Familial association of Polycystic Ovary Syndrome (PCOS) in women attending the Gynaecological Endocrinology Clinic at Groote Schuur Hospital.

Dissertation in part fulfillment of the requirements for Masters in Philosophy in Reproductive Medicine, University of Cape Town

Sascha Edelstein

THE GENETIC BASIS OF THE POLYCYSTIC OVARY SYNDROME IN SOUTH AFRICA

A Collaborative study between the Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Cape Town and the Nuffield Department of Obstetrics and Gynaecology, University of Oxford

REC: 008/1995 and 137/2002

THE GENETIC BASIS OF THE POLYCYSTIC OVARY SYNDROME IN SOUTH AFRICA

NAME: ……...

HOSP NO: ...……

ADDRESS: ……

TELEPHONE NOs. ……

……..

DOB: ……..

AGE AT PRESENTION
[i.e. AGE AT INITIAL CONSULTATION] ……..

DATE OF COMPLETION OF FORM:…….....

FORM COMPLETED BY:…….

STUDY NO: **LABORATORY NO:**

PATIENT CATEGORY:	☐ 0 Proband ☐ 1 Sister ☐ 2 Mother ☐ 3 Daughter ☐ 4 Father

PATIENT STATUS:	☐ 0 PCOS ☐ 1 PCO ☐ 2 Neither PCO/PCOS

INDEX PATIENT (NAME AND NO.):

Only for index patient **Recruited Y / N**

Sister -	1	…….	☐
	2	…….	☐
	3	…….	☐
	4	……..	☐
	5	……..	☐
	6	……..	☐
	7	……..	☐
Mother -			☐
Father -			☐

Fill in reference no.

eg.	*PCOG1*	=	*index subject*
	PCOG1M	=	*mother*
	PCOG1F	=	*father*
	PCOG 1(1), 1(2), etc	=	*sister*

ETHNICITY:	☐ 1 Black South African ☐ 2 Black African (specify) ☐ 3 Coloured (SA) ☐ 4 Mixed Race	☐ 5 Indian ☐ 6 Chinese ☐ 7 White ☐ 8 Other (specify)

PRESENTING COMPLAINT:

1. Presenting complaint

   □ 0 Amenorrhoea

   □ 1 Menstrual dysfunction

   □ 2 Hirsutism

   □ 3 Acne

   □ 4 Hyperandrogenemia

   □ 5 Infertility

   □ 6 Weight gain

   □ 7 Recurrent miscarriage

   □ 8 Family history

   □ 9 Family study

   □ 10 Other problems (please specify)

MENSTRUAL HISTORY AND CONTRACEPTION

2. Age of menarche: _____

3. Have you ever used any type of contraception?

   □ 0 No [go to question 6]

   □ 1

4. Are you currently using any type of hormonal contraception?

   □ 0 No [go to question 6]

   □ 1

5. What type of hormonal contraception are you using?

□ 0 Combined oral contraceptive pill

□ 1

□ 2 Injectable

□ 3 Intra-uterine system

6. Are you currently using any form or hormone replacement therapy?

□ 0 No

□ 1 Yes

When you were in <u>each</u> of the following age groups (until the age you are now), what were your periods like when you were **<u>NOT</u>** using hormonal contraception?
[Please give answers that apply to you as closely as possible, or otherwise indicate that you cannot remember]

MENSTRUAL HISTORY			
	UP TO 20 YRS	**21-35 YRS**	**36 YRS AND OVER**
When you were NOT using hormonal contraceptive	□ 0 Not applicable *(I was always on the Pill)*	□ 0 Not applicable *(I was always on the Pill)*	□ 0 Not applicable *(I was always on the Pill)*
Were you having regular monthly periods?	□ 0 No □ 1 Yes □ 2 Can't remember	□ 0 No □ 1 Yes □ 2 Can't remember	□ 0 No □ 1 Yes □ 2 Can't remember
How many periods did you have per year?	□ 0 Nil □ 1 If not monthly < 6 □ 2 If not monthly > 6 □ 3 If not monthly >1	□ 0 Nil □ 1 If not monthly < 6 □ 2 If not monthly > 6 □ 3 If not monthly >12	□ 0 Nil □ 1 If not monthly < 6 □ 2 If not monthly > 6 □ 3 If not monthly >12
How many days of bleeding did you usually have each period? *(bleeding requiring a tampon or sanitary pad)*	…. Days or tick √ □ 0 Too irregular to say □ 1 Can't remember	…. Days or tick √ □ 0 Too irregular to say □ 1 Can't remember	…. Days or tick √ □ 0 Too irregular to say □ 1 Can't remember
How heavy was your menstrual flow usually?	□ 0 Light □ 1 Moderate □ 2 Heavy (clots or flooding) □ 3 Can't remember	□ 0 Light □ 1 Moderate □ 2 Heavy (clots or flooding) □ 3 Can't remember	□ 0 Light □ 1 Moderate □ 2 Heavy (clots or flooding) □ 3 Can't remember

PREGNANCY HISTORY

8 Have you every been pregnant [including miscarriages, ectopic pregnancy or TOP]

□ 0 No [go to question 9]

□ 1 Yes

If Yes:
Please answer the following questions for each of your pregnancies (if you had more than 5, add a page]

PREGNANCY HISTORY					
	1st	**2nd**	**3rd**	**4th**	**5th**
How old were you at the start of the pregnancy?	……..years old	……..years old	…….years old	…….years old	…….. years old
What was the outcome of this pregnancy?	□0 Live birth □1 Still birth □2 Ectopic □3 Miscarriage □4 Termination	□0 Live birth □1 Still birth □2 Ectopic □3 Miscarriage □4 Termination	□0 Live birth □1 Still birth □2 Ectopic □3 Miscarriage □4 Termination	□0 Live birth □1 Still birth □2 Ectopic □3 Miscarriage □4 Termination	□0 Live birth □1 Still birth □2 Ectopic □3 Miscarriage □4 Termination
How many weeks were you pregnant for? (Fullterm=40w)	□0 Less than 37 □1 37 or more	□0 Less than 37 □1 37 or more	□0 Less than 37 □1 37 or more	□0 Less than 37 □1 37 or more	□0 Less than 37 □1 37 or more
If this pregnancy resulted in a birth, was the delivery vaginal or via Caesarean section?	□0 Vaginal birth □1 Caesarean □2 Not applicable	□0 Vaginal birth □1 Caesarean □2 Not applicable	□0 Vaginal birth □1 Caesarean □2 Not applicable	□0 Vaginal birth □1 Caesarean □2 Not applicable	□0 Vaginal birth □1 Caesarean □2 Not applicable
If this pregnancy resulted in a birth, what was your baby's birthweight?	…… ……grams or tick √ □0 Not applicable □1 Can't remember	…… ……grams or tick √ □0 Not applicable □1 Can't remember	…… ……grams or tick √ □0 Not applicable □1 Can't remember	…… ……grams or tick √ □0 Not applicable □1 Can't remember	…… ……grams or tick √ □0 Not applicable □1 Can't remember
Did you suffer any of the following complications of pregnancy? (Please tick all that apply)	□0 Diabetes in Pregnancy □1 High blood pressure □2 Pre-Eclampsia □3 Polyhydramnios	□0 Diabetes in Pregnancy □1 High blood pressure □2 Pre-Eclampsia □3 Polyhydramnios	□0 Diabetes in Pregnancy □1 High blood pressure □2 Pre-Eclampsia □3 Polyhydramnios	□0 Diabetes in Pregnancy □1 High blood pressure □2 Pre-Eclampsia □3 Polyhydramnios	□0 Diabetes in Pregnancy □1 High blood pressure □2 Pre-Eclampsia □3 Polyhydramnios

9. Have you ever tried to get pregnant for more than 12 consecutive months without success?

□ 0 No [go to question 11]

□ 1 Yes

10. Did you or your partner have any test(s) to discover the cause of the fertility problem?

□ 0 No [go to question 11]

□ 1 Yes

If **yes:** What were you or your partner diagnosed with? **(Please tick all that apply):**	□ 0 Endometriosis □ 1 Polycystic ovary syndrome □ 2 Pelvic inflammatory disease/pelvic infection □ 3 Uterine fibroids □ 4 Blocked tubes □ 5 Irregular or no ovulation □ 6 Poor sperm count or quality □ 7 Other problems (please specify)

Weight Changes:

11. Has your weight changed over the past five years?

WEIGHT CHANGE			
	Same	**Lost weight**	**Gained weight**
Five Years	□0	□1	□2

12. If your weight has remained unchanged please skip to question 13.

WEIGHT CHANGE					
	Lost > 13 kg	**Lost up 13 kg**	**Gained up to 6.5 kg**	**Gained up to 13 kg**	**Gained more than 13 kg**
Five Years	□0	□1	□2	□3	□4

Skin:

13. Do you suffer from excess hair growth?

□ 0 No [please go to question 14]

□ 1 Yes

[If yes please list areas affected at the different ages listed]

HAIR GROWTH			
	Up to 20yrs	**21-35 yrs**	**36 yrs and over**
Face	□0	□0	□0
Back	□1	□1	□1
Arms	□2	□2	□2
Chest	□3	□3	□3
Abdomen	□4	□4	□4
Legs	□5	□5	□5

14. Do you suffer from alopecia (thinning of scalp hair)

□ 0 No

□ 1 Yes

15. Have you ever been diagnosed by a doctor as having acanthosis nigricans?

□ 0 No

□ 1 Yes

[If yes : please list areas affected …….]

16. Do you or have you ever suffered from acne?

□ 0 No

□ 1 Yes

Medical History:

17. Do you suffer from diabetes mellitus?

□ 0 No [please go to question 20]

□ 1 Yes

18. If you have diabetes how old were you when the diagnosis was made?

…… years old

19. If you do have diabetes, how is it controlled?

□ 0 Lifestyle/diet

□ 1 Oral agents (metformin, glibenclamide)

□ 2 Insulin

20. Do you suffer from hypertension (high blood pressure)?

□ 0 No [please go to question 23]

□ 1 Yes

21. If you have hypertension, how old were you when the diagnosis was made?

…… years old

22. If you do have hypertension, how is it controlled?

□ 0 Lifestyle/diet

□ 1 Diuretics

□ 2 Antihypertensive

23. Do you suffer from high cholesterol (dyslipidaemia)?

□ 0 No [please go to question 26]

□ 1 Yes

24. If you have high cholesterol, how old were you when the diagnosis was made?

…… years old

25. If you do have high cholesterol, how is it controlled?

□ 0 Lifestyle/diet

□ 1 Statins

Family History:

26. How many full siblings do you have?

…….. sisters ……. brothers

Half siblings?

Same father: …… sisters …… brothers

Same mother: …… sisters …… brothers

27. Check whether or not any of the following conditions have occurred among your blood relatives. If it has occurred please tick the appropriate box

FAMILY MEDICAL HISTORY					
CONDITION	**Mother**	**Father**	**Female Sibling**	**Male Sibling**	**Other relatives**
Hirsutism	□0	□1	□2	□3	□4
Infertility	□0	□1	□2	□3	□4
Male balding under the age of 30	□0	□1	□2	□3	□4
Diabetes	□0	□1	□2	□3	□4
Heart Disease	□0	□1	□2	□3	□4
Heart attack	□0	□1	□2	□3	□4
High Blood Pressure	□0	□1	□2	□3	□4
High Cholesterol	□0	□1	□2	□3	□4
Blood clots in the lungs or legs	□0	□1	□2	□3	□4
Breast Cancer	□0	□1	□2	□3	□4
Uterine Cancer	□0	□1	□2	□3	□4
More than 3 miscarriages	□0	□1	□2	□3	□4

28. Have you ever used any of the following medication as treatment for PCOS ? e.g Acne

If **yes** please indicate when and for how long *[Please tick all that apply]*

(*This question is not applicable to controls)

	Up to 20 yrs	**21-35 yrs**	**36 yrs and over**
Diane	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months
Combined Oral Contraceptive Pill	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months
Metformin	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months
Cyproterone Acetate	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months
Laser Treatment / Electrolysis / Bleaching/ Waxing	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months	□$_0$ No □$_1$ Yes If Yes, ……. …..months
Acne Treatments Specify	□$_0$ No □$_1$ Yes If Yes, ……. …..months …… …… …… …… ……	□$_0$ No □$_1$ Yes If Yes, ……. …..months …… …… …… ……	□$_0$ No □$_1$ Yes If Yes, ……. …..months …… …… …… …… ……
Ovulation Induction Specify	□$_0$ No □$_1$ Yes If Yes, ……. …..months …… …… …… …… ……	□$_0$ No □$_1$ Yes If Yes, ……. …..months …… … …… …… ……	□$_0$ No □$_1$ Yes If Yes, ……. …..months …… …… …… …… ……

EXAMINATION (WOMEN)

DATE:

BP (Supine) 1. 2. Average:

Weight: Height: BMI:

Waist: Hips: WHR:

Acanthosis nigricans - □ 1 Yes □ 2 No

Hirsutism -
□ 0 No □ 1 Mild □ 2 Moderate □ 3 Severe

Ferriman Gallwey Score - □□ (Date:)

Acne -
□ 0 No □ 1 Mild □ 2 Moderate □ 3 Severe

Signs of Virilization -

Clitoromegaly	□ 1	Yes	□ 2	No	□3	N/A
Muscle Bulk	□ 1	Yes	□ 2	No	□3	N/A
Voice changes	□ 1	Yes	□ 2	No	□3	N/A
Other	□ 1	Yes	□ 2	No	□3	N/A

Specify: _____

Cardiovascular:

Respiratory:

Abdomen:

VE:

□ 0 Not done

□ 1 Normal

□ 2 Abnormal Specify: _____

Other abnormalities O/E:

Index patient: Date of original exam: Date of genetic bloods:

EXAMINATION (MEN)

DATE:

BP (Supine) 1. 2.

Weight: Height: BMI:

Balding: □ 0 No

□ 1 Yes

Age of onset:

SPECIAL INVESTIGATIONS (Women):

1. **Ultrasound** (TV / TA) Date:

   Left ovary, PCO Y / N

   Right ovary, PCO Y / N

   Other findings (include ovarian volume):

2. **Blood (fasting)** Date:

   (hormonal contraception/HRT: endocrine profile N/A)

   a. Endocrine

   FSH ……

   LH ……

   Testosterone ……

   17 α OHP ……

   SHBG ……

   DHEAS ……

   Estradiol ……

   Day 21 progesterone ……

   FAI ……

b. Metabolic (Fasting)

Glucose		……
Insulin		……
G/I ratio		……
FIRI		……
HOMA		……
Lipids -	Cholesterol	……
	Trigliserides	……
	LDL	……
	HDL	……

c. Genetic

DNA → laboratory

THE GENETIC BASIS OF THE POLYCYSTIC OVARY SYNDROME IN SOUTH AFRICA

Checklist

1. Explain study and consent to patient. Ensure family can be recruited.

2. Get informed consent

3. Complete questionnaire.

4. Examination of patient/subject

5. Arrange for ultrasound before starting study to confirm PCO. Include non-PCO sisters provided at least one sister-pair with PCO.

6. Organise fasting bloods:

   - Endocrine: Clotted blood (yellow top, gel tube) Spin and aliquot x5
   - Lipids: Clotted tube (yellow top and gel tube)
   - Glucose: Grey top (fluoride tube)
   - Insulin: Clotted tube (yellow top and gel tube)
   - DNA: 30 ml EDTA (10 ml, purple top, plain tube)

7. Enter all data into spreadsheet

DECLARATION:

This is to certify that the work contained in this dissertation is the original work of the candidate and work by others has been acknowledged as such.

This work was carried out while a subspecialist trainee in the Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Cape Town in part fulfilment of the requirements for a Masters in Philosophy in Reproductive Medicine , University of Cape Town.

Signed: _____ Dated in Cape Town: _____

Supervisor:

Prof ZM van der Spuy _____

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- Assoc Prof L Myer, for his invaluable advice and assistance with statistical analysis.
- The patients of the Groote Schuur Hospital Gynaecological Endocrinology Clinic and their families who agreed to participate in this study.
- The members of the Reproductive Medicine Unit who have contributed to the database, and in particular, Sisters S van Zijl and A Hoffman who presently maintains the PCOS patient database and Drs J van den Bergh and F Wilson who each assisted in recruiting patients and completing patient questionnaires.
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ABSTRACT:

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy in women of reproductive age, affecting 5-10% of women in the general population. Patients present with menstrual disturbances, infertility and clinical hyperandrogenism. While the pathophysiology is not completely delineated, a strong familial association has been demonstrated, suggesting a genetic component.

From January 2007 until February 2009, a total of 83 probands were recruited from the Gynaecological Endocrinology Clinic (GEC) at GSH. These were all women with PCOS according to the Rotterdam criteria who presented for management at the GEC. With their consent, first degree female family members were contacted and 57 mothers, 108 sisters and 8 daughters agreed to participate in the study.

Participants underwent a detailed history, a physical examination, venepuncture for hormonal and metabolic analysis, and either trans-abdominal or trans-vaginal ultrasound to assess for polycystic ovary morphology. The statistical package SPSS v17 was used for analysis (SPSS Inc., Chicago, Illinois). Two separate analyses were performed. Demographic details, anthropomorphic features, metabolic and hormone profile were compared first between probands, PCOS, PCO and unaffected sisters and second between mothers with PCOS and mothers without PCOS.

The prevalence of PCOS amongst first degree female relatives was high, with 19.6% of mothers, 45.4% of sisters and 55.6% of daughters assessed as affected. We were unable to demonstrate a statistically significant correlation between abnormal total testosterone or free androgen index in probands with an elevated mean total testosterone or free androgen index in family members. Hirsutism was a common clinical finding in our study population (present in 65% probands, 43% of mothers, 42% of sisters and 33% of daughters) and was a predominant feature in the phenotypic presentation of these women. The study population was overweight and had an increased WHR. An adverse lipid profile was found in mothers and

sisters of probands with PCOS. The presence of this adverse lipid profile was independent of PCOS status, age, BMI or insulin resistance, suggesting an additional factor predisposing these women to dyslipidaemia.

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LIST OF ABBREVIATIONS:

17-OHP	17-Hydoxy progesterone
AE-PCOS	Androgen Excess and PCOS Society
ASRM	American Society for Reproductive Medicine
ASN	Androgen secreting neoplasm
BMI	Body mass index
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulphate
E2	Estradiol
ESHRE	European Society for Human Reproduction and Embryology
FAI	Free androgen index
FSH	Follicle stimulating hormone
GEC	Gynaecological Endocrinology Clinic
G:I	Glucose:insulin ratio
GSH	Groote Schuur Hospital
HA	Hyperandrogenemia
HAIRAN	Hyperandrogenic insulin resistant acanthosis nigricans syndrome
HDL	High density lipoprotein

HOMA	Homeostatic model assessment of insulin resistance
IH	Idiopathic hyperandrogenism
LDL	Low density lipoprotein
LH	Luteinizing hormone
mFG	Modified Ferriman-Gallwey score
NCAH	Non-classical adrenal hyperplasia
NIH	National Institute of Health
PCO	Polycystic ovary
PCOS	Polycystic ovary syndrome
PMPB	Premature male pattern balding
SHBG	Sex hormone binding globulin
T	Testosterone
TC	Total cholesterol
TG	Triglyceride
WHR	Waist hip ratio

INTRODUCTION AND LITERATURE REVIEW:

Polycystic ovary syndrome (PCOS) is the commonest endocrinopathy in women of reproductive age, affecting 5-10% of women in the general population (Knochenhauer et al, 1998; Asuncion et al, 2000). Patients present with menstrual disturbances, infertility and clinical hyperandrogenism. While the pathophysiology is not completely delineated, a strong familial association has been demonstrated, suggesting a genetic component. Earlier association studies suggested an autosomal dominant mode of inheritance (Carey et al, 1993; Govind et al, 1999), although recently a more complex mode of inheritance has been postulated (Franks and McCarthy, 2004).

Past association studies have been confounded by the definitions used for PCOS, sample size and the absence of a clearly defined male phenotype (Amato and Simpson, 2004). Debate remains as to how PCOS should best be defined, with recent input from the Androgen Excess and PCOS Society (2009) recommending that PCOS be conceptualized as primarily an androgen excess disorder, in keeping with National Institute of Health (NIH) 1990 criteria.

PCOS is the commonest diagnosis in women presenting with androgen excess (Azziz et al, 2004; Carmina et al, 2006). In addition, hyperandrogenemia is present in 75% of women with PCOS (Huang et al, 2009). Despite the limitations of family association studies, there is evidence of a genetic basis for hyperandrogenemia in PCOS (Legro et al, 1998). Family studies have shown that sisters of women with PCOS have high prevalence of hyperandrogenism, higher than the general population, even in the absence of PCOS.

Study aim:

i) To establish the prevalence of PCOS among first degree female relatives in our patient population group.

ii) To define the phenotypic presentation(s) of women with PCOS in our study population.

iii) To compare metabolic and hormonal parameters between probands and first degree relatives.

iv) To assess whether hyperandrogenemia has a predilection for first degree relatives of probands with hyperandrogenemia themselves.

Defining the syndrome:

The National Institutes of Health (NIH) 1990 expert conference identified the Polycystic Ovary Syndrome (PCOS) as an androgen excess disorder of exclusion, with an ovarian aetiology and/or consequences. The NIH criteria are:

i) hyperandrogenism and/or hyperandrogenemia,

ii) menstrual dysfunction, and

iii) the exclusion of other known disorders of androgen excess (Knochenhauer et al, 1998).

The joint meeting of the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) in Rotterdam (2003) revised the definition of PCOS. This meeting concluded that PCOS be defined when at least two of the following three features are present:

i) oligo and/or anovulation,

ii) clinical and/or biochemical signs of hyperandrogenism, and

iii) the presence of polycystic ovaries.

This group also recommended that other androgen excess disorders be excluded before diagnosing PCOS (ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004).

While the Rotterdam criteria are in widespread use, there is still ongoing debate regarding the diagnostic criteria of PCOS. Most recently, the Androgen Excess and PCOS Society (AE-PCOS) published their task force report which concludes that PCOS should be defined by the presence of

i) hyperandrogenism (clinical and/or biochemical),

ii) ovarian dysfunction (oligo-anovulation and/or polycystic ovaries) and

iii) the exclusion of related disorders (Azziz et al, 2009).

The NIH and AE-PCOS definitions both have androgen excess as an essential criterion for the diagnosis of PCOS, whereas the Rotterdam criteria do not require androgen excess in the presence of ovulatory dysfunction and polycystic ovary (PCO) morphology. Much of the debate focuses on the inclusion of ultrasound morphology as an independent criterion, especially as polycystic ovaries are present in approximately 20% of apparently normal women of reproductive age (Polson et al, 1988; Clayton et al, 1992; Farquhar et al, 1994; Botsis et al, 1995; and Cresswell et al, 1997). The impact of using a broader definition is that it can lead to a significant increase in the population considered to be affected, but conversely the narrow definitions may exclude important clinical variants of PCOS.

Consensus on the ultrasound criteria for defining PCO was reached at the joint ESHRE/ASRM meeting, Rotterdam 2003. These include the presence of 12 or more follicles (each 2-9mm diameter) or increased ovarian volume (>10cm^3) on one occasion in one ovary. The definition does not apply to women on the oral contraceptive pill, nor can an assessment be made in the presence of a dominant follicle or corpus

luteum cyst. Technical recommendations are made, including that assessment should be performed by appropriately trained personnel; scanning should be done in the early follicular phase in regularly menstruating women (or days 3-5 after a progestogen-induced bleed); and the transvaginal approach is preferred in obese women (Balen et al, 2003).

Androgen excess disorders:

Androgen excess refers to elevated androgen levels (hyperandrogenemia) or increased androgen action (clinical hyperandrogenism) and is a common endocrine disorder, affecting approximately 7% of women in the reproductive age (Azziz et al, 2004; Carmina et al, 2006). Androgen excess can be due to disorders with specific identifiable cause and those where the diagnosis is one of exclusion. Identifiable disorders include non-classic congenital adrenal hyperplasia (NCAH), hyperandrogenic insulin-resistant acanthosis nigricans syndrome (HAIRAN) and androgen-secreting neoplasm (ASN). Disorders of exclusion include PCOS, idiopathic hirsutism and idiopathic hyperandrogenism. Idiopathic hirsutism includes those patients who have hirsutism, but normal androgen levels, ovulatory cycles and normal ovaries on ultrasound. Idiopathic hyperandrogenism refers to those patients who have clinical hyperandrogenism and elevated serum androgens, but ovulatory cycles and normal ovaries on ultrasound (Azziz et al, 2004).

Carmina et al (2006) reported the prevalence of different androgen excess disorders in a cohort of 950 women who presented with clinical hyperandrogenism to their institution at the University of Palermo, Italy between 1980 and 2004. Records were retrospectively evaluated and diagnoses assigned based on current criteria, using clinical assessment, total and free testosterone (T), dehydroepiandrosterone sulphate (DHEAS), 17 hydroxy-progesterone (17-OHP), progesterone and pelvic ultrasound. In this large study, the majority of women had PCOS - 72.1**%** of 950 patients were diagnosed with PCOS according to

the Rotterdam 2003 criteria. Of these, 538 (56.6%) were anovulatory and were considered affected by classic PCOS (consistent with NIH 1990 criteria), whereas 147 (15.5%) were ovulatory and were considered affected by mild ovulatory PCOS. Idiopathic hyperandrogenism (IH) (ovulatory and normal ovaries) was present in 15.8%, idiopathic hirsutism (ovulatory, normal ovaries and normal androgens) in 7.6%, non-classic adrenal hyperplasia (NCAH) in 4.3% and androgen-secreting neoplasm (ASN) in 0.2% (Carmina et al, 2006).

Azziz and colleagues (2004) based in Birmingham, Alabama prospectively evaluated 873 women presenting with androgen excess to their clinical service between 1987 and 2002. In this cohort, only 7% were found to have specific androgen excess disorders such as NCAH, HAIRAN and androgen secreting neoplasm (ASN). PCOS (according to NIH 1990 criteria) accounted for 82% of the 873 women presenting with clinical hyperandrogenism.

The prevalence of PCOS in the above two large studies differs somewhat, being 72.1% of 950 patients with Carmina and colleagues, but 82% of 873 patients with the Azziz group. In their discussion, Carmina et al (2006) suggest an explanation for this apparent discrepancy, namely that the setting and selection criteria differed between these two groups. Azziz et al. used the more limited NIH 1990 criteria, but were based in an Obstetrics and Gynaecology Department and included ovulatory dysfunction independent of clinical hyperandrogenism as a selection criterion. Carmina's group used Rotterdam 2003 criteria, but was based in a Medical Endocrine Department and only included women complaining of clinical hyperandrogenism (Carmina et al, 2006). Despite this discrepancy, both studies found PCOS to account for the vast majority of cases of androgen excess.

Hyperandrogenemia:

Serum testosterone circulates bound to SHBG and other proteins, such as albumin. It is only the unbound or free fraction that is metabolically active. Assessment of free testosterone is thus more important when diagnosing hyperandrogenic disorders, such as PCOS. The measurement of free testosterone by direct radioimmunoassay is, however, highly inaccurate. Measurement of free testosterone by using extraction and chromatography or gas/liquid chromatography-mass spectrometry is more accurate, but is restricted to research settings due to the cost and complexity of such procedures. The diagnostic accuracy of measuring serum testosterone is enhanced by simultaneous measurement of SHBG and then calculating the free fraction (i.e. free androgen index) (Stanczyk, 2006).

DHEAS is an important testosterone precursor that is formed from dehydroepiandrosterone (DHEA), predominantly in the adrenals. DHEA is derived from both the adrenals (approximately 80%) and the ovary (approximately 20%). DHEAS can thus be elevated in PCOS, when ovarian DHEA production is increased. The advantage of measuring DHEAS is that it has a long half-life and is relatively stable throughout the day and menstrual cycle (Stanczyk, 2006).

In order to establish the predictive value of hyperandrogenemia (defined as at least one androgen value above the 95th percentile of normal controls) in diagnosing PCOS, Haung et al (2009) determined the prevalence of supra-normal total T (>3.1nmol/L), free T (>0.023nmol/L) and DHEAS (>7.47umol/L) in a group of 720 women with PCOS. All were patients attending the Birmingham Hospital at the University of Alabama and diagnosed with PCOS according to NIH 1990 criteria. None were premenarchal or postmenopausal, had undergone hysterectomy or oophorectomy, or had received hormonal treatment within 3 months before evaluation. Normative values for androgens were derived from 98 healthy women from the same geographical area attending for pre-employment medical examination. The control group was non-hirsute, did not have acne, had regular menstrual cycles, was not on hormonal

treatment and was aged 18-45 years. In the cohort of PCOS patients, the overall prevalence of hyperandrogenemia was 75.3%. Supranormal levels of free T were present in 57.5% (n=412), elevated total T in 33.0% (n=236) and elevated DHEAS in 32.7% (n=234). Elevated DHEAS as a sole finding was present in 13.8% (n=98) of patients, whereas elevated total T as the sole abnormality was present in only 2.2% (n=16) of patients (Haung et al, 2009).

Putting aside the debate regarding the inclusion of ultrasound as a diagnostic criterion for PCOS, Legro et al (1998) suggest that biochemical phenotyping has several advantages over ultrasound. As a diagnostic tool, serum androgens are simpler to collect, easier to standardize (by using control groups to establish normal values) and are objective (do not rely on operator interpretation).

The AE-PCOS Task Force, however, recommend that serum levels should be used in conjunction with clinical assessment when diagnosing hyperandrogenism, as between 20-40% of women with PCOS have androgen levels within the normal range (Azziz et al, 2009).

Clinical hyperandrogenism:

Hyperandrogenism can present as hirsutism, acne, and androgenic alopecia or male pattern balding (Souter et al, 2004). Significant virilization suggests an androgen secreting neoplasm (ASN) - ovarian or adrenal in origin, androgenic substance abuse or severe insulin resistance and is not usually a feature of PCOS. Androgen excess affects approximately 7% of reproductive-age women, with the most common diagnosis being PCOS (Azziz et al, 2004; Carmina et al, 2006).

Women with hirsutism:

Hirsutism is defined as the presence of terminal hair in women in a male-like distribution and affects approximately 6-7% of reproductive-aged women (Souter et al, 2004). Numerous scoring systems are available, although most authors use a modification of the method proposed by Ferriman and Gallwey (Ferriman and Gallwey, 1961). For the modified Ferriman-Gallwey score (mF-G), nine body areas are assigned a score of 0-4 based on the density of terminal hairs, a score of 0 representing absence of terminal hairs and a score of 4 extensive terminal hair growth. Using this approach, variable cut off values have been reported. Hirsutism has been defined as mFG score >5 (Kahsar-Miller et al, 2001; Carmina et al, 2006), mFG >6 (Chang et al, 2005 and Huang et al, 2009), or a mFG >7 (Legro et al, 1998 and Govind et al, 1999).

While hirsutism may be the most prominent clinical feature of androgen excess, not all women with hirsutism have androgen excess. Conversely, not all patients with androgen excess have hirsutism. Idiopathic hirsutism is used to define those women with clinical hirsutism, but normal serum androgens (normal free T and DHEAS) and normal ovulatory function. This condition occurs due to increased peripheral action of 5α-reductase, which promotes the peripheral androgen effect within the hair follicle through the local conversion of T and androstenedione to dihydrotestosterone. In addition to exaggerated local 5α-reductase activity, functional alterations in the local androgen receptor have been demonstrated in these women (Azziz et al, 2000).

The majority of women with hirsutism do have an androgen excess disorder, the commonest being PCOS. In their systematic review of 18 prevalence studies with a total of 6281 women with PCOS, Azziz et al (2009) report the prevalence of hirsutism to be 75% in women with PCOS (Azziz et al, 2009).

It appears that even minimal unwanted hair growth is associated with androgen excess and PCOS. Souter et al (2004) evaluated 188 consecutive women attending their gynae-endocrine clinic between 1995 and 2002 that presented with unwanted hair and had a mF-G score of 1-5. They found a prevalence of androgen excess within this cohort to be 54.3% (n=102). Of these, 94 (92% of patients with androgen excess, 50% of the patient cohort) had PCOS (NIH 1990 criteria). These data support the routine endocrine evaluation of patients presenting with minimal unwanted hair growth, even when the mF-G score does not indicate frank hirsutism (Souter et al, 2004).

The prevalence of hirsutism is affected by ethnic variations. Hirsutism does not appear to differ between white and black women, however is less frequent in East Asian women. Despite these variations, race-specific normative ranges have not been established (DeUgarte et al, 2006).

Women with acne:

The prevalence of PCOS among women who present only with acne is not well defined. Most studies of acne patients have not reported adequately regarding PCOS status. The prevalence of PCOS amongst patients with severe acne has been reported to be between 19-37%. In Azziz et al's prospective analysis of 873 women presenting with androgen excess, acne was present in 14.5% of the 716 women with PCOS (Azziz et al, 2004). Carmina et al (2006) do not report the prevalence of acne amongst women with PCOS, but in their cohort of 950 women with androgen excess 12.6% had acne (Carmina et al, 2006).

Acne is, however, a relatively common clinical feature, particularly in younger women. Approximately 20% of women in their midteens and 15% in their early 20s complain of acne, thus the degree to which PCOS elevates the risk of acne above the general population is not clear. Future population studies of

acne patients, particularly those without other evidence of hyperandrogenism, will better define the prevalence of PCOS amongst acne patients (Souter et al, 2004).

Burke and Cunliffe (1984) described two scoring systems for assessing acne. These included a grading system, where three sites – face, back and chest were graded from 0 to 10; 0 reflecting no acne and 10 representing most severe acne. Alternatively a counting system was used, where lesions (divided into inflamed and non-inflamed) are counted (Burke and Cunliffe, 1984). In clinical practice, acne is frequently only assessed subjectively as mild, moderate or severe.

Women with alopecia:

Female androgenic alopecia refers to slowly progressive hair loss that is characterized by diffuse reduction in the volume and density of hair. The two most frequent patterns of hair loss are progressive thinning of hair at the crown with preservation of the frontal hairline and balding with bi-temporal recession.

The pathogenesis of androgenic alopecia involves local androgen production at hair follicles. Excessive in situ conversion of testosterone to dihydrotestosterone occurs because of hyperactivity of 5a-reductase and 17β-hydroxysteroid dehydrogenase enzymes. In addition, there is apparent over-expression of androgen receptors in balding compared with non-balding scalps (Yildiz, 2006).

The prevalence of PCOS among women with alopecia is also not clear. In one report of 109 consecutive premenopausal women whose presenting complaint was alopecia, with or without hirsutism, the incidence of PCOS was found to be 36.5%. Of the 40 women with PCOS, nine (22.5%) had no other sign of hyperandrogenism at the time of presentation (Futterweit et al, 1988).

Overall, it would appear that the proportion of women with alopecia only who have PCOS is considerably less than that of women with hirsutism, with or without alopecia. Current data would suggest that a majority of patients with hirsutism (75%–80%) have PCOS (NIH 1990 criteria); between 20-40% of patients with only persistent acne, and 10% of those women with only alopecia will have PCOS.

Women with menstrual and ovulatory dysfunction:

Oligomenorrhea can be defined as menstrual cycles at >35-day intervals or <10 bleeds per year, and polymenorrhea as cycles <25 days duration. The menstrual dysfunction of PCOS is most frequently oligo- or amenorrhea, but a smaller proportion present with polymenorrhea. In their systematic review of 20 studies, Azziz et al (2009) report the prevalence of oligo- or amenorrhea in 6978 women with PCOS to be 79.1%. The prevalence of PCOS (NIH 1990 criteria) among women with menstrual dysfunction in the general population was found to be between 14.6%–22.8% (Azziz et al, 2009). The prevalence of menstrual dysfunction in women with PCOS decreases with age. Elting and colleagues (2000) interviewed 205 women with PCOS who were not on any hormonal treatment and found a significant linear relationship between increasing age and the resumption of regular menses. Weight loss, BMI, hirsutism, ethnic origin and history of smoking were all found not to be influenced by aging. They suggest that the decline in antral follicle count with ageing may explain the occurrence of regular menses in older women with PCOS (Elting et al, 2000). Because this age-related change may affect the prevalence of PCOS (Rotterdam criteria) it is important to control for age in studies

Phenotype and genotype:

PCOS is a heterogeneous syndrome, with variable expression of four key features among affected individuals. The key features are menstrual and ovulatory dysfunction, hyperandrogenemia (HA), hirsutism and polycystic ovaries (PCO). Insulin resistance and hyperinsulinaemia are seen as secondary metabolic complications of PCOS that are also present to a variable degree. A temporal component is also important, with environmental factors such as body weight and lifestyle choices modifying the presentation of clinical features over time. Using these four key features, 16 possible permutations of the PCOS phenotype exist (Azziz et al, 2009). The NIH criteria do not include PCO morphology and are thus limited to 6 phenotypes. The recent AE-PCOS 2009 criteria differ from the Rotterdam criteria in that PCO morphology and oligomenorrhoea are not included in the former, but are part of the latter definition (see Table 1 below).

Obesity:

Obesity refers to excess body weight, with an abnormally high proportion of body fat. Body mass index (BMI), determined by calculating weight (in kilograms) divided by height in metres squared, provides a comparative measure that is standardized for height. The American NIH defines a BMI of 25-29.9kg/m^2 to be overweight and BMI of ≥30kg/m^2 as obese. BMI does not, however, account for the distribution of adipose tissue. To account for the distribution of adiposity, waist circumference and waist/hip ratios (WHR) are used in both clinical practice and research. For women, visceral obesity is defined as a waist circumference >88cm by American norms, or >80cm by the International Diabetes Federation, with a WHR >0.85 (Sowers, 2003; Milewicz and Jedrzejuk, 2007).

The reported prevalence of obesity in women with PCOS depends on the diagnostic criteria used, as well as the geographic and racial group being studied. However, in most studies a large proportion of women with PCOS are

Table 1: Potential PCOS phenotypes (adapted from Azziz et al, 2009)				
	Phenotype	**NIH criteria 2000**	**Rotterdam 2003 criteria**	**AE-PCOS 2009 criteria**
1	HA + hirsutism + oligo-amenorrhoea + PCO	√	√	√
2	HA + hirsutism + oligo-amenorrhoea	√	√	√
3	HA + oligo-amenorrhoea + PCO	√	√	√
4	HA + oligo-amenorrhoea	√	√	√
5	Hirsutism + oligo-amenorrhoea + PCO	√	√	√
6	Hirsutism + oligo-amenorrhoea	√	√	√
7	HA + hirsutism + PCO		√	√
8	Hirsutism + PCO		√	√
9	HA + PCO		√	√
10	Oligo-amenorhoea + PCO		√	
11	HA + hirsutism			
12	PCO			
13	Oligo-amenorrhoea			
14	Hirsutism			
15	HA			
16	None			
HA – hyperandrogenism; PCO – polycystic ovary				

overweight (BMI>25) or obese (BMI>30). In our GEC at GSH, over the past 10 years 885 women with PCOS have been prospectively included in our database. Most of these women had an abnormal BMI, with 22% being overweight (BMI 25-30), 41% obese (BMI 30-40) and 14% being morbidly obese (BMI>40) (Edelstein, 2008).

The clinical features of PCOS are strongly influenced by obesity. Obese PCOS women have significantly lower SHBG plasma levels and consequently higher free androgens, with more hirsutism and menstrual irregularities compared

with non-obese PCOS women. This was demonstrated by Edelstein (2008) who compared 33 PCOS women with lean BMI (<20); 121 PCOS women with normal BMI (20-25); and 125 PCOS women with morbid obesity BMI (>40). A highly significant inverse correlation of SHBG with BMI was found. The mean SHBG was 22.3nmol/L in the morbidly obese compared with 44.9nmol/L in the normal BMI group (P<0.001), with a mean FAI 12.0 and 7.2 respectively (P<0.001) (Edelstein, 2008).

Obesity also impacts on the insulin-glucose system in PCOS women. Both fasting and glucose-stimulated insulin concentrations are significantly higher in obese compared with non-obese PCOS women. In the same study, Edelstein found significantly higher fasting insulin in morbidly obese compared with normal BMI PCOS women, with a mean fasting insulin 31.8mIU/L and 10.8mIU/L respectively (P<0.001). Similarly the G:I ratio was also influenced by BMI, being 0.21 and 0.63 respectively (P<0.001) (Edelstein, 2008).

The pattern of fat distribution, rather than obesity per se, contributes to the expression of metabolic abnormalities. Lord et al, 2006 found visceral adiposity in women with PCOS to be strongly related to insulin resistance, as well as other parameters of metabolic syndrome. They investigated a group of 40 women with anovulation and PCO, with a mean age of 29 years, attending a reproductive medicine clinic in Derriford Hospital, South England. In this group, 89% had a BMI >25 and 83% had a waist circumference >88cm. Visceral adiposity was strongly correlated with fasting insulin and insulin resistance; triglycerides and HDL; and SHBG. Of interest, in this study waist circumference (rather then WHR or BMI) had the strongest correlation with visceral adiposity and insulin resistance (Lord et al, 2006).

Insulin resistance:

In order to provide guidance on the definition, pathophysiology and clinical implications, in 1997 the American Diabetes Association convened an expert panel to develop a consensus statement on insulin resistance (American Diabetes Association, 1997). This panel defined insulin resistance as an impaired biological response to exogenous

or endogenous insulin. This effect is not confined to glucose homeostasis, but impacts on all of the biological actions of insulin, including lipid and protein metabolism, vascular endothelial function and gene expression. Several research setting procedures have been developed to test for insulin resistance, however there is no standard, universally accepted measure to define insulin resistance. From a clinical perspective, the most practical way to assess insulin resistance is to measure fasting plasma insulin and glucose levels. Very high fasting insulin levels in the setting of normal glucose levels are indicative of insulin resistance, however the cut-off between normal and abnormal is not well defined (American Diabetes Association, 1997).

Various methods are used to quantify insulin resistance. In the research setting, dynamic insulin/glucose sampling techniques are used, whereby serum insulin/glucose can be sampled regularly over a period of time (e.g. every 5min for 90min) in order monitor dynamic changes. However, these methods are intensive, time-consuming and costly. Simpler indices derived from fasting insulin, glucose and serum cholesterol have been proposed to determine insulin resistance. These include the homeostasis model assessment (HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI) and revised-QUICKI (R-QUICKI).

HOMA has been shown to be a reliable, simple and reproducible measure of insulin resistance. Bonora and colleagues (2000) compared HOMA to the glucose clamp technique in 115 subjects with various degrees of glucose tolerance and insulin sensitivity. These authors demonstrated a strong correlation between clamp-measured total glucose disposal and HOMA-estimated insulin sensitivity ($P < 0.0001$), with no substantial differences based on age, gender, BMI, pre-existing diabetes or hypertension. They thus concluded that HOMA can be reliably used to assess insulin resistance in studies in which only a fasting blood sample is available.

Hyperinsulinemia has numerous effects on adrenal and gonadal hormones and is thought to stimulate both intraovarian androgen synthesis and enhance adrenal androgen secretion. Insulin resistance is also associated with an atherogenic lipid profile, with increased triglycerides (TG) and reduced high density lipoprotein (HDL) cholesterol levels (Strowizki et al, 2002).

Hyperinsulinemia is thought to play a key role in the hyperandrogenism of PCOS. This occurs both as a direct action on ovarian androgen production, as well as indirectly by reducing SHBG levels. Since testosterone is highly bound to SHBG, its reduction leads to increased tissue availability of circulating testosterone.

Lipid profile:

Dyslipidaemia is defined as a clinically significant change in serum lipids and lipoproteins that predisposes the individual to coronary artery disease and related conditions. The most common lipid abnormality is hypercholesterolaemia, defined as TC > 7.5mmol/L, whereas the normal levels should be <5.0mmol/L. The desired lipid profile is to have HDL >1.2mmol/L and LDL <3.0mmol/L. Hypertriglyceridaemia is defined as TG >5.0mmol/L (Berger and Marais, 2000).

Women with PCOS are at increased risk of atherosclerotic vascular disease compared with age-matched normal controls, due to a male type lipid profile. Wild et al (1985), in a case-control study, found women with PCOS (n=29) (NIH criteria) to have higher TG, higher very low density lipoprotein (VLDL) cholesterol and lower HDL cholesterol than healthy age-matched controls (n=30) (Wild et al, 1985).

Genetics of PCOS

The fact that cases of PCOS cluster in families suggest a genetic component in the aetiology of PCOS. Earlier association studies proposed an autosomal dominant mode of inheritance (Carey et al, 1993; Govind et al, 1999), although recently a more complex mode of inheritance has been postulated (Franks and McCarthy, 2004).

The well established biochemical abnormalities of PCOS have lead researchers to utilize the candidate gene approach in identifying possible genetic defects underlying the inheritance of PCOS. Association

studies have utilized both case-control and family-based association methodologies. Research has focused on genes involved in the biosynthesis and metabolism of androgens, luteinizing hormone and its receptor, cholesterol side chain cleavage, 17-alpha hydroxylase, insulin secretion and action, insulin receptor and folliculogenesis. To date few studies have been sufficiently powered to provide conclusive evidence for a genetic defect leading to the metabolic abnormalities associated with PCOS (Franks and McCarthy, 2004). A recent systematic review of functional genetic polymorphisms in PCOS concluded that "several genes involved in ovarian function and metabolism are associated with increased susceptibility to PCOS, but none is strong enough to correlate alone with susceptibility to the disease" (Simoni et al, 2008).

Familial association studies

Kahsar-Miller et al (2001) found the prevalence of PCOS amongst mothers and sisters of patients with PCOS to be significantly higher than that of the general population of reproductive age women. Consent was obtained from 195 consecutive patients with PCOS to contact their first degree female relatives and of these, the relatives of 93 probands agreed to participate. PCOS was diagnosed according to the NIH 1990 criteria (i.e. the presence of ovulatory disorders and hyperandrogenemia and/or hirsutism). In their study, the rate of PCOS amongst first-degree female relatives was 24% for mothers and 32% for sisters.

A segregation (sibship) ratio was calculated in the subset of premenopausal untreated sisters. Power analysis suggested that a total of 310 untreated sisters would be required to calculate inheritance, however only 50 sisters were studied. Due to the small size of the sibling group, these researchers were unable to determine whether the observed 0.40 segregation ratio was in keeping with that expected if PCOS was autosomal dominant (0.50) or recessive (0.25) (Kahsar-Miller, 2001).

Total testosterone, free testosterone and DHEAS were measured in all premenopausal untreated relatives. The mean androgen levels among unaffected mothers or sisters were lower than those of probands or affected first degree relatives, but were similar to normal controls (Kahsar-Miller, 2001).

Govind and colleagues (1999) analysed 29 families with PCOS and 10 control families. For this case control study conducted in the UK, probands were recruited prospectively from infertility and endocrine clinics and PCOS was defined by the presence of PCO on ultrasound, menstrual irregularity or hirsutism on history and/or elevated total testosterone and/or androstenedione (Rotterdam 2003 criteria). Controls were recruited from healthy staff at the North Staffordshire Hospital in England and all had no PCO on ultrasound, no evidence of hyperandrogenism, regular menstrual cycles and had never sought treatment for menstrual disturbances, hirsutism or infertility (Govind, 1999).

Affected status in family members was solely assigned based on ultrasound findings in women (the presence of PCO bilaterally) and on visual inspection of photographs for premature male pattern baldness (PMPB) (fronto-parietal balding before the age of 30) in male relatives. These authors found ultrasound to be a highly sensitive predictor of PCOS, with 30 out of 32 women with irregular menses and hirsutism having PCO on ultrasound (94% sensitivity). Of all the women with PCO morphology, 64 of 71 (90%) had at least one clinical feature of PCOS (menstrual irregularity, hirsutism or acne) and 46 (65%) had 2 features.

The proportion of PCO-positive sisters with oligomenorrhoea was significantly higher than PCO-negative sisters (present in 54% of affected sisters versus 16% of unaffected sisters). Hirsutism (defined as mF-G score >7) was more common in affected sisters (43%) versus unaffected sisters (22%), however did not reach statistical significance.

The prevalence of PMPB was 22% in PCOS proband families compared with 5% in control families. Mean serum testosterone was significantly greater in men with PMPB compared with unaffected men.

Biochemical analysis found no significant difference in serum testosterone, DHEAS, SHBG, FSH or LH between affected and unaffected sisters. Androstenedione was, however, significantly higher in PCO affected sisters, with median (range) value of 9.3nmol/L (3.7-17.0nmol/L) compared with 6.1nmol/L (4.7-10.0nmol/L) in unaffected sisters (p=0.004). The difference in median DHEAS values between affected and unaffected sisters almost reached statistical significance, being 7.0umol/L (2.2-11.0umol/L) and 5.9umol/L (2.0-7.8umol/L) respectively (p = 0.06) (Govind, 1999).

A segregation analysis was also undertaken. Of the 29 PCOS proband families, 15 of 29 mothers (52%), 6 of 28 fathers (21%), 35 of 53 sisters (66%), and 4 of 18 brothers (22%) were assigned affected status. First degree female relatives of probands had a 61% chance of being affected and first degree male relatives had a 22% chance of being affected. Considering the total of 71 postpubertal siblings of PCOS probands, 39 were assigned affected status (PCO or PMPB) consistent with an autosomal dominant pattern of inheritance (Govind et al, 1999).

Hyperandrogenemia and first degree relatives:

Legro and colleagues (1998) found a familial aggregation of hyperandrogenemia in PCOS kindreds. They conducted a familial association study in Pennsylvania, USA. The sisters of 80 probands known with PCOS were contacted and 115 were included in the study. In addition, 43 age-, ethnicity-, and weight-matched healthy controls were recruited to establish normative androgen values. The control women had regular menses, no hirsutism or acne, no history of hypertension or diabetes mellitus and were not using any medication known to affect sex hormone metabolism.

These researchers found that 22% of the 115 sisters had PCOS (diagnosed by the combination of elevated serum androgens and ≤6 menses per year). A further 24% had hyperandrogenemia only and regular menses (termed hyperandrogenemic sisters), thus a total of 46% of sisters had hyperandrogenemia (total testosterone or free testosterone greater than 2SD above the control mean). Sisters of PCOS women had a 3-fold greater risk of being hyperandrogenemic compared with controls (46% vs. 14%, P<0.0001).

A sub-analysis of the sisters showed that probands and PCOS sisters had significantly higher BMI than unaffected sisters (mean BMI 33.3, 33.6 and 26.0 respectively) and that hyperandrogenemic sisters had mean BMI (28.8) between that of the unaffected and PCOS sisters. Of interest, the control group had a mean BMI (31.7), which is greater than hyperandrogenemic sisters. It is possible that within families there is genetic predispositions to develop hyperandrogenemia and that with the influence of increasing BMI these women become anovulatory and are thus classed as PCOS. From this data, it would suggest that predisposed women become affected at a lower BMI than normal controls.

With respect to androgens, these researchers found a bimodal androgen distribution amongst the sisters, but a unimodal distribution in the controls. The bimodal distribution reflects the divide between unaffected sisters versus those sisters with either PCOS or hyperandrogenemia alone. Both free testosterone and DHEAS were elevated in the probands with PCOS (free T 1.05nmol/L, DHEAS 6.93umol/L), PCOS sisters (free T 0.91nmol/L, DHEAS 7.1umol/L) and hyperandrogenemic non-PCOS sisters (free T 0.81nmol/L, DHEAS 8.1umol/L). There was no statistically significant difference between unaffected sisters (free T 0.21nmol/L, DHEAS 4.38umol/L) and controls (free T 0.32nmol/L, DHEAS 4.48umol/L). Legro et al (1998) suggest that the bimodal distribution of androgens may be due to a monogenic trait controlled by two alleles at an autosomal locus. They comment further that if this were so, then a possible candidate gene would need to involve regulation of both ovarian and adrenal steroidogenesis, as DHEAS levels were also increased.

Yildiz et al (2003) assessed the prevalence of both insulin resistance and hyperandrogenemia in first degree relatives of women with PCOS. This study, conducted in Ankara, Turkey, recruited 102 family members of 52 patients known with PCOS (NIH 1990 criteria). A further 82 age- and weight-matched healthy controls, without a history of diabetes mellitus or PCOS were recruited as controls. They also found statistically significant higher androgens in sisters of women with PCOS compared with normal controls. This study does not disaggregate which sisters themselves would be diagnosed as PCOS, but for the group of 19 sisters mean total testosterone were 3.9nmol/L., androstenedione 8.6nmol/L and DHEAS 6.36umol/L. For the control group of 31 women, the mean results were 2.2nmol/L, 5.9nmol/L and 4.96umol/L respectively – all significantly lower (P= <0.001, <0.01, <0.05 respectively) (Yildiz et al, 2003).

Pitfalls in family association studies:

Family association studies are noted to have several problems. The most prominent being the assignment of affected status (i.e. PCOS diagnosis). PCOS is a heterogeneous disorder, with different phenotypic presentations even within families. Researchers still often do not agree on the diagnostic criteria to be used. The Rotterdam 2003 Criteria are used by some researchers, while others prefer the NIH 1990 definition. In some cases assignment of affected status is based solely on the presence of polycystic ovaries on ultrasound, despite the high prevalence of PCO in the general population. Thus comparison between studies is difficult to perform and requires careful review of the assignment of the diagnosis of PCOS.

Some studies have not interviewed and examined family members directly, rather have used questionnaires or interviews with PCOS patients to gain family histories. Such an approach relies on the memory of the interviewee and is subject to recall bias and is problematic.

PCOS, as an ovulatory disorder, is associated with infertility, thus limiting the availability of large pedigrees for linkage analysis. Further, it is a disorder of reproductive life, with a temporal variation in symptoms. Assignment of affected status in postmenopausal women again relies on patient recall of symptoms, as ultrasound and biochemical analysis is less helpful in this age group. Finally, while premature male pattern balding has been used in some studies, a male phenotype is not uniformly accepted.

Thus phenotypic heterogeneity, inconsistent diagnostic criteria, temporal variation in symptoms, and the absence of a male phenotype all hamper research (Azziz et al, 2009)

PATIENTS & METHODS:

Probands and first degree female relatives:

Probands were patients attending the Gynaecological Endocrinology Clinic at Groote Schuur Hospital with a diagnosis of PCOS as defined by the Rotterdam 2003 criteria (ESHRE/ASRM, 2004). Women were referred to this clinic with menstrual irregularity, reproductive failure and/or clinical hyperandrogenism, as well as having had an ultrasound confirming polycystic ovary morphology after presenting to a gynaecology outpatient clinic. From January 2007 until February 2009, a total of 83 probands were prospectively recruited from our database of PCOS patients.

With their consent, attempts were made to include all first degree female relatives of probands in the study (i.e. mothers, and post-pubertal sisters and daughters). Attempts to recruit male family members met with very limited success and were not pursued.

Clinical evaluation:

Subjects were requested to attend the unit following an overnight fast. A detailed history was taken, which included enquiry into menstrual patterns, reproductive function, signs of clinical hyperandrogenism (hirsutism and/or acne), stability of body weight over time, personal and family medical history, the use of any prescription medication, contraceptive use and present or past treatment for PCOS (see APPENDIX 1 – CLERKING SHEET).

All subjects then underwent a physical examination, which included anthropomorphic measures (weight, height, waist and hip circumference), two manual supine blood pressure readings, assessment for clinical features of hyperandrogenism (acne: mild, moderate, severe) hirsutism (defined as F-G score ≥7) and hyperinsulinemia (acanthosis nigricans). Virilisation was also excluded by looking for the presence of clitoromegaly, increased muscle bulk and/or voice changes.

Unless contra-indicated, transvaginal ultrasound was performed and PCO defined according to the criteria described by Balen et al (2003). In those women in whom transvaginal ultrasound was not possible, transabdominal ultrasound was performed.

Samples for hormonal and metabolic analysis were obtained by venepuncture. This assessment included the measurement of glucose, insulin, lipid profile (triglycerides, total cholesterol, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) fractions), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), total testosterone (total T), 17α-hydroxyprogesterone (17-OHP), dihydroepiandrostene sulphate (DHEAS) and sex-hormone binding globulin (SHBG). The endocrine profile was not assessed in those women using hormonal treatment (either contraception or hormonal replacement) at the time of assessment or for twelve weeks prior to assessment. Derived indices included the free androgen index (FAI), the homeostasis model assessment (HOMA) and glucose:insulin ratio (G:I ratio). For the purposes of this study, FSH, LH, E2 and 17-OHP are not analysed or reported.

Designation of PCOS status:

Family members were designated as affected if they fulfilled the Rotterdam 2003 criteria for diagnosis. If they only had PCO on ultrasound, they were classified as PCO.

Clinical hyperandrogenism was established using a F-G score ≥7 (Hatch et al, 1981) and/or moderate-severe acne in untreated women. Biochemical hyperandrogenism was defined as total testosterone >2.9nmol/L or FAI >5.9.

Other conditions known to cause hyperandrogenism, such as Cushing's syndrome, androgen secreting neoplasm, and pituitary tumours were excluded when clinically suspected. In those patients where there

was clinical suspicion, a normal 17-αOH progesterone response after ACTH stimulation test excluded non-classical congenital adrenal hyperplasia (NCAH).

Menstrual irregularity included oligomenorrhoea or amenorrhoea. Oligomenorrhoea was established on history as being <6 menstrual periods per annum in women not on any form of hormonal contraception. Amenorrhoea was the absence of menses for >3 months.

Definition of derived indices:

Body mass index (BMI):

BMI = weight in kilograms/(height in meters)^2

Waist hip ratio (WHR):

WHR = waist circumference/hip circumference (cm)

Free Androgen Index (FAI):

FAI = total T × 100/SHBG

Glucose-Insulin ratio (GI):

GI = fasting glucose/fasting insulin

Homeostatic model assessment of insulin resistance (HOMA):

HOMA = (fasting glucose (mmol/L)× fasting insulin (µU/ml)/22.5

Hormone assays:

Commercially available kits (Roche Modular E170) were used for hormonal and metabolic analysis.

Glucose was analyzed by using an enzymatic colourimetric method, utilizing glucose oxidase.

Triglyceride and total cholesterol were determined by using enzymatic coluorimetric assay. Triglyceride analysis utilizing lipase and glycerokinase, while total cholesterol analysis utilized cholesterol oxidase. HDL-cholesterol was determined after precipitation of apolipoprotein-B containing lipoproteins. The LDL-cholesterol was calculated using Friedewald's formula (LDL=TC-(VLDL/2.25)).

Insulin, DHEAS, testosterone and SHBG were all calculated by using electrochem-iluminescence immunoassay. Insulin and SHBG analysis utilized a sandwich assay. DHEAS and testosterone analysis utilized a competitive assay.

Assay analysis and intra-assay coefficients of variation are summarized in table 2 below. Assay coefficient of variation is expressed as a percentage of inter-assay variation at a lower-limit and an upper-limit total assay mean.

Table 2: Analysis method, principle of analysis, analyser used, total inter-assay coefficient of variation for assays performed.					
Assay	**Test method**	**Principle of analysis**	**Unit**	**Total assay mean**	**Assay coefficient of variation**
Testosterone	Electrochem-iluminescence	Competitive assay	nmol/L	1.7 28.2	5.6% 2.8%
SHBG	Electrochem-iluminescence	Sandwich assay	nmol/L	13.7 42	1.8% 2.1%
DHEAS	Electrochem-iluminescence	Competitive assay	umol/L	2.5 20.4	2.5% 2.4%
Glucose	Enzymatic colourimetric	Glucose oxidase	mmol/L	6.8	1.8%
Insulin	Electrochemiluminescence	Sandwich assay	mU/L	6.9 55	4.9% 3.4%
Total cholesterol	Enzymatic colourimetric	Cholesterol oxidase	mmol/L	5.4	1.7%
Triglycerides	Enzymatic colourimetric	Lipase; glycerokinase	mmol/L	2.5	1.8%
HDL	Enzymatic colourimetric	Cholesterol oxidase	mmol/L	0.8 1.7	1.9% 1.3%
LDL	Calculated – Friedewald	LDL=TC-(VLDL/2.25)			
*Coefficient of variation reported as percentage variation for low and high mean assay levels					

Statistical analysis:

The raw data from clerking sheets were captured in duplicate, using Microsoft Excel. Microsoft Excel Compare was then run to identify any discrepancies in the data captured and the data were then 'cleaned' by referring back to original patient clerking notes whenever a discrepancy was identified.

The statistical package SPSS v17 was used for analysis (SPSS Inc., Chicago, Illinois). For descriptive details, the frequencies function was used to produce mean, median, standard deviation and range as applicable. For categorical variables, cross-tabulation function was used, with chi-square and Fisher's exact tests used to calculate statistical significance. For continuous variables mean, median, standard deviation and range was calculated for statistical significance. For biological variables that were not normally distributed, non-parametric Kruskall Wallis and Mann-Whitney tests were used and results were reported as median, with inter-quartile range. Statistical significance for all tests was set at $P<0.05$.

Two separate analyses were performed. Demographic details, anthropomorphic features, metabolic and hormone profile were compared first between probands, PCOS, PCO and unaffected sisters and second between mothers with PCOS and mothers without PCOS. Global p-values (rather than every possible two-way comparison of different patient groups) were used to compare measures across patient categories to help minimize the impact of type 1 error on interpretation of results.

Summary findings of the statistical analyses are presented in the Results Section below in tabular form. The full statistical analyses are included as APPENDIX 2.

RESULTS:

Summary demographics for study population:

From January 2007 until February 2009, a total of 83 probands were recruited from the Gynaecological Endocrinology Clinic (GEC) at GSH. These were all women with PCOS according to the Rotterdam criteria who presented for management at the GEC. With their consent, first degree female family members were contacted and 57 mothers, 108 sisters and 8 daughters agreed to participate in the study. The characteristics of the study group are summarized in table 3 below.

The mean age of probands and their sisters was 28.1 years and 29.7 years respectively, reflecting women of a reproductive age. However, the age range for both these groups was wide, with probands ranging from 12-47 years and their sisters ranging from 10-66 years. All of the daughters included in the study were post-menarche, with a mean age of 17.4 years and age range of 12-22 years. The mean age of mothers recruited was 54.4 years, however, the age range of 25-76 years was wide. Thus while the majority of mothers were peri- and post-menopausal women, a proportion were still women in their reproductive years.

As reflected in our clinic attendances of the probands, the vast majority of women recruited were of Coloured ethnicity, with 87.9% of probands, 89.5% of mothers, 91.7% of sisters and 100% of daughters being from this ethnic group. Women of a Caucasian ethnicity accounted for 8.4% of probands, 7% of mothers and 3.7% of sisters. Indian ethnicity accounted for 2.4% of probands, 3.5% of mothers and 3.7% of sisters. One (1.2%) women of Black African ethnicity and her 2 (1.9%) sisters were included in the study.

The study population was overweight and had an increased WHR. Mean BMI for probands and mothers was 32.8 and 32.2 respectively, categorized as obese. Of note, even the daughters were overweight, with

a mean BMI of 29.3. Mean WHR for all patient categories was greater than 0.85, indicating visceral distribution of adiposity in these women.

By design 100% of the probands had PCOS according to the Rotterdam criteria. The prevalence of PCOS among first degree female relatives was high, with 19.6% of mothers, 45.4% of sisters and 55.6% of daughters assessed as affected (see Table 3).

Distribution of phenotypes among study population (see Table 4):

Very few women in this study had all 4 phenotypic features of PCOS (hyperandrogenism, hirsutism, oligo-amenorrhoea and PCO). Only 9 (10.8%) of probands, 2 (1.9%) sisters and 1(11.1%) daughter demonstrated this phenotypic pattern (see Table 4).

Hirsutism was a common clinical feature of the study population. 35(42.2%) probands had hirsutism + oligo-amenorrhoea + PCO and a further 19(22.9%) had hirsutism + PCO. Hirsutism + PCO were present in 5(8.9%) mothers, 31(28.7%) sisters and 3(33.3%) daughters. Hirsutism alone was present in 19(33.9%) mothers and 14(13.8%) sisters.

Isolated hyperandrogenemia (HA) was not a feature of the study population. HA + hirsutism + PCO was present in 5(6%) probands and 2(1.9%) sisters. HA + PCO + oligo-amenorrhoea was present in 2(1.4%) probands and 2(1.9%) sisters. HA + PCO was present in 1(0.9%) sister. None of the study participants had either HA alone or HA + hirsutism.

PCO as the sole feature without clinical or biochemical abnormality was present in 26 (24.1%) of sisters.

Twenty-one (37.5%) mothers, 17(15.7%) sisters and 3(33.3%) daughters were not affected at all.

Table 3: Summary demographics and phenotypes of study population				
Group	**Probands**	**Mothers**	**Sisters**	**Daughters**
Total no. studied	83	57	108	8
Ethnicity: Coloured	73 (87.9%)	51 (89.5%)	98 (91.7%)	8 (100%)
Caucasian	7 (8.4%)	4 (7%)	4 (3.7%)	0
Indian	2 (2.4%)	2 (3.5%)	4 (3.7%)	0
Black African	1 (1.2%)	0	2 (1.9%)	0
Age (mean ± SD)	28.1 ±8.6	54.4 ±10.2	29.7 ±10.9	17.4 ±3.02
Age (median)	26	54	29	17.5
Age range (min-max)	12-47	25-76	10-66	12-22
BMI (mean ± SD)	32.8 ±8.8	32.2 ±6.3	28.9 ±7.9	29.3 ±6.7
WHR (mean ± SD)	0.89 ±0.009	0.90 ±0.01	0.87 ±0.007	0.91 ±0.018
Total with PCOS (Rotterdam)	83 (100%)	11 (19.6%)	49 (45.4%)	5 (55.6%)
Total with PCOS (NIH)	47 (56.6%)	4 (7%)	12 (11.1%)	1 (11.1%)
Total with hirsutism only	0	19 (33.9%)	14 (13.8%)	0
Total with hyperandrogenism only	0	0	0	0
Total with oligomenorrhoea only	0	1 (1.8%)	2 (1.9%)	1 (11.1%)

Table 4: Distribution of phenotypes among study population (n and %)					
	Phenotype	**Probands (n=83)**	**Mothers (n=57)**	**Sisters (n=108)**	**Daughters (n=8)**
1	**HA + hirsutism + oligo-amenorrhoea + PCO**	9 (10.8%)	0	2 (1.9%)	1 (11.1%)
2	**HA + hirsutism + oligo-amenorrhoea**	0	0	0	0
3	**HA + oligo-amenorrhoea + PCO**	2 (2.4%)	0	2 (1.9%)	0
4	**HA + oligo-amenorrhoea**	0	0	0	0
5	**Hirsutism + oligo-amenorrhoea + PCO**	35 (42.2%)	3 (6.8%)	6 (5.6%)	0
6	**Hirsutism + oligo-amenorrhoea**	1 (1.2%)	1 (1.8%)	2 (1.9%)	0
7	**HA + hirsutism + PCO**	5 (6.0%)	0	2 (1.9%)	0
8	**Hirsutism + PCO**	19 (22.9%)	5 (8.9%)	31 (28.7%)	3 (33.3%)
9	**HA + PCO**	0	0	1 (0.9%)	0
10	**Oligo-amenorhoea + PCO**	12 (14.5%)	3 (5.4%)	3 (2.8%)	0
11	**HA + hirsutism**	0	0	0	0
12	**PCO**	0	4 (7.1%)	26 (24.1%)	1 (11.1%)
13	**Oligo-amenorrhoea**	0	1 (1.8%)	2 (1.9%)	0
14	**Hirsutism**	0	19 (33.9%)	14 (13.8%)	0
15	**HA**	0	0	0	0
16	**None**	0	21 (37.5%)	17 (15.7%)	3 (33.3)

Clinical findings in PCOS, PCO and remaining phenotypes in sisters compared with probands:

Probands and sisters were all of a similar age, with unaffected sisters being slightly older than probands [32.1years and 28.2 years respectively (NS)] (see table 5 below). There was a statistically significant

difference in BMI between patient groups, with probands having the highest BMI, than sisters with PCOS, sisters with PCO and unaffected sisters - BMI of 32.8, 30.4, 28.2 and 27.3 respectively (P=0.006). All of the above patient groups had an unfavourable adipose distribution, with WHR > 0.85. Menstrual irregularity was present in 59(71.1%) probands and 15(30.6%) sisters with PCOS.

Hirsutism was, by definition, more prevalent amongst probands and sisters with PCOS. Other features of clinical hyperandrogenism were also prevalent among probands and PCOS, PCO and unaffected sisters. Acne was present in 43(51.8%) probands, 23(45.9%) sisters with PCOS, 12(46.2%) sisters with PCO and 9(27.3%) unaffected sisters. Alopecia was present in 40(48.2%) probands, 15(30.6%) sisters with PCOS, 9(34.6%) sisters with PCO and 3(9.1%) unaffected sisters. The clinical feature of hyperinsulinemia and acanthosis nigicans was significantly more prevalent among probands and occurred in 35(42.2%) of probands compared with 8(16.3%) PCOS sisters and 1(3.8%) PCO sisters (P <0.001).

Early onset male pattern balding (EOMB) was not reported by a large proportion of the study population. For probands, the history of EOMB in a male sibling and father was given by 4(4.8%) and 3(3.6%) respectively. In sisters with PCOS 3(6.1%) and 2(4.1%) gave this history respectively. Only 2(7.7%) of sisters with PCO gave a history of PMPB in their male siblings.

Table 5: Clinical findings in PCOS, PCO and unaffected sisters compared with probands (mean ± standard deviation)					
	Probands (n=83)	**Sisters with PCOS (n=49)**	**Sisters with PCO (n=26)**	**Unaffected sisters (n=33)**	**P-value**
Age	28.1 ± 8.6	27.9 ± 9.1	29.9 ± 11.7	32.1 ± 12.5	0.217
BMI	32.8 ± 8.8	30.4 ± 7.7	28.2 ± 8.7	27.3 ± 7.6	0.006
Waist circumference	100.9 ± 19.9	95.9 ± 16.5	90.8 ± 18.4	91.2 ± 18.5	0.025
Hip circumference	113.4 ± 17.3	110.7 ± 17.2	103.7 ± 15.2	104.7 ± 16.3	0.019
WHR	0.89 ± 0.08	0.86 ± 0.06	0.87 ± 0.08	0.87 ± 0.08	0.420
Oligo-amenorrhoea (n)	59 (71.1%)	15 (30.6%)	0	2 (6.1%)	<0.001
Hirsutism (n)	69 (83.1%)	43 (87.8%)	0	14 (45.2%)	<0.001
Ferriman Gallwey Score	12.9 ± 6.5	11.9 ± 5.2	4.23 ± 1.9	6.5 ± 4.1	<0.001
Acne (n)	43 (51.8%)	23 (46.9%)	12 (46.2%)	9 (27.3%)	0.120
Alopecia (n)	40 (48.2%)	15 (30.6%)	9 (34.6%)	3 (9.1%)	<0.001
Acanthosis nigricans (n)	35 (42.2%)	8 (16.3%)	1 (3.8%)	0	<0.001
Family history PMPB (male sibling)	4 (4.8%)	3 (6.1%)	2 (7.7%)	0	0.466
Family history PMPB (father)	3 (3.6%)	2 (4.1%)	0	0	0.731

Biochemical findings in PCOS, PCO and remaining phenotypes in sisters compared with probands:

While the median testosterone and DHEAS were within normal limits, the probands had significantly higher androgens than any category of sister. Median total T and inter-quartile range for probands, sisters with PCOS, sisters with PCO and unaffected sisters was 2.1nmol/L(1.5-2.9), 1.4nmol/L(0.8-2.4),

1.3nmol/L(0.85-1.9) and 1.1nmol/L(0.5-1.8) respectively (P<0.001) (see table 6 below). Results for DHEAS were 7.3nmol/L(4.2-8.1), 4.2nmol/L(2.4-7.3), 5.8nmol/L(2.5-6.8) and 3.1nmol/L(2.0-6.4) respectively (P=0.062). As anticipated, median FAI of 8.0 (4.1-12.8) was above normal for the probands. However, median FAI of 2.6(1.8-11.1) for sisters with PCOS was within normal limits and was similar to median FAI of 2.7(2.1-4.0) for sisters with PCO and median FAI 1.9(0.8-3.5) for unaffected sisters.

Analysis of the lipid profiles did not find a significant difference between patient categories. Median TC was similar for probands, sisters with PCOS, sisters with PCO and unaffected sisters. However, median fasting TC ranged between 4.7 to 4.9mmol/L, just below the upper limit of normal, which is a TC<5mmol/L. Median LDL for both sisters with PCOS and unaffected sisters were above the upper limit of normal (<3.0mmol/L) being 3.2mmol/L(2.5-3.8) and 3.3mmol/L(2.9-3.6) respectively. While there was no statistically significant difference between patient categories, median fasting HDL was below the lower limit of normal (<1.2mmol/L) for both sisters with PCOS and unaffected sisters being 1.1mmol/L(0.9-1.6) and 1.1mmol/L(0.9-1.3) respectively. Median fasting TG were normal for all these patient categories and there was no statistical significant difference between patient categories.

Metabolic parameters showed a bimodal distribution, with probands and sisters with PCOS having similar measures of glucose, insulin and insulin resistance. There was no significant difference for fasting glucose between patient categories and median fasting glucose for all patient categories was within normal. Probands and sisters with PCOS had elevated median insulin, being 16.3mU/L(9.6-30.9) and 16.6mU/L(8.9-24.5) respectively. Concordant with these insulin levels, these patient categories also had measures of insulin resistance. The median G:I ratio was 0.32(0.18-0.52) and 0.32(0.2-.0.58) and the median HOMA 3.1(1.8-7.3) and 3.5(1.5-5.2) for the probands and sisters with PCOS respectively. These values suggest insulin resistance, with a GI ratio >0.45 and HOMA<1 being normal. Sisters with PCO and unaffected sisters had lower median insulin levels, at 10.3mU/L(6.1-17.4) and 11.5mU/L(6.5-15.4)

respectively. Thus these categories of patients had median G:I ratios that were low normal, at 0.47(0.3-0.8) and 0.46(0.31-0.67) respectively. Median HOMA for sisters with PCO and unaffected sisters was 1.6(0.6-3.6) and 1.9(0.7-3.5), which was lower than probands and sisters with PCOS, but still abnormal.

Table 6: Biochemical findings in PCOS, PCO and unaffected sisters compared with probands (median, inter-quartile range)

	Probands (n=83)	Sisters with PCOS (n=49)	Sisters with PCO (n=26)	Unaffected sisters (n=33)	P-value
Total testosterone (nmol/L)	2.1 (1.5-2.9)	1.4 (0.8-2.4)	1.3 (0.85-1.9)	1.1 (0.5-1.8)	<0.001
DHEAS (nmol/L)	7.3 (4.2-8.1)	4.2 (2.4-7.3)	5.8 (2.5-6.8)	3.1 (2.0-6.4)	0.062
SHBG (mmol/L)	24.5 (17.7-45.5)	34.6 (23-71.2)	46.5 (39.3-59.1)	50.6 (32.8-71.2)	<0.001
FAI	8.0 (4.1-12.8)	2.6 (1.8-11.1)	2.7 (2.1-4.0)	1.9 (0.8-3.5)	<0.001
TC (mmol/L)	4.7 (4.0-5.2)	4.9 (4.3-5.7)	4.7 (3.8-5.6)	4.9 (4.4-5.4)	0.2
LDL (mmol/L)	2.75 (2.23-3.4)	3.2 (2.5-3.8)	3.0 (2.0-3.4)	3.3 (2.9-3.6)	0.32
HDL (mmol/L)	1.3 (1.03-1.5)	1.1 (0.9-1.6)	1.2 (0.9-1.93)	1.1 (0.8-1.3)	0.35
TG (mmol/L)	1.0 (0.7-1.45)	1.1 (0.73-1.6)	0.9 (0.6-1.3)	1.1 (0.8-1.4)	0.766
Glucose (mmol/L)	4.9 (4.6-5.4)	5.1 (4.7-5.4)	4.8 (4.5-5.1)	4.7 (4.5-5.1)	0.145
Insulin (mU/L)	16.3 (9.6-30.9)	16.6 (8.9-24.5)	10.3 (6.1-17.4)	11.5 (6.5-15.4)	0.13
G:I ratio	0.32 (0.18-0.52)	0.32 (0.2-0.58)	0.47 (0.3-0.8)	0.46 (0.31-0.67)	0.022
HOMA	3.1 (1.8-7.3)	3.5 (1.5-5.2)	1.6 (0.6-3.6)	1.9 (0.7-3.5)	0.001

Clinical findings in mothers with PCOS compared with mothers without PCOS:

Mothers with PCOS and those without PCOS were all of a similar age, with mean age of 54.7 years and 54.3 years respectively (see table 7 below). The age range was wide, with mothers with PCOS ranging from 35 to 68 years. The age range for mothers without PCOS was even wider, being 25 to 76 years. However, mean age for both groups of mothers falls within peri-menopausal to menopausal age range.

There was no statistically significant difference in BMI between the PCOS and non-PCOS mothers, however the mean BMI for both groups was obese at 32.1 and 32.3 respectively. The WHR for both groups was also abnormal, at 0.92 and 0.90 respectively, suggesting visceral distribution of adipose tissue. Seven mothers (58.3%) with PCOS reported oligo-amenorrhoea during their reproductive life. This was significantly higher than reported by one mother (2.3%) without PCOS ($p<0.001$).

Hirsutism was a common finding in both groups of mothers, occurring in 9 mothers (75%) with PCOS and 19 mothers (42.2%) without PCOS. The mean Ferriman-Gallwey (FG) score was, by definition, higher in those mothers with PCOS compared with mothers without PCOS, at FG score 10.3 and 6.4 respectively ($P=0.001$). Acne and alopecia were common features in both groups of mothers. Four mothers (33.3%) with PCOS had acne and three had alopecia (25%). Nine mothers (20%) without PCOS had acne and nineteen had alopecia (42.2%). Acanthosis nigricans was not reported in any of the mothers assessed.

Neither group of mothers reported a family history of PMPB, either in their siblings or fathers.

Table 7: Clinical findings in mothers with PCOS compared with mothers without PCOS (mean, standard deviation)			
	Mothers with PCOS (n=12)	Mothers without PCOS (n=45)	P-value
Age	54.7 (±8.9)	54.3 (±10.6)	0.911
BMI	32.1 (±7.1)	32.3 (±6.1)	0.914
Waist circumference	103.7 (±12.0)	103.9 (±15.6)	0.917
Hip circumference	113.6 (±15)	115.5 (±13.6)	0.677
WHR	0.92 (±0.056)	0.90 (±0.074)	0.446
Oligo-amenorrhoea (n)	7(58.3%)	1 (2.3%)	<0.001
Hirsutism (n)	9(75%)	19(42.2%)	0.056
Ferriman-Gallwey Score	10.3 (±4.2)	6.4 (±3.1)	0.001
Acne (n)	4(33.3%)	9(20%)	0.44
Alopecia (n)	3(25%)	19(42.2%)	0.335
Acanthosis nigricans (n)	0	0	N/A
Family history PMPB (male sibling)	0	0	N/A
Family history PMPB (father)	0	0	N/A

Biochemical findings in mothers with PCOS compared with mothers without PCOS:

Androgens in both groups of mothers were in keeping with their peri-/post-menopausal age group. Median total T was 0.6nmol/L (0.1-1.7) and 0.7nmol/L (0.4-1.1) respectively (see table 8 below). Similarly, median DHEAS was 2.3nmol/L (1.0-3.6) and 2.0nmol/L (1.1-3.4) respectively. The median FAI was within normal and did not differ significantly for both groups of mothers, at 2.0 (0.2-4.9) and 1.4 (0.7-3.4) (P=0.916).

Analysis of the lipid profiles did not find a significant difference between both groups of mothers. Median fasting TC was 5.6mmol/L (5.0-6.2) and 5.7mmol/L (5.0-6.3) respectively. Both groups of mothers had

median fasting TC that was above normal (normal fasting TC<5mmol/L). Again, median fasting LDL was above the upper limit of normal (normal LDL <3.0mmol/L) being 3.6mmol/L (3.2-4.2) and 3.7mmol/L (2.8-4.3) respectively. Further, median fasting HDL was below the lower limit of normal (normal HDL >1.2mmol/L) for both groups of mothers, at 1.2mmol/L (0.8-1.3) and 0.9mmol/L (0.8-1.2) respectively. Median fasting TG were normal for both groups of mothers, at 1.4mmol/L (1.1-2.7) and 1.5mmol/L (1.2-2.1) respectively.

Table 8: Biochemical findings in mothers with PCOS compared with mothers without PCOS (median, inter-quartile range)

	Mothers with PCOS (n=12)	Mothers without PCOS (n=45)	P-value
Total testosterone (nmol/L)	0.6 (0.1-1.7)	0.7 (0.4-1.1)	0.89
DHEAS (nmol/L)	2.3 (1.0-3.6)	2.0 (1.1-3.4)	0.87
SHBG (mmol/L)	34.6 (27.4-59.6)	34.5 (23.1-63.5)	0.983
FAI	2.0 (0.2-4.9)	1.4 (0.7-3.4)	0.916
TC (mmol/L)	5.6 (5.0-6.2)	5.7 (5.0-6.3)	0.915
LDL (mmol/L)	3.6 (3.2-4.2)	3.7 (2.8-4.4)	0.914
HDL (mmol/L)	1.2 (0.8-1.3)	0.9 (0.8-1.2)	0.913
TG (mmol/L)	1.4 (1.1-2.7)	1.5 (1.2-2.1)	0.926
Glucose (mmol/L)	5.6 (4.8-9.0)	5.5 (5.2-6.8)	0.869
Insulin (mU/L)	17.4 (8.6-30)	13.1 (6.2-23.9)	0.521
G:I ratio	0.49 (0.2-0.68)	0.41 (0.21-0.91)	0.691
HOMA	2.14 (0.29-9.24)	1.98 (0.72-5.62)	0.561

Mean androgens of first degree relatives stratified by abnormality within probands:

In order to investigate whether the families of probands with abnormal androgens are predisposed to hyperandrogenemia, median total T and FAI of the first degree female relatives (mothers, sisters,

daughters) are stratified according to whether the proband had normal or abnormal total T or FAI (see table 9 below). The only statistically significant difference found was for daughters of probands with abnormal FAI. These daughters had mean FAI that was significantly higher than daughters of probands with normal FAI. However, due to the small sample size of daughters, we should be cautious when interpreting these results.

Table 9: Mean androgens of first degree relatives stratified by abnormality within proband

	Proband total T			**Proband FAI**		
	Normal	Abnormal	P-value	Normal	Abnormal	P-value
Mother	0.65 ± 0.46	1.0 ± 0.58	0.081	2.28 ± 1.9	2.56 ± 2.4	0.73
Sister	1.46 ± 0.97	1.95 ± 1.88	0.149	3.77 ± 4.1	5.47 ± 5.9	0.22
Daughter	1.6 ± 1.35	1.17 ± 0.67	0.623	11.3 ± 9.3	2.2 ± 0.66	0.03

DISCUSSION:

Summary of key findings:

This study demonstrated a strong familial association of PCOS, with PCOS (Rotterdam criteria) being present in 45.4% sisters, 19.6% mothers, 55.6% daughters. The commonest phenotypes in our study population included women with hirsutism. For probands 42.2% had the phenotype hirsutism + oligomenorrhoea + PCO. The phenotype hirsutism + PCO were present in 28.7% of sisters and 33.3% of daughters. Isolated hirsutism was present in 33.9% of mothers. Other features of clinical hyperandrogenism were prevalent in our study population. Acne was a common finding, present in 51.8% of probands, 46.9% of sisters with PCOS, 46.2% of sisters with PCO and 27.3% of unaffected sisters. Acne was also present in 33.3% of mothers with PCOS and 20% of mothers without PCOS. Alopecia was reported in 48.2% of probands, 30.6% of sisters with PCOS, 34.6% of sisters with PCO, 9.1% of unaffected sisters, 25% of mothers with PCOS and 42.2% of mothers without PCOS.

In contrast to the findings of clinical hyperandrogenism, elevated median total T and/or DHEAS was not a feature of our study population, although probands did have a total T of 2.1nmol/L which was significantly higher than any of the sisters ($P<0.001$). Median FAI was significantly higher than any subgroup of sister analyzed, although the FAI range for sisters with PCOS extended into abnormal levels. The median androgens of the mothers were within the range expected of post-menopausal women and were low-normal. Median FAI was also normal for both groups of mothers assessed.

We were unable to demonstrate a statistically significant increase in androgens in the first degree relatives of probands with abnormal androgens.

Family history of PMPB was not a feature of our study population. PMPB in a male sibling was reported by 4.8% of probands, 6.1% of sisters with PCOS and 7.7% of sisters with PCO. PMPB in father was reported in

3.6% of probands and 4.1% of sisters with PCOS. Neither PMPB in sibling or father was reported by either group of mothers analysed.

The study population were women with mean BMI above 25. Probands and sisters with PCOS differed from the sisters with PCO and unaffected sisters, by both having BMI>30. Probands, sisters with PCOS, sisters with PCO and unaffected sisters all had WHR >0.85. Insulin levels mirrored the BMI and adverse WHR, and were higher in probands and sisters with PCOS than sisters with PCO or unaffected sisters. G:I ratio and HOMA were both significantly more adverse in probands and sisters with PCOS, compared with sisters with PCO and unaffected sisters. Both groups of mothers analysed had mean BMI>30 and WHR>0.85. There was no statistically significant difference in insulin levels between mothers with PCOS compared with mothers without PCOS and both of these groups of women had elevated BMI and WHR. The G:I ratio was at the lower limit of normal in mothers with PCOS and just below normal in mothers without PCOS. HOMA was elevated for both groups of mothers.

Interestingly, the lipid profiles were equally unfavourable in sisters with PCOS and unaffected sisters, being worse than probands or sisters with PCO. However, these differences did not reach statistical significance. Both groups of mothers had equally unfavourable lipid profiles, which was worse than their daughters. The adverse lipid profile in the mothers is most likely an effect of ageing.

Discussion of key findings:

Our study found a higher prevalence of PCOS among sisters, but a slightly lower prevalence in mothers compared with Kahsar Miller (2001). Kahsar-Miller and colleagues reported that 32% of sisters and 24% of mothers were affected with PCOS (Kahsar-Miller, 2001).We used the Rotterdam criteria to define PCOS, whereas Kahsar-Miller and colleagues used more limited NIH criteria. If we use NIH criteria to define PCOS, then fewer first degree relatives in our study would be diagnosed with PCOS. Still the high

prevalence of PCOS among first degree female relatives in our study supports the assumption that a genetic predisposition plays a role in the expression of PCOS. This is supported by the findings of Govind et al (1999).

The assignment of PCOS in mothers was difficult. The mean age of mothers in our study was 54.4 (±10.2) years. Many were peri- or post-menopausal women, as reflected in the low levels of androgens in both PCOS and non-PCOS mothers. In addition, some were on hormone replacement therapy, thus were excluded from hormonal analysis. Ultrasound is also more difficult in this age group, as ovarian volume decreases with age. Furthermore, menstrual irregularity is affected by recall bias in the post-menopausal women who were asked to recall their menstrual pattern during reproductive age, which could have been 15 years or more prior to our assessment.

Our study did not directly assess male first degree relatives. Participants were asked about the medical history of family members, including the presence of premature male pattern balding (PMPB) in either male siblings or fathers. This finding was universally low for all groups of patients in our study. Four (4.8%) probands, 3 (6.1%) sisters with PCOS and 2 (7.7%) sisters with PCO reported PMPB in a male sibling. Three (3.6%) probands and 2 (4.1%) sisters with PCOS reported PMPB in their father. Govind et al (1999) report 6 out of 28 fathers and 4 out of 18 male siblings of probands with PCOS to be affected with PMPB, or 21.4% fathers and 22.2% of male siblings affected. Govind et al (1999) assessed the male relatives directly and used earlier photographs of them when they were older than 30 years at the time of assessment. We relied solely on history, which is subject to recall bias and participant interpretation. The mean age of probands and sisters in our study was 28.1 years and 29.7 years respectively, thus a proportion could have male siblings <30 years of age. Further, many women in our study population do not have a sustained relationship with their fathers, thus may not be aware of the presence of PMPB in their fathers. Still, the finding of PMPB in male relatives was lower than expected in our study.

Huang and colleagues found a prevalence of hyperandrogenemia in PCOS women to be 75.3%, with supranormal free T present in 57.5%, elevated total T in 33% and DHEAS in 32.7% of patients with PCOS (Huang, 2009). Further, Legro and colleagues demonstrated a familial aggregation of hyperandrogenemia in PCOS family members.

In our study, probands had a median (inter-quartile range) FAI of 8.0 (4.1-12.8). Thus, by definition, at least 50% of the probands in our study population had abnormal FAI (>5.9). Median DHEAS of probands in our study group is similar to the mean reported by Legro et al (1998), being 7.3µmol/L. But the finding of relative absence of hyperandrogenemia in the family members of our study is in contrast to that of Legro et al, 1998. In their study PCOS sisters had mean (±SD) total T 2.87nmol/L (±0.95) and DHEAS 7.2µmol/L (±3.8). While we report median rather than mean values, androgen levels in the sisters with PCOS in our study population is lower (median total T 2.1nmol/L, median DHEAS 4.2µmol/L and median FAI 2.6). Thus our findings do not support those of Legro et al, 1998, who suggest that hyperandrogenemia could be used to identify which family members are affected with PCOS. We did not perform free T analysis, thus cannot compare this measure. Perhaps, if we were able to analyze free T we would be able to demonstrate a stronger familial association of hyperandrogenemia in our study population. FAI is recognised as a surrogate measure of free T, but we did not demonstrate elevated FAI in any group of sister or mother analysed.

A likely explanation for the difference in androgen levels in the sisters with PCOS in our study compared with the findings of Legro et al (1998) is the definition of PCOS used. While we used Rotterdam criteria (to define both probands and affected sisters), Legro et al (1998) used the more limited NIH criteria. The NIH criteria, by definition, require hyperandrogenemia (or hyperandrogenism) to make the diagnosis.

Hirsutism was a common feature in our study population. In their review, Azziz et al (2009) report the prevalence of hirsutism among women with PCOS to be 75%. We used a FG score of ≥7 to define

hirsutism and found 83.1% of our probands, 87.8% of sisters with PCOS and 75% of mothers with PCOS to be hirsute. Huang et al (2009), who used the same score to define hirsutism, report a prevalence of 72.2% among their population of women with PCOS. This finding of a high prevalence of hirsutism among women with PCOS is not surprising, but reminds us of the importance of this clinical feature of hyperandrogenism as a presenting symptom in women with PCOS.

Acne and alopecia were also common features in our study population. Azziz et al (2004) found acne to be present in 14.5% of 716 women with PCOS, whereas Carmina et al (2006) report that 12.6% of 950 women with androgen excess had acne. We found acne to be present in 43 (51.8%) of probands, 23 (46.9%) of sisters with PCOS, 12 (46.2%) of sisters with PCO and 9 (27.3%) of unaffected sisters.

In our study, alopecia was reported by 40 (48.2%) probands, 15 (30.6%) sisters with PCOS, 9 (34.6%) sisters with PCO, 3 (9.1%) unaffected sisters, 3 (25%) mothers with PCOS and 19 (42.2%) of mothers without PCOS. Futterweit et al (1998) report that 22.5% of women with PCOS had isolated alopecia at the time of presentation. While our study shows a significant increase in alopecia among probands compared with sisters, the prevalence is higher than expected. We suggest that there may be an over-reporting of alopecia in our study as participants may have misinterpreted the question 12 (see APPENDIX 1):

12. Do you suffer from alopecia (thinning of scalp hair)

□ 0 No

□ 1 Yes “

We recognize that “thinning of scalp hair” is a common complaint that is not always a reflection of alopecia. “Thinning of scalp hair” is too sensitive and too subjective a marker of alopecia that has lead to an over-reporting of this condition in our study population.

Dyslipidaemia is a known metabolic association in PCOS. Birdsall et al (1997) found an adverse lipid profile in women with PCOS, compared with age and BMI-matched controls. In their study, women with PCOS had mean fasting total cholesterol of 6.0mmol/L, triglyceride of 1.95mmol/L, HDL of 1.29mmol/L and LDL of 3.9mmol/L. These were women of a peri-menopausal age group, with a mean age of 52 years (Birdsall et al, 1997). This compares with our finding in mothers of probands, who also displayed dyslipidaemia with adverse total cholesterol, LDL and HDL. However, both PCOS affected and non-PCOS mothers had dyslipidaemia, which suggests that factors other than PCOS status influenced these parameters. Both age and elevated BMI are likely to have contributed to the adverse lipid profile in mothers.

Our findings in the younger group of women (probands and their sisters) are also interesting. While the difference did not reach statistical significance, sisters with PCOS and unaffected sisters had similar (adverse) lipid profiles, which was more unfavourable than probands or sisters with PCO. These findings cannot be entirely explained by obesity, as probands had significantly higher BMI than unaffected sisters (mean BMI 32.8 and 27.3 respectively, P = 0.006). However, there was no statistically significant difference in WHR between these two groups, both having adverse WHR. Thus unaffected sisters, while being less obese than their sisters with PCOS, still had an adverse adipose distribution. Still the impact of adverse WHR is not played out on all metabolic parameters and insulin levels do not explain the adverse lipid profile in unaffected sisters. Unaffected sisters had median insulin of 11.5mU/L, lower than 16.3mU/L in probands. The median G:I ratio of unaffected sisters was 0.46, the lower limit of normal. This G:I ratio is higher than that of probands (median G:I 0.32), suggesting that hyperinsulinaemia or insulin resistance are not responsible for the difference in lipid profile between unaffected sisters and probands. These findings suggest a polygenic mode of inheritance, with certain family members being affected with PCOS phenotype, while others have an adverse lipid profile.

Our study population was overweight and had an adverse WHR.

This study is the first PCOS familial association study of its type in South Africa. The Coloured ethnic group that makes up the majority of our study, have not been studied before and differ from the ethnic groups studied by either Govind et al or Kahsar-Miller et al.

Strengths and limitations of study:

By design, this study used first degree relatives (primarily sisters) of patients with PCOS (probands) to compare the prevalence of PCOS phenotypes, as well as hormonal and metabolic characteristics of probands and their first degree relatives. This is a strength when trying to discern the impact of the syndrome (PCOS) versus the role of inherent genotypic expression on phenotype. Using random (age- and/or weight-matched controls) does not control for familial genotypic expression.

The study size of 83 probands, with 108 sisters, 57 mothers and 8 daughters is reasonable. This compares well with other family association studies in the literature, which have reported on 29 probands (Govind et al, 1999) and 91 probands (Kahsar et al, 2001) respectively. Due to the relatively small number of daughters recruited in this study, they are not included in the statistical analyses.

Some of the limitations of this study include the absence of age- and BMI-matched non-PCOS controls, which would help to discern the impact of these parameters (age and BMI) on the hormonal and metabolic characteristics of our study population. Furthermore, our analysis does not use age-adjusted values, thus the influence of age on hormonal and metabolic findings within groups is not controlled for. In recognition of age as an important factor, probands are compared to sisters (who have a similar mean age) and mothers with PCOS are compared with mothers without PCOS (also with similar mean age).

For this study, the assignment of menstrual irregularity was based on self-reporting by study participants and did not include the use of mid-luteal progesterone level. Self-reporting can be subject to recall bias, especially in the older post-menopausal women who may not accurately recall her menstrual history. For

this study oligomenorrhoea was defined as less than 6 menses per year. This definition may exclude some women with menstrual cycle >35 days but having >6 menses per annum, thus potentially reducing the number of women assigned as PCOS affected. However, this stricter definition was thought to include those women with a definite history of menstrual irregularity.

The presence of acne and alopecia were both assessed by self-reporting. For alopecia the trigger-phrase "thinning of scalp hair" was given in the questionnaire to assist participants in reporting this clinical feature. However, "thinning of scalp hair" may be too sensitive as a trigger-phrase and thus may have lead to over-reporting of alopecia. Both acne and alopecia should be assessed by the researcher to improve on the specificity of the diagnoses.

The study did not directly assess male first degree relatives and reporting of PMPB in this study was universally low. Assessment of male phenotype is important when undertaking a family association study, especially as we understand that the inheritance of PCOS can be through fathers and can affect brothers. However, in our setting male family members have been extremely difficult to recruit, in part because they are at work and also because they do not perceive any value in participating in research within a Department of Obstetrics and Gynaecology Department. The assessment of male phenotype was not adequate in our study, but reflects on similar experience in recruiting men in previous family studies.

Relevance to clinical practice:

Hirsutism, more than hyperandrogenemia, characterizes our PCOS population. It would follow that women presenting with hirsutism should be assessed for other features of PCOS, as this may be one of the more common presenting manifestations of hyperandrogenism and thus PCOS.

A detailed family history should be taken for all women diagnosed with PCOS. Female first degree relatives should also be assessed, due to the high prevalence of PCOS within first degree relatives. Early assessment may offer an opportunity to provide life-style modification, before metabolic derangements develop.

The high prevalence of dyslipidaemia within our study population also suggests that we should always investigate for dyslipidaemia amongst PCOS patients and their families.

Relevance for future research:

The prevalence of PCOS among first degree female relatives in our study supports further research into genetic abnormalities that may predispose individuals to develop PCOS. A multi-factorial multi-gene inheritance is likely.

From our study data, the use of hyperandrogenemia is not adequate as a marker of affected status for linkage analysis. Perhaps if the more sensitive free T was calculated, then these findings would differ. Alternatively, hyperandrogenemia as a marker of affected status may be more useful if NIH limited criteria are used to define PCOS.

The findings of adverse lipid profiles in PCOS and PCOS unaffected sisters suggests that a polygenic mode of inheritance may exist. A more detailed examination of those families with dyslipidaemia would be useful to delineate contributory factors, including familial association of dyslipidaemia independent of PCOS.

CONCLUSION:

The results of our analysis clearly demonstrate a strong familial association of PCOS among first degree female relatives of women with PCOS. We were unable to demonstrate a statistically significant correlation between abnormal total testosterone or free androgen index in probands with an elevated mean total testosterone or free androgen index in family members. Hirsutism was a common clinical finding in our study population and was a predominant feature in the phenotypic presentation of these women.

An adverse lipid profile was found in mothers and sisters of probands with PCOS. The presence of adverse lipid profile was independent of PCOS status, age, BMI or insulin resistance, suggesting an additional factor predisposing these women to dyslipidaemia.

Future detailed analysis of those families with hyperandrogenemia, insulin resistance and dyslipidaemia may help to explain the contributory influences that these metabolic abnormalities have on each other.

While many genetic studies into PCOS have been undertaken in the past, none have yet involved South African women. The results of genetic studies are keenly anticipated, as these may assist in defining defective metabolic pathways in South African women with PCOS. Should such research yield results, this would also assist therapeutics that could target defective pathways.

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APPENDIX 1: CLERKING SHEET

APPENDIX 2: STATISTICAL ANALYSIS