1 mile)
2. What is your usual **pace** of walking? (Please check one.)
   a. ___ Casual or strolling (less than 2 mph) b. ___ Average or normal (2 to 3 mph)
   c. ___ Fairly brisk (3 to 4 mph) d. ___ Brisk or striding (4 mph or faster)
3. How many flights or stairs to you climb up each day? ___ **flights/day** (Let 1 flight = 10 steps.)
4. List any sports or recreation you have actively participated in during the past year. Please remember seasonal sports or events.

Sport, Recreation, or Other Physical Activity	Number of **Times/year**	Average **Time/Episode** Hours	Minutes	Years Participation
a.				
b.				
c.				
d.				
e.				
f.				

5. Which of these statements best expresses your view? (Please check one.)
   a. ___ I take enough exercise to keep healthy. b. ___ I ought to take more exercise c ___ Don't know
6. At least once a week, do **you** engage in regular activity akin to brisk walking, **jogging**, bicycling, swimming, etc. long enough to work up a sweat, get your heart thumping, or get out of breath?
   ___ No Why not? ________________ Yes How many times per week? ___ Activity: ____________
7. **When** you are exercising in your usual fashion, how would you rate your level of exertion (degree of effort)? (Please circle one number.)



8. On a usual weekday and a weekend day, how much time do you spend on the following activities? Total for each day should add to 24 hours.

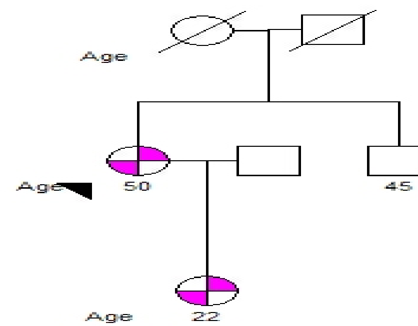
	Usual Weekday Hours/Day	Usual Weekend Day Hours/Day
a. Vigorous activity (digging in the garden, strenuous sports, jogging, aerobic dancing, sustained swimming, brisk walking, heavy carpentry, bicycling on hills, etc.)		
b. Moderate activity (housework, light sports, regular walking, golf, yard work, lawn mowing, painting, repairing, light carpentry, ballroom dancing, bicycling on level ground, etc.)		
c. Light activity (office work, driving car, strolling, personal care, standing with little motion, etc.)		
d. Sitting activity (eating, reading, desk work, watching TV, listening to radio, etc.)		
e. Sleeping or reclining		

APPENDIX B

FAMILY PEDIGREES

B.1 FAMILY 1

Family 1



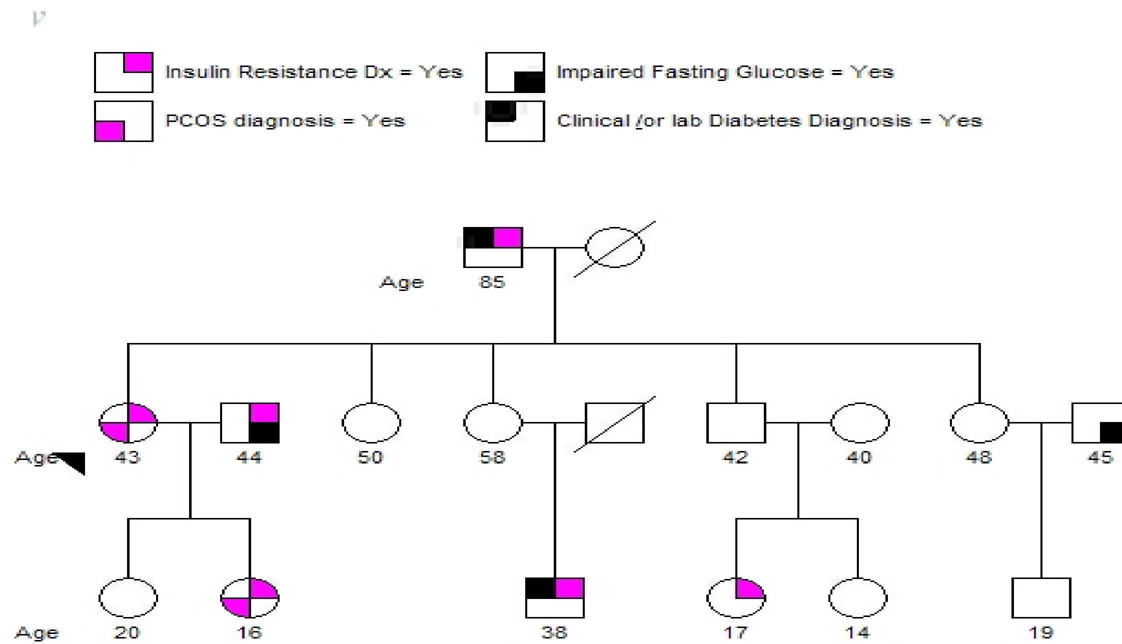
B.2 FAMILY 2

Family 2

Clinical or lab Diabetes Diagnosis = Yes
Insulin Resistance Dx = Yes
PCOS diagnosis = Yes
Impaired Fasting Glucose = Yes

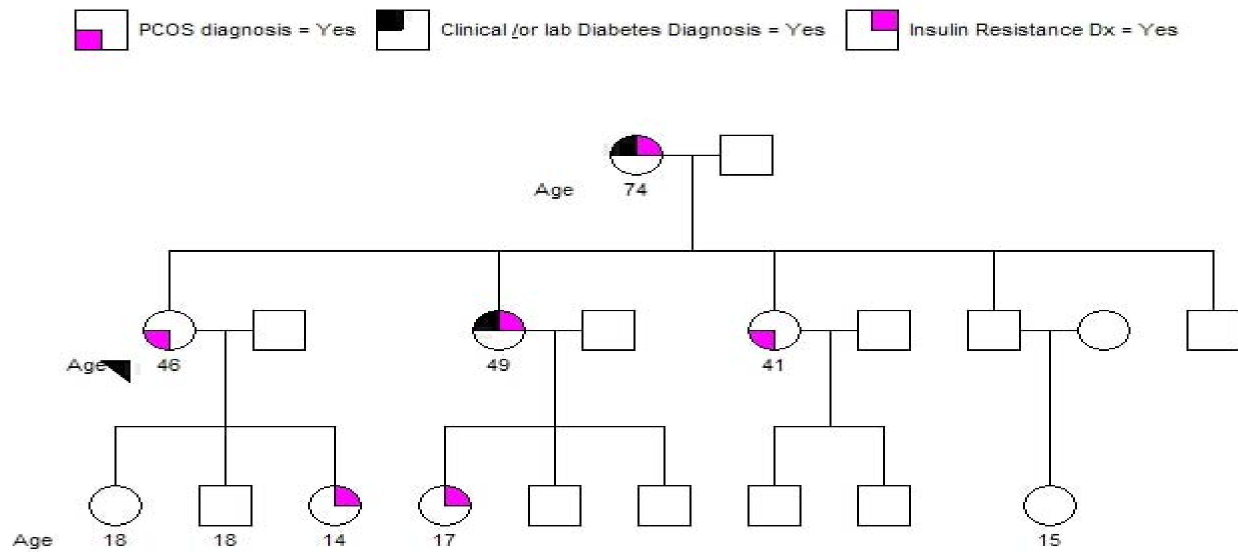
B.3 FAMILY 3

Family 3



B.4 FAMILY 4

Family 4



B.5 FAMILY 5

Family 5

Insulin Resistance Dx = Yes

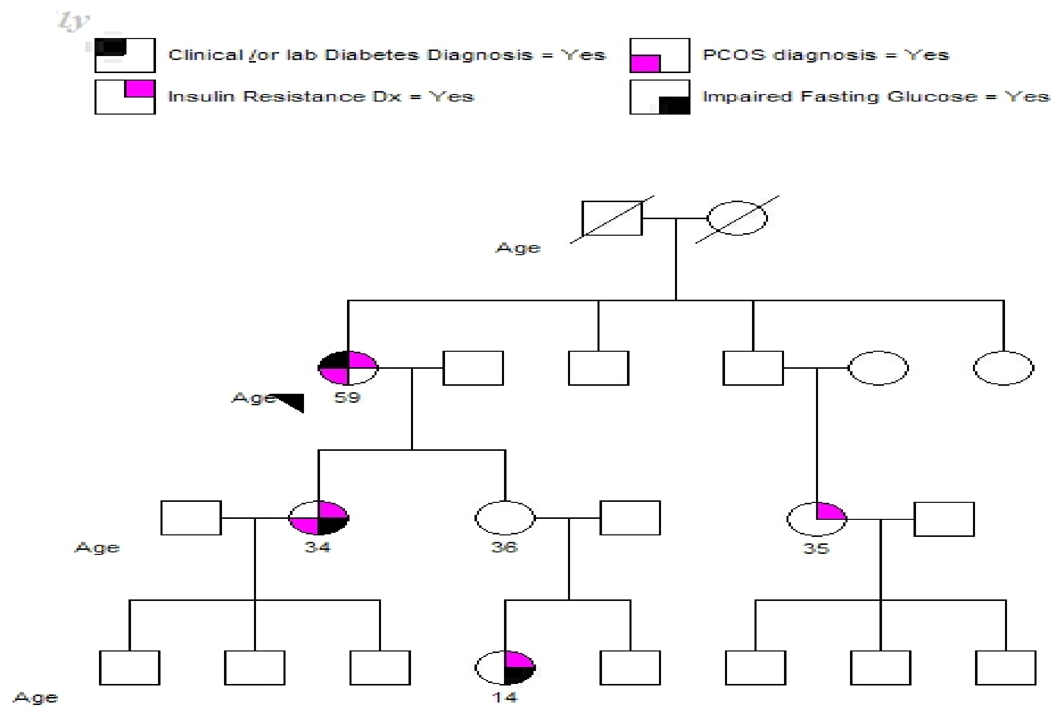
Impaired Fasting Glucose = Yes

PCOS diagnosis = Yes

Clinical /or lab Diabetes Diagnosis = Yes

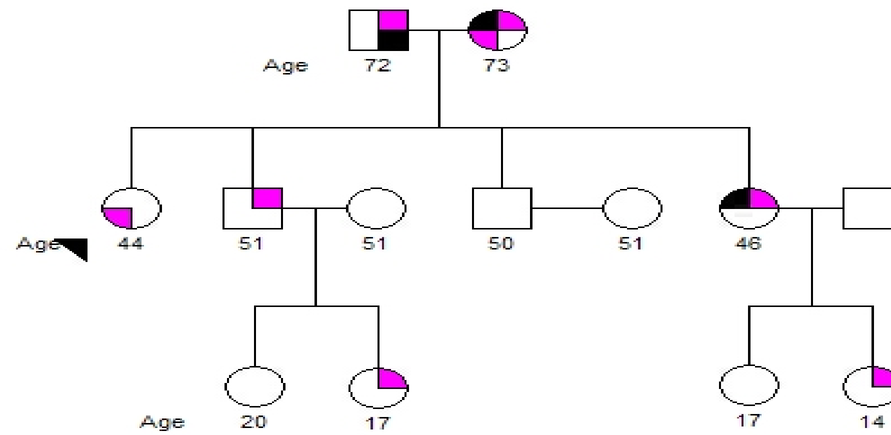
B.6 FAMILY 6

Family 6

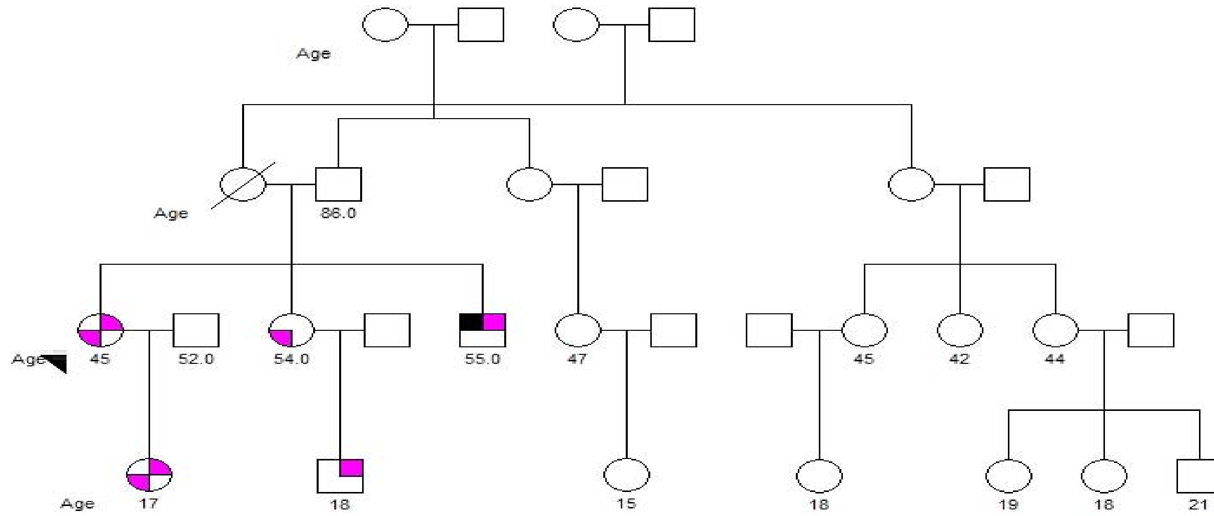


B.7 FAMILY 7

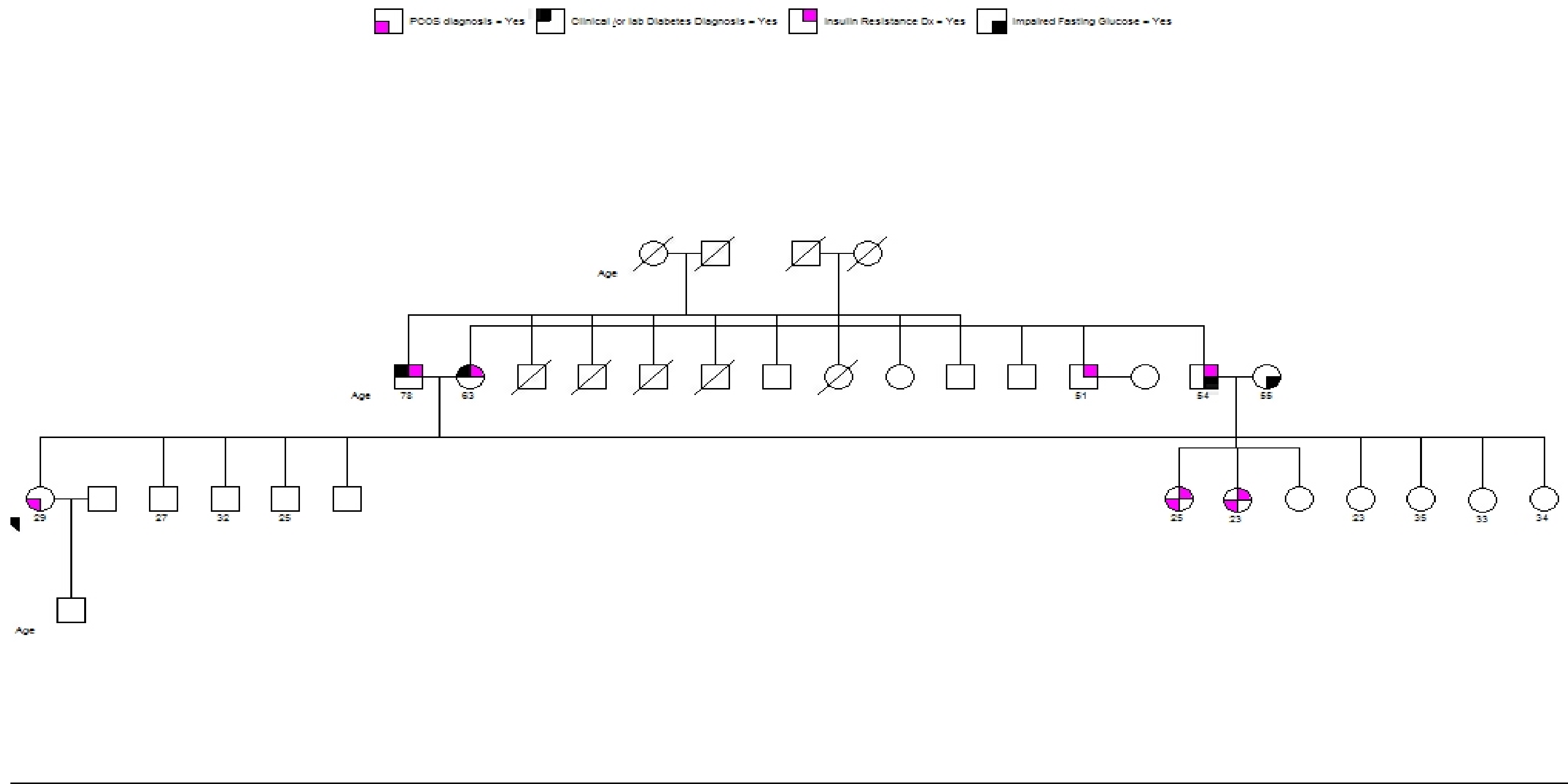
Family 7



B.8 FAMILY 8



B.9 FAMILY 9



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