Influence of a positive family history of both type 2 diabetes and PCOS on metabolic and endocrine parameters in a large cohort of PCOS women

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Abstract

Objective: There is evidence suggesting a strong genetic background of polycystic ovary syndrome (PCOS). We aim to study the metabolic and endocrine characteristics of PCOS women with and without a family history (FHx) of type 2 diabetes mellitus (T2DM) and PCOS.

Design: Cross-sectional study.

Methods: We analysed the association of T2DM FHx and PCOS FHx with metabolic and endocrine parameters in 714 PCOS women.

Results: A positive FHx of T2DM and PCOS were prevalent in 36.8 and 21.4% of PCOS women respectively. We found an independent association of T2DM FHx with central fat accumulation, obesity, prediabetes, metabolic syndrome (MS), insulin resistance, low HDL and elevated blood pressure ($P<0.05$ for all). PCOS FHx was independently associated with prediabetes ($P<0.05$). We observed an independent association of PCOS FHx with clinical and biochemical hyperandrogenism ($P<0.05$ for all), whereas there was no independent association of T2DM FHx with hyperandrogenism. PCOS women with a positive FHx of both T2DM and PCOS had an adverse metabolic and endocrine profile including a linear increase in risk of obesity, central fat accumulation, MS, prediabetes and low HDL ($P<0.05$ for all).

Conclusions: Our findings suggest that the assessment of FHx might allow risk stratification of PCOS women, which is important considering the high prevalence of PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disease in women of reproductive age, affecting up to 20% of all women when using Rotterdam criteria (1). There are several lines of evidence suggesting a strong genetic background of PCOS, which is also reflected by a familial accumulation of PCOS cases (2, 3, 4, 5). Thus, a positive FHx of PCOS is considered as a risk factor for PCOS development. PCOS women present not only with hyperandrogenism but also affected by an increased metabolic risk which might be related to obesity as well as to genetic and environmental factors (6). There is accumulating evidence suggesting that central obesity is the key factor in the development of metabolic and cardiovascular risk in PCOS women (7, 8, 9). In this context, several indices such as lipid accumulation product (LAP) (8, 10) and visceral adiposity index (VAI) (11) have been shown to be associated with an increased risk of insulin resistance, prediabetes and type 2 diabetes mellitus (T2DM) in PCOS as well as in other cohorts. Further, there is

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evidence showing that a positive family history (FHx) of T2DM, as a reflection of genetic risk, is associated with an increased risk of the development of T2DM in PCOS women (12, 13, 14). In PCOS women, obesity and T2DM-related genes (3, 15) as well as genetic polymorphisms related to hyperandrogenism (16) have been shown to be associated with PCOS phenotype, suggesting an important genetic background. Apart from this, it has not been investigated so far whether a positive FHx of T2DM and PCOS contribute equally to metabolic and endocrine disturbances in PCOS women or whether there are differences regarding the impact of T2DM and PCOS FHx on metabolic and endocrine parameters. Further, most previous studies investigating the association of T2DM FHx with PCOS phenotype included women from the USA who are more obese and have a higher prevalence of metabolic disturbances compared with European cohorts (12, 13, 14).

The aim of the study was to:

i) study the metabolic and endocrine characteristics of central European PCOS women with and without a FHx of T2DM and PCOS respectively; and
ii) investigate the association of central fat accumulation reflected by LAP and VAI with a positive FHx of T2DM and PCOS respectively.

Subjects and methods

Subjects

The study cohort consisted of 714 Caucasian White women with PCOS, aged 16–45 years, and 139 BMI-matched control women within the same age range. PCOS women were routinely referred to our outpatient clinic for evaluation of PCOS from 2006 to 2012. PCOS women consulted our outpatient clinic for PCOS-related symptoms such as hirsutism, acne, obesity, infertility or menstrual irregularities or were referred to our outpatient clinic by gynaecologists for further endocrine and metabolic evaluation because of polycystic ovaries. PCOS was diagnosed using the Rotterdam criteria (17). Two out of the following three characteristics are required to confirm the diagnosis: clinical and/or biochemical signs of hyperandrogenism, oligo- and/or anovulation and polycystic ovaries (by ultrasound). Hyperandrogenism was defined by the clinical presence of hirsutism (modified Ferriman–Gallwey (FG) score ≥ 6), acne or alopecia and/or elevated androgen levels (normal range of total testosterone: <0.77 ng/ml, free testosterone: <0.013 ng/ml, and free androgen index (FAI): <5.5). Oligo- and/or anovulation were defined by the presence of oligomenorrhoea or amenorrhoea. Polycystic ovarian morphology was examined by ultrasound (17). Hyperprolactinaemia, Cushing's syndrome, congenital adrenal hyperplasia and androgen-secreting tumours were excluded by specific laboratory analysis (prolactin, cortisol, adrenocorticotropic hormone (ACTH) and 17α-OH progesterone).

Healthy women, who were routinely referred to our outpatient clinic for thyroid evaluation between 2009 and 2010, were included in the study as a control group. All control women had normal thyroid function, regular menstrual cycles, normal serum androgens and no clinical signs of hyperandrogenism. PCOS and control women did not take any medication known to affect endocrine parameters, carbohydrate metabolism or serum lipid profile for at least 3 months before entering the study.

The study protocol was approved by the ethics committee of the Medical University of Graz and written informed consent was obtained from each patient.

Procedures

We assessed FHx of PCOS using questionnaires. PCOS women were asked the following questions: have your mother or sisters been diagnosed with PCOS? Do your mother or sisters have clinical (hirsutism or acne) or biochemical hyperandrogenism? Do your mother or sisters have menstrual irregularities or PCO? Similarly, FHx of T2DM was assessed using questionnaires asking whether T2DM is prevalent in brothers, sisters, parents or grandparents of PCOS women and control women respectively.

Standard anthropometric data (height, weight, waist circumference (WC) and hip circumference, blood pressure (BP)) were obtained from each subject. WC was measured in a standing position midway between the lower costal margin and the iliac crest. Hip circumference was measured in a standing position at the maximum circumference over the buttocks. The BMI was calculated as the weight in kilograms divided by the square of height in meters. Hirsutism was quantified with the modified FG score.

Basal blood samples for hormonal (total testosterone, sex-hormone-binding globulin (SHBG), androstenedione, DHEAS, free tri-iodothyronine (fT_3), free thyroxine (fT_4), TSH, 17α-OH progesterone, prolactin, cortisol, ACTH), glucose, HbA1c and serum lipids were collected between 0800 and 0900 h after an overnight fast in the follicular phase in women with regular menses or at random in women with menstrual irregularities. Free testosterone values were calculated from total testosterone, SHBG and albumin according to Vermeulen (18). The FAI was

calculated as total testosterone (nmol/l)/SHBG (nmol/l)× 100. All participants underwent a fasting 75 g oral glucose tolerance test (OGTT). Blood samples were drawn after 30, 60 and 120 min for glucose and insulin determination. The area under the glucose response curve (AUCgluc) and the area under the insulin response curve (AUCins) were calculated according to the trapezoidal method.

Obesity was defined as BMI $\geq 30\ kg/m^2$. LAP was calculated using the formula (WC (cm) − 58)×(triglycerides (TG) (mmol/l)). Elevated LAP was defined as >44.1 as described previously in a large PCOS cohort as best cut-off for detecting disturbed glucose tolerance (8). VAI was calculated using the formula (WC (cm)/(36.58 +(1.89 × BMI (kg/m^2)))) × (TG (mmol/l)/0.81) × (1.52/HDL (mmol/l)). Elevated VAI was defined as VAI >1.675 as described previously (11). Insulin resistance was estimated using the homeostatic model assessment-insulin resistance (HOMA-IR) and was assumed for levels >2.5. Quantitative insulin sensitivity check index (QUICKI) was used for estimation of insulin sensitivity (11). We further calculated insulin sensitivity index Matsuda (ISI (Matsuda)) as an insulin sensitivity index that reflects a composite estimate of hepatic and muscle insulin sensitivity determined from OGTT data (ISI (Matsuda) = $10\,000/\sqrt{((FG \times \text{fasting insulin}) \times (\text{glucose}_{OGTTmean} \times \text{insulin}_{OGTTmean}))}$) (19). It has been shown that ISI (Matsuda) correlates reasonably well with the estimates of whole-body insulin sensitivity determined by the glucose clamp. The fasting component reflects hepatic insulin sensitivity, whereas the mean of the dynamic data primarily represents skeletal muscle insulin sensitivity. This partitioning concept has recently been validated using glucose clamp studies (20).

Prediabetes and T2DM were defined according to the American Diabetes Association (ADA) (21). Elevated TG levels were defined as TG >150 mg/dl and low HDL as HDL <50 mg/dl. Elevated BP was defined as systolic BP >130 mmHg and/or diastolic BP >85 mmHg (22, 23). The MS was defined by the National Cholesterol Education Program and the Adult Treatment Panel-III in women presenting at least three of the following criteria: WC >88 cm, HDL cholesterol <50 mg/dl, TG level >150 mg/dl, raised blood pressure (systolic >130 mmHg, diastolic >85 mmHg), and raised FG (>110 mg/dl) or prevalent T2DM.

Biochemical analyses

Total testosterone, insulin, prolactin, TSH, fT_3, fT_4 and cortisol (Siemens, Erlangen, Germany) were measured by luminescence immunoassay (intra- and interassay coefficients of variation (CV values) of <10%). SHBG was measured by luminescence immunoassay (Roche) with an intra- and interassay CV of 1.3 and 2.1% respectively. Albumin was measured by photometric assay (Roche). DHEAS (LDN Labor Diagnostika Nord GmbH, Nordhorn, Germany), androstenedione and 17α-OH progesterone (DiaMetra, BioVendor, Brno, Czech Republic) were measured by ELISA with intra- and interassay CV of <10%.

Statistical analyses

Data are presented as median with interquartile range unless otherwise stated. The distribution of data was analysed by descriptive statistics and Kolmogorov–Smirnov test. All parameters were found to be non-normally distributed. Kruskal–Wallis test (adjusted for multiple comparisons), Mann–Whitney *U* test and χ^2 test were used for comparisons between groups. We calculated binary logistic regression analyses using all-factors models with various binary metabolic variables as dependent variables and FHx of T2DM (or FHx of PCOS), age and testosterone as independent variables. Moreover, we calculated binary logistic regression analyses using biochemical and clinical hyperandrogenism as dependent variable and age, BMI and T2DM FHX (or PCOS FHx) as independent variables. Age, BMI and total testosterone were log-transformed and rechecked for normal distribution before being entered in logistic regression analyses. The variables followed normal distribution after log-transformation. We further carried out subgroup analyses of obese and non-obese women, repeating the analyses as described earlier.

All statistical procedures were carried out with SPSS version 20 (SPSS, Inc.). *P* value <0.05 was considered statistically significant.

Results

A positive FHx of T2DM and a positive PCOS FHx were prevalent in 36.8 and 21.4% of PCOS women respectively. Conversely, 35.1% of women with normal glucose tolerance had a positive FHx of T2DM, whereas 48.7% of PCOS women with prediabetes had a positive FHx of T2DM (odds ratio (OR) 1.75 (1.08–2.84); $P=0.023$) and 44.4% of PCOS women with T2DM had a positive FHx of T2DM ($P=0.639$). Baseline characteristics of subjects stratified by FHx of T2DM and PCOS are shown in Tables 1 and 2. Hyperandrogenism, menstrual irregularities and PCO were present in 50.6% of PCOS women, 32.9% had hyperandrogenism and menstrual irregularities, 9.8% had

Table 1 Characteristics of PCOS women stratified by FHx of T2DM. Analyses were carried out using Mann–Whitney *U* test or χ^2 test.

	All women ($n=714$)		**FHx of T2DM**				
			Negative ($n=451$)		Positive ($n=263$)		
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	***P* value**
Age (years)	27	23–32	26	23–31	27	24–31	0.121
BMI (kg/m^2)	24.2	21.3–30.1	23.8	20.8–29	25.2	21.9–32.1	0.001
WC (cm)	82	72–96	80	72–94	86	73–100	0.011
WHR	0.79	0.73–0.87	0.79	0.73–0.86	0.80	0.74–0.88	0.119
Systolic BP (mmHg)	118	110–130	117	110–127	120	110–133	0.008
Diastolic BP (mmHg)	80	74–89	80	73–87	81	75–90	0.129
Fasting glucose (mg/dl)	85	79–90	84	79–90	85	79–91	0.097
Glucose 2-h (mg/dl)	95	81–115	93	78–110	99	85–123	0.001
AUCgluc	172.3	151.3–198.8	170.0	150.3–191.3	178.6	152.9–210.1	0.010
HbA1c (%)	5.2	5.0–5.3	5.2	5.0–5.3	5.1	5.0–5.3	0.137
Fasting insulin (μU/ml)	6.2	3.8–9.8	6.0	3.7–9.5	6.4	4.0–10.8	0.119
Insulin 2-h (μU/ml)	33.4	20.0–58.0	31.3	18.5–55.5	37.4	21.7–61.8	0.009
AUCins	61.6	39.4–96.7	57.8	36.8–96.7	65.6	44.4–97.9	0.106
HOMA-IR	1.27	0.80–2.17	1.26	0.77–2.04	1.30	0.82–2.73	0.061
QUICKI	0.37	0.34–0.40	0.37	0.34–0.40	0.37	0.33–0.40	0.119
ISI (Matsuda)	7.34	4.52–11.34	7.83	4.90–11.74	6.76	4.13–10.62	0.020
LAP	19.7	10.3–41.3	18.4	9.8–35.5	21.9	10.8–50.4	0.022
VAI	0.89	0.62–1.53	0.86	0.58–1.47	0.98	0.65–1.86	0.015
TC (mg/dl)	178	156–199	177	155–199	178	159–199	0.563
TG (mg/dl)	75	54–103	75	54–103	75	55–103	0.345
HDL (mg/dl)	64	53–76	66	54–78	61	49–74	0.001
LDL (mg/dl)	100	83–118	98	82–117	103	86–120	0.046
Testosterone (ng/ml)	0.60	0.46–0.79	0.60	0.45–0.80	0.60	0.46–0.78	0.739
SHBG (nmol/l)	45.4	29.8–63.0	47.1	31.8–66.3	41.4	27.2–58.8	0.004
FAI	4.96	3.16–7.72	4.80	2.96–7.39	5.21	3.47–7.96	0.069
Free testosterone (ng/ml)	0.009	0.007–0.0013	0.009	0.006–0.0013	0.009	0.007–0.014	0.287
FG score	6	2–11	6	2–11	6	2–12	0.800
Positive FHx of PCOS (%)	21.4		16.1		30.6		<0.001
Obesity (%)	25.3		22.0		30.9		0.009
LAP elevated (%)	23.5		19.9		28.9		0.012
VAI elevated (%)	23.0		20.1		27.4		0.037
Prediabetes (%)	12.8		10.4		16.9		0.022
T2DM (%)	1.5		1.3		1.8		0.638
MS (%)	11.5		8.6		16.4		0.006
TG elevated (%)	10.4		9.1		12.6		0.167
HDL low (%)	18.8		14.8		25.8		0.001
BP elevated (%)	37.4		34.0		42.6		0.035
Menstrual irregularities (%)	85.5		85.6		85.3		0.393
PCO (%)	56.8		55.9		58.2		0.582
Clinical hyperandrogenism (%)	72.7		73.5		71.5		0.609
Biochemical hyperandrogenism (%)	51.0		52.8		47.9		0.252

PCOS, polycystic ovary syndrome; FHx, family history; T2DM, type 2 diabetes mellitus; WC, waist circumference; WHR, waist-to-hip ratio; BP, blood pressure; AUC, area under the curve; HOMA, homeostatic model assessment; QUICKI, quantitative insulin sensitivity check index; ISI, insulin sensitivity index; LAP, lipid accumulation product; VAI, visceral adiposity index; SHBG, sex hormone-binding globulin; FAI, free androgen index; FG, Ferriman–Gallwey; TC, total cholesterol; TG, triglycerides; MS, metabolic syndrome; PCO, polycystic ovaries.

menstrual irregularities and PCO and 6.8% had hyperandrogenism and PCO.

Binary logistic regression analyses investigating the association of various metabolic parameters with FHx of T2DM and PCOS are presented in Table 3. We found an independent association of T2DM FHx with elevated LAP, obesity, prediabetes, MS, insulin resistance, low HDL and elevated BP. Further, PCOS FHx was independently associated with prediabetes.

Binary logistic regression analyses investigating the association of hyperandrogenism with FHx of T2DM and PCOS are shown in Table 4. We observed an independent

Table 2 Characteristics of PCOS women stratified by FHx of PCOS. Analyses were carried out using Mann–Whitney *U* test or χ^2 test.

	FHx of PCOS				
	Negative ($n=500$)		Positive ($n=136$)		
	Median	Interquartile range	Median	Interquartile range	***P* value**
Age (years)	27	23–31	27	23–31	0.587
BMI (kg/m^2)	23.9	21.1–29.3	25.3	22.1–31.2	0.025
WC (cm)	81	72–95	85	75–100	0.038
WHR	0.79	0.73–0.86	0.80	0.74–0.88	0.166
Systolic BP (mmHg)	117	110–130	116	110–132	0.443
Diastolic BP (mmHg)	80	72–88	80	74–86	0.790
Fasting glucose (mg/dl)	84	79–90	87	82–93	0.001
Glucose 2-h (mg/dl)	95	81–114	102	85–124	0.021
AUCgluc	170.8	150.8–192.0	187.8	154.8–209.8	0.013
HbA1c (%)	5.1	5.0–5.3	5.2	4.9–5.3	0.560
Fasting insulin (μU/ml)	6.0	3.8–9.6	6.9	4.5–11.5	0.057
Insulin 2-h (μU/ml)	31.3	18.5–55.5	37.4	21.7–61.8	0.481
AUCins	61.1	39.1–92.3	70.9	42.7–108.2	0.141
HOMA-IR	1.26	0.77–2.00	1.46	0.97–2.64	0.020
QUICKI	0.37	0.34–0.40	0.36	0.33–0.39	0.027
ISI (Matsuda)	7.28	4.65–11.46	6.56	3.70–10.14	0.072
LAP	19.1	10.0–38.3	23.3	11.4–53.0	0.053
VAI	0.89	0.63–1.48	0.99	0.63–1.77	0.272
TC (mg/dl)	178	158–198	179	154–206	0.645
TG (mg/dl)	75	55–101	78	56–115	0.303
HDL (mg/dl)	65	53–77	64	53–77	0.707
LDL (mg/dl)	99	82–116	102	84–121	0.379
Testosterone (ng/ml)	0.62	0.49–0.80	0.63	0.48–0.81	0.555
SHBG (nmol/l)	46.4	31.0–63.5	39.9	25.4–55.1	0.001
FAI	4.79	2.96–7.39	5.68	3.70–9.43	0.004
Free testosterone (ng/ml)	0.009	0.006–0.0013	0.011	0.008–0.015	0.012
FG score	6	2–11	9	4–15	<0.001
Positive FHx of T2DM (%)	32.2		52.2		<0.001
Obesity (%)	22.9		28.9		0.147
LAP elevated (%)	21.2		29.7		0.049
VAI elevated (%)	21.6		27.3		0.182
Prediabetes (%)	11.4		21.6		0.005
T2DM (%)	1.2		2.5		0.301
MS (%)	10.8		14.1		0.307
TG elevated (%)	9.6		13.4		0.194
HDL low (%)	18.2		20.9		0.486
BP elevated (%)	34.5		36.4		0.703
Menstrual irregularities (%)	85.1		86.8		0.631
PCO (%)	57.0		55.9		0.816
Clinical hyperandrogenism (%)	69.9		81.7		0.008
Biochemical hyperandrogenism (%)	50.6		52.5		0.711

PCOS, polycystic ovary syndrome; FHx, family history; WC, waist circumference; WHR, waist-to-hip ratio; BP, blood pressure; AUC, area under the curve; HOMA, homeostatic model assessment; QUICKI, quantitative insulin sensitivity check index; ISI, insulin sensitivity index; LAP, lipid accumulation product; VAI, visceral adiposity index; SHBG, sex hormone-binding globulin; FAI, free androgen index; FG, Ferriman–Gallwey; TC, total cholesterol; TG, triglycerides; T2DM, type 2 diabetes mellitus; MS, metabolic syndrome; PCO, polycystic ovaries.

association of PCOS FHx with clinical hyperandrogenism, hirsutism, elevated FAI levels and free testosterone levels, whereas there was no independent association of T2DM FHx with clinical or biochemical hyperandrogenism.

We carried out further analyses stratifying PCOS women into three groups: no positive FHx (53.3%), positive FHx of T2DM or PCOS (35.3%9) and positive FHx of T2DM and PCOS (11.2%) (Table 5). PCOS women with a positive FHx of both T2DM and PCOS had an adverse metabolic and endocrine profile. Moreover, we found a linear increase in risk of obesity, central fat accumulation, MS, prediabetes and low HDL. Binary logistic regression analyses using various metabolic disturbances as dependent variables and FHx (three groups: no positive FHx (reference); positive FHx of T2DM or PCOS; positive FHx of T2DM and PCOS), age and testosterone as independent variables are shown in Fig. 1.

Table 3 Multivariate adjusted binary logistic regression analyses for various metabolic risk factors using FHx of T2DM and PCOS as dependent variables. Analyses were carried out by binary logistic regression analyses using all-factors models with metabolic risk factors as binary dependent variables and positive FHx of T2DM or PCOS as explanatory variables (a negative FHx was defined as reference). Analyses were adjusted for age and testosterone.

	Positive FHx of T2DM[a]		Positive FHx of PCOS[b]	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
LAP elevated	1.58 (1.07–2.34)	0.020	1.46 (0.92–2.31)	0.107
VAI elevated	1.47 (1.00–2.17)	0.052	1.32 (0.83–2.09)	0.240
Obesity	1.57 (1.11–2.23)	0.012	1.30 (0.84–2.00)	0.242
Prediabetes	1.71 (1.05–2.78)	0.031	2.08 (1.21–3.56)	0.008
T2DM	1.31 (0.35–4.94)	0.695	1.90 (0.44–8.18)	0.389
MS	2.07 (1.20–3.57)	0.009	1.21 (0.66–2.21)	0.543
Insulin resistance	1.69 (1.13–2.53)	0.010	1.47 (0.93–2.31)	0.101
TG elevated	1.46 (0.86–2.47)	0.158	1.39 (0.77–2.49)	0.277
HDL low	2.01 (1.34–3.02)	0.001	1.20 (0.75–1.93)	0.453
Elevated BP	1.45 (1.03–2.04)	0.034	0.89 (0.58–1.38)	0.603

FHx, family history; T2DM, type 2 diabetes mellitus; PCOS, polycystic ovary syndrome; LAP, lipid accumulation product; VAI, visceral adiposity index; MS, metabolic syndrome; TG, triglycerides; BP, blood pressure.
[a]Data available in 714 women.
[b]Data available in 636 women.

Non-obese women

We carried out subgroup analyses of non-obese (BMI $<30\ kg/m^2$) and obese (BMI $\geq 30\ kg/m^2$) PCOS women and metabolic characteristics are shown in Table 6. We found a significantly higher prevalence of PCOS FHx ($P=0.016$) and decreased HDL in non-obese PCOS women with a positive T2DM FHx, whereas the remaining associations were attenuated (data not shown). Non-obese PCOS women with a positive PCOS FHx had a higher prevalence of T2DM FHx ($P=0.016$), higher FG ($P=0.004$) and FG scores ($P=0.027$) and lower SHBG levels ($P=0.018$). We observed a trend towards a higher prediabetes prevalence ($P=0.062$), AUCgluc ($P=0.052$) and FAI levels ($P=0.071$) in non-obese PCOS women with a positive PCOS FHx. Further, non-obese PCOS women with a positive FHx of T2DM and PCOS were significantly younger ($P=0.027$), had higher BMI ($P=0.025$) and FG ($P=0.030$) levels, a higher prevalence of prediabetes ($P=0.025$) and lower SHBG levels ($P=0.007$) and showed a trend towards higher AUCgluc levels ($P=0.053$).

Binary logistic regression analyses using metabolic and endocrine parameters as dependent variables were attenuated (except decreased HDL (OR for T2DM FHx: 2.12, 1.15–3.91; $P=0.015$) and hirsutism (OR for PCOS FHx: 1.59, 1.00–2.52; $P=0.050$)).

Obese women

In obese PCOS women, a positive T2DM FHX was significantly associated with a higher prevalence of PCOS FHx ($P<0.001$), prediabetes ($P=0.040$), lower SHBG ($P=0.034$) and HDL ($P=0.030$) levels, and showed a trend towards higher FG ($P=0.060$), 2-h glucose ($P=0.058$) and testosterone levels ($P=0.073$). A positive PCOS FHx was significantly associated with a higher prevalence of T2DM FHx ($P<0.001$) and clinical hyperandrogenism ($P=0.020$), higher levels of 2-h glucose (0.007), FAI ($P=0.001$) and FG score ($P<0.0019$ and lower SHBG levels (0.001)). Further, FT levels tended to be higher in PCOS women with a positive PCOS FHx ($P=0.084$). Moreover, a positive FHx of T2DM and PCOS was significantly associated with higher FG ($P=0.024$) and FAI ($P=0.014$) levels and a higher prevalence of prediabetes ($P=0.047$) and lower SHBG levels ($P=0.002$). Further, we observed a trend towards higher TG levels ($P=0.062$) and elevated VAI ($P=0.074$).

Table 4 Multivariate adjusted binary logistic regression analyses for hyperandrogenism using FHx of T2DM and PCOS as dependent variables. Analyses were carried out by binary logistic regression analyses using all-factors models with hyperandrogenism as binary dependent variable and positive FHx of T2DM or PCOS as explanatory variables (a negative FHx was defined as reference). Clinical hyperandrogenism was defined as the presence of hirsutism and/or acne and/or alopecia. Biochemical hyperandrogenism was defined as elevated testosterone and/or free testosterone and/or FAI. Analyses were adjusted for age and BMI.

	Positive FHx of T2DM[a]		Positive FHx of PCOS[b]	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Clinical hyperandrogenism	0.84 (0.57–1.24)	0.378	1.85 (1.13–3.03)	0.015
Hirsutism	0.81 (0.57–1.15)	0.235	1.80 (1.19–2.73)	0.006
Biochemical hyperandrogenism	0.77 (0.54–1.09)	0.143	1.02 (0.66–1.57)	0.935
Testosterone high	0.85 (0.58–1.24)	0.396	1.01 (0.65–1.56)	0.973
Free testosterone high	0.90 (0.56–145)	0.671	1.68 (1.01–2.80)	0.044
FAI high	0.90 (0.62–1.31)	0.592	1.64 (1.06–2.53)	0.025

FHx, family history; T2DM, type 2 diabetes mellitus; PCOS, polycystic ovary syndrome; FAI, free androgen index.
[a]Data available in 714 women.
[b]Data available in 636 women.

Table 5 Characteristics of PCOS women stratified by FHx of T2DM and PCOS respectively, comparing PCOS women without a positive FHx with women with a positive FHx of T2DM or PCOS and to women with a positive FHx of T2DM and PCOS.

	Negative FHx ($n=339$)		**Positive FHx of T2DM or PCOS** ($n=226$)		**Positive FHx of T2DM and PCOS** ($n=71$)		
	Median	Interquartile range	Median	Interquartile range	Median	Interquartile range	***P* value**
Age (years)	26	22–31	27	24–31	27	23–31	0.196
BMI (kg/m^2)	23.9	20.9–29.1	24.1	21.3–29.0	27.6†,‡	23.1–33.6	<0.001
WC (cm)	80	72–93	82	72–95	88†,‡	78–105	0.007
WHR	0.78	0.73–0.86	0.79	0.74–0.87	0.81	0.75–0.89	0.245
Systolic BP (mmHg)	116	109–128	119	110–130	120	111–138	0.108
Diastolic BP (mmHg)	80	72–87	80	73–90	80	75–88	0.753
Fasting glucose (mg/dl)	84	78–90	86*	79–91	87†	82–93	0.004
Glucose 2-h (mg/dl)	94	81–111	97	82–123	107†,‡	90–126	0.003
AUCgluc	170.0	150.3–189.0	172.0	151.8–204.0	189.5†	164.5–212.0	0.004
HbA1c (%)	5.1	5.0–5.3	5.1	5.0–5.3	5.1	4.9–5.4	0.706
Fasting insulin (μU/ml)	6.0	3.7–9.5	6.3	3.8–10.0	7.4†,‡	5.3–13.4	0.035
Insulin 2-h (μU/ml)	32.4	20.0–56.0	34.2	20.0–57.0	44.1	23.4–72.5	0.099
AUCins	57.4	37.4–92.4	64.1	39.3–91.4	71.8	47.1–118.4	0.117
HOMA-IR	1.27	0.76–2.04	1.26	0.80–2.28	1.68†,‡	1.11–2.95	0.009
QUICKI	0.37	0.34–0.40	0.37	0.34–0.40	0.35‡	0.33–0.38	0.017
ISI (Matsuda)	7.72	4.88–11.95	6.90	4.27–10.77	5.85†	3.09–9.48	0.025
LAP	18.7	10.0–35.7	19.3	10.3–39.7	26.5†,‡	13.2–63.0	0.017
VAI	0.88	0.62–1.49	0.88	0.63–1.45	1.10‡	0.75–2.32	0.067
TC (mg/dl)	178	156–199	179	158–202	176	156–202	0.840
TG (mg/dl)	75	56–105	75	54–98	85‡	62–126	0.083
HDL (mg/dl)	67	53–78	63	54–76	59†	46–74	0.043
LDL (mg/dl)	98	80–117	101	85–117	97	84–120	0.212
Testosterone (ng/ml)	0.62	0.49–0.80	0.62	0.47–0.80	0.61	0.47–0.81	0.969
SHBG (nmol/l)	47.6	31.1–66.3	44.3	29.2–62.0	33.0†,‡	24.4–48.5	<0.001
FAI	4.67	2.84–7.29	4.98	3.36–7.75	5.78†,‡	4.21–9.76	0.006
Free testosterone (ng/ml)	0.009	0.006–0.012	0.010	0.007–0.014	0.010	0.008–0.015	0.072
FG score	6	2–11	6	3–11	8†	4–13	0.040
Obesity (%)	21.6		23.5		38.6		0.010
LAP elevated (%)	20.4		22.9		35.9		0.035
VAI elevated (%)	21.7		20.5		35.3		0.035
Prediabetes (%)	10.4		14.4		26.2		0.005
T2DM (%)	1.4		1.0		3.1		0.498
MS (%)	8.3		14.0		17.6		0.041
TG elevated (%)	9.0		10.4		16.9		0.143
HDL low (%)	15.8		19.5		31.0		0.011
BP elevated (%)	33.7		37.2		41.3		0.479
Menstrual irregularities (%)	85.4		85.0		87.3		0.885
PCO (%)	57.8		52.7		64.8		0.168
Clinical hyperandrogenism (%)	71.5		71.5		81.2		0.242
Biochemical hyperandrogenism (%)	52.7		48.3		51.6		0.617

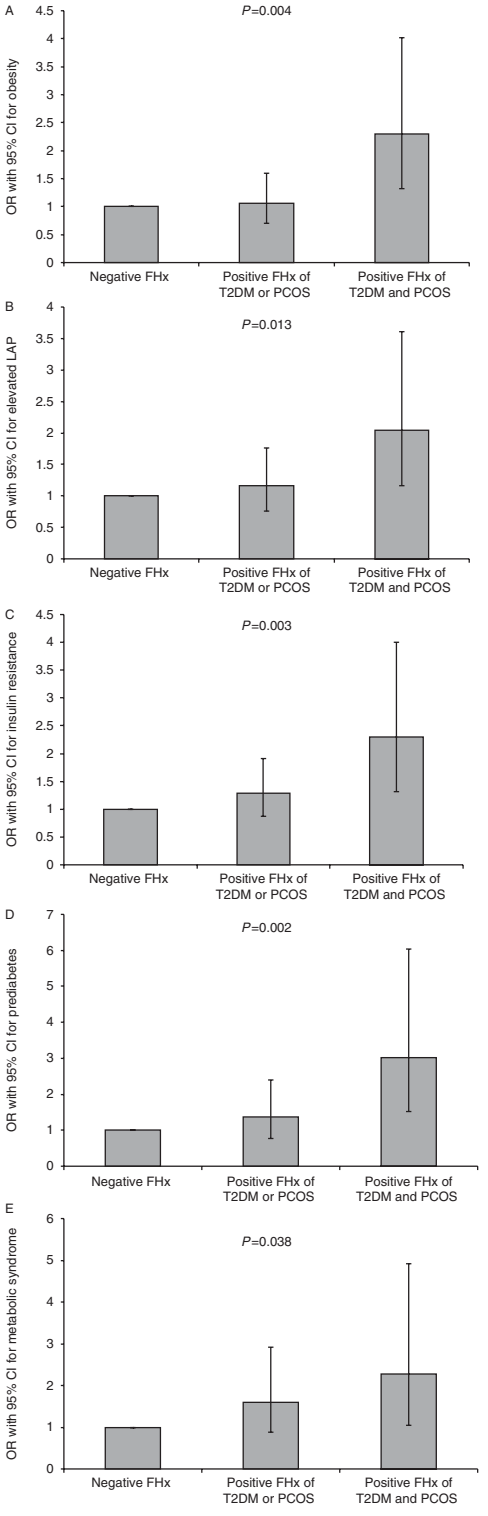
*$P<0.05$ for negative FHx vs positive FHx of T2DM or PCOS (Mann–Whitney *U* test); $^{\dagger}P<0.05$ for negative FHx vs positive FHx of T2DM and PCOS (Mann–Whitney *U* test); $^{\ddagger}P<0.05$ for positive FHx of T2DM or PCOS vs positive FHx of T2DM and PCOS (Mann–Whitney *U* test). PCOS, polycystic ovary syndrome; FHx, family history; T2DM, type 2 diabetes mellitus; WC, waist circumference; WHR, waist-to-hip ratio; BP, blood pressure; AUC, area under the curve; HOMA, homeostatic model assessment; QUICKI, quantitative insulin sensitivity check index; ISI, insulin sensitivity index; LAP, lipid accumulation product; VAI, visceral adiposity index; SHBG, sex hormone-binding globulin; FAI, free androgen index; FG, Ferriman–Gallwey; TC, total cholesterol; TG, triglycerides; MS, metabolic syndrome; PCO, polycystic ovaries.

Binary logistic regression analyses using metabolic variables as dependent variables were attenuated (except prediabetes (OR for T2DM FHx: 2.26, 1.03–4.98; $P=0.040$)). In contrast, binary regression analysis remained stable for PCOS FHx and clinical hyperandrogenism (OR 4.11, 1.16–14.58; $P=0.019$), hirsutism (OR 3.66, 1.31–10.20; $P=0.013$), elevated FAI (OR 2.95, 1.03–8.44; $P=0.044$) and elevated FT (OR 2.32, 1.02–5.27; $P=0.044$).

Healthy women

Baseline characteristics of the control group have been reported previously (24). The prevalence of T2DM FHx was

significantly lower in control women (16.2%, $P<0.001$) compared with PCOS women. We found no significant differences in metabolic or endocrine parameters between healthy women with and without T2DM FHx ($P>0.100$ for all, data not shown).



Discussion

We present evidence that a positive FHx of T2DM is independently associated with metabolic disturbances such as central fat accumulation, obesity, prediabetes, MS, insulin resistance dyslipidaemia and high BP in a large cohort of PCOS women. A positive FHx of PCOS, however, was independently associated with clinical and biochemical hyperandrogenism and prediabetes. Of note, PCOS women with a positive FHx of both T2DM and PCOS had the highest prevalence of metabolic disturbances and hyperandrogenism.

Our results indicate that FHx of T2DM and PCOS have a different influence on PCOS phenotype. FHx of T2DM was independently associated with obesity, central fat accumulation, MS, IR and prediabetes, dyslipidaemia and arterial hypertension. In contrast, PCOS FHx was only related to prediabetes but showed no significant independent association with other metabolic parameters. These findings might have several implications for clinical practice as well as for future research. First and clinically most important, the simple assessment whether a relative is affected by T2DM or not might allow risk stratification of PCOS women. It might help identify PCOS women at high metabolic risk in whom further evaluation including a regular follow-up as well as intensified treatment are indicated. Second, a positive FHx of T2DM might identify a distinct subgroup of PCOS women affected by central fat

Figure 1

OR with 95% CI for obesity, central fat accumulation, insulin resistance, prediabetes and MS in PCOS women stratified by positive FHx of T2DM and PCOS. Binary logistic regression analyses using all-factors models with obesity, prediabetes and MS as dependent variables adjusted for age and testosterone (negative FHx of both diseases was defined as reference). Negative FHx: $n=336$; positive FHx of T2DM or PCOS: $n=226$; positive FHx of T2DM and PCOS: $n=71$. OR, odds ratio; MS, metabolic syndrome; PCOS, polycystic ovary syndrome; FHx, family history; T2DM, type 2 diabetes mellitus; LAP, lipid accumulation product. (A) OR with 95% CI for obesity depending on a positive FHx of T2DM and PCOS. (B) OR with 95% CI for elevated LAP depending on a positive FHx of T2DM and PCOS. (C) OR with 95% CI for insulin resistance depending on a positive FHx of T2DM and PCOS. (D) OR with 95% CI for prediabetes depending on a positive FHx of T2DM and PCOS. (E) OR with 95% CI for MS depending on a positive FHx of T2DM and PCOS.

Table 6 Metabolic characteristics of non-obese and obese PCOS women. Analyses were carried out using χ^2 test.

	Non-obese PCOS women ($n=533$)	Obese PCOS women ($n=181$)	*P* value
LAP elevated (%)	6.6	70.9	<0.001
VAI elevated (%)	11.4	56.6	<0.001
Prediabetes (%)	8.2	25.7	<0.001
T2DM (%)	0.4	4.5	<0.001
MS (%)	2.7	41.3	<0.001
Insulin resistance (%)	9.7	60.0	<0.001
TG elevated (%)	5.8	24.5	<0.001
HDL low (%)	10.3	45.9	<0.001
Elevated BP (%)	27.3	66.2	<0.001

PCOS, polycystic ovary syndrome; LAP, lipid accumulation product; VAI, visceral adiposity index; T2DM, type 2 diabetes mellitus; MS, metabolic syndrome; TG, triglycerides; BP, blood pressure.

accumulation and metabolic disturbances. Those women differ from women without a positive FHx of T2DM, which might be related to either genetic or environmental factors. Of note, it has been shown that T2DM-related genetic variants are also associated with PCOS phenotype and risk (3, 15). To our knowledge, it has not been investigated so far whether PCOS women with a positive FHx of T2DM have a higher prevalence of T2DM-related genetic variants than PCOS women with a negative T2DM FHx. Thus, future genetic studies on PCOS phenotype should also analyse the association of positive T2DM FHx with those genetic variants. We found an about 1.7-fold increased risk of prediabetes and insulin resistance and twofold increased risk of MS and dyslipidaemia in PCOS women with a positive FHx of T2DM. Thus, our findings are consistent with a genetic basis for metabolic disturbances in PCOS, which has been suggested previously (15, 25, 26). Interestingly, PCOS women with a positive T2DM FHx were more likely to have a positive PCOS FHx suggesting a common genetic background of both diseases. The hypothesis of a strong genetic background of PCOS and T2DM is supported by a study among 200 parents of PCOS women and 120 parents of healthy young women showing that parents of PCOS women are more insulin resistant and have a higher prevalence of T2DM (27). Similarly, a higher prevalence of T2DM and insulin resistance has been found in 102 first-degree relatives of PCOS women compared with 82 healthy controls without PCOS FHx (28).

Prediabetes and MS were prevalent in 16.9 and 16.4% of women with a positive FHx of T2DM respectively, whereas only 10.4 and 8.6% of PCOS women without FHx of T2DM presented with prediabetes and MS respectively. Legro *et al*. (12) observed a borderline significant difference in disturbed glucose metabolism that was prevalent in 52.6% of PCOS women with a FHx of T2DM and in 34% of PCOS women with negative FHx (among 254 women with PCOS). The study was conducted in the US and the obesity prevalence was much higher (mean BMI 35.9 kg/m^2) than in the present study among Austrian PCOS women with a median of BMI 24.2 kg/m^2. Further, Ehrmann *et al*. (13) found a 2.6-fold higher prevalence of first-degree relatives with T2DM in 12 PCOS women with T2DM compared with 67 PCOS women with normal glucose tolerance (83 vs 31%, $P<0.01$). That study again differed from our cohort with respect to BMI (mean BMI 33.4, 36.9 and 41.0 kg/m^2 for women with normal glucose tolerance, IGT and T2DM respectively) (13). A larger study including 408 women with PCOS found a positive FHx of T2DM in 44% of the 16 diabetic PCOS women, 39% of the 94 women with impaired glucose tolerance and 21% of the 298 women with normal glucose tolerance ($P<0.01$) (14). Conversely, the prevalence of IGT and T2DM was significantly higher in PCOS women with a positive FHx of T2DM, which is in line with the findings of our study. Again, that study was conducted in the US and included women with a mean BMI of 36.2 kg/m^2 (14).

Interestingly, Ehrmann *et al*. (29) found that a positive FHx of T2DM in first-degree relatives defines a subset of PCOS women with a greater prevalence of insulin secretory defects that might be associated with an increased risk for developing T2DM in later life. Similarly, T2DM FHx has been shown to be associated with insulin resistance in 112 PCOS women from Spain (30) as well as in 37 Indian PCOS women (31). This notion is supported by our finding suggesting an independent association of T2DM FHx with insulin resistance in PCOS women.

Our results showing an increased metabolic risk of PCOS women with a positive FHx of both T2DM and PCOS are of high clinical impact. The prevalence of MS in that subgroup was 18%, 26% presented with prediabetes, 35% had central fat accumulation and 38% were obese. In clinical routine the simple question whether relatives are affected by T2DM and/or PCOS might be a useful screening tool to identify PCOS women at high metabolic risk. In our opinion, a 2-h OGTT should be carried out in all women with PCOS (32), which has also been suggested by the new Endocrine Society Clinical Practice Guideline (33). However, there might be situations when this is not possible and this time-consuming test is available only in a subgroup of PCOS women. Given the fact that PCOS has a

very high prevalence, there is a need for simple and cheap screening tools to identify PCOS women at high metabolic risk. Those women should receive adequate diagnostic testing such as 2-h OGTT and intensive treatment such as lifestyle counselling and insulin-sensitising drugs. Of note, the recent Androgen Excess Society statement suggests that 2-h OGTT should be carried out in all PCOS women with a positive FHx of T2DM, recognising the importance of positive FHx as a risk factor for the development of disturbed glucose metabolism (23).

PCOS and the MS appear to be interrelated, although they are distinct entities. Women with PCOS are commonly affected by MS, while women with MS may display reproductive or endocrine features of PCOS. Thus, one might speculate on a mutual pathophysiologic relationship and potentially significant clinical sequelae. This relationship seems, however, to be largely mediated via obesity as only 2.7% of non-obese, but 41.3% of obese PCOS women in our study were affected by the MS. This is in accordance with relatively lean PCOS cohorts from Southern Europe showing a prevalence of MS between 8 and 25% (34, 35). In contrast, the estimated prevalence of the MS in the USA is 33–47% and 13.7 times more likely in women with PCOS in the highest vs the lowest BMI (23, 36). This notion is supported by a study among 469 South Asian PCOS women with increasing MS prevalence from the lowest (6.7%) to highest (42.0%) quartiles of BMI and a relatively high overall prevalence of 36%. Further, central obesity and visceral fat accumulation have been associated as key factors in the development of the MS, and the prevalence of the MS is low if there is no excessive abdominal adiposity (8, 9). Moreover, non-alcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of MS and it has been shown that 89% of PCOS women with MS have elevated fatty liver index indicating NAFLD (24). Besides obesity and insulin resistance, an inflammatory atherothrombotic state with elevated proinflammatory substances (such as C-reactive protein, fibrinogen, white blood cells, plasminogen activator inhibitor-1, and endothelin-1) has also been suggested in MS, which impair endothelial function, reduce vasoreactivity and promote subclinical atherosclerosis (23). Interestingly, it has also been demonstrated that genetic variants involved in atherothrombotic diseases and blood coagulation, such as the F13A1 gene, are associated with the metabolic phenotype in PCOS (37). Thus, a common genetic background of PCOS and the MS might be related to obesity as well as to other factors such as an atherothrombotic state.

We found no significant association of T2DM FHx with metabolic or endocrine parameters in control women. This might be related to the smaller sample size or to the fact that the prevalence of T2DM FHx was lower in healthy women (16.2 vs 36.8%). Further, the prevalence of metabolic disturbances is low in those healthy young women (24). Considering the lack of significance one might also speculate that the impact of T2DM FHx is larger in PCOS compared with BMI-matched women without hyperandrogenaemia.

Considering the higher prevalence of obesity in PCOS women with T2DM FHx, we tried to explore whether our findings are related to a genetic background or rather to lifestyle factors following a non-genetic model. The attenuation of some of our results in subgroup analyses of obese and non-obese women is in favour of a non-genetic model. This weakening might, however, also be related to the smaller sample size and the low prevalence of metabolic disturbances in non-obese women. Interestingly, most associations of PCOS FHx and metabolic and endocrine parameters remained stable in obese and non-obese women, suggesting that this relationship is mediated via genetic rather than lifestyle factors. Further, as some of our results remained significant and due to the fact that we found no significant association in healthy women, one should also consider the possibility of an important impact of FHx and therefore genetics in PCOS women. Further, the assumption of a common genetic background of PCOS and T2DM is strengthened by our results showing that both obese as well as non-obese PCOS women with a positive T2DM FHx are more likely to have a positive PCOS FHx.

Interestingly, a positive FHx of PCOS was closely related to biochemical and clinical hyperandrogenism in our PCOS cohort. In contrast, when analysing metabolic parameters, the only independent association was found with prediabetes. Thus, our findings suggest that women with a positive FHx of PCOS might constitute a distinct subgroup of PCOS women with an increased risk of hyperandrogenism but a relatively low risk of insulin resistance or MS. Thus, future genetic studies should also focus on the relationship of hyperandrogenism-related genotypes with PCOS FHx.

One limitation of our study is the fact that we assessed FHx of T2DM and PCOS via questionnaires, but we did not obtain information via medical records of family members. Further, relatives of PCOS women were not tested individually. Thus, we cannot exclude under- or over-reporting of PCOS or T2DM and one might consider a recall bias. As the diagnosis of PCOS might have been missed in mothers of PCOS women at their premenopausal age, it is likely that the prevalence of positive PCOS

FHx is underestimated in the present study. However, as a clear definition of postmenopausal PCOS is missing, we are unable to make a definite diagnosis of PCOS in most mothers of the present PCOS population. Further, the use of patient recall rather than direct testing of family members is a common approach in studies assessing FHx and this is especially true for studies including a large sample size.

Nevertheless, it is possible that the use of questionnaires to assess FHx has a negative impact on the reliability of our findings. It is worth mentioning that our definition of a positive FHx of T2DM was not only restricted to first-degree relatives but also included grandparents. Thus, our results might differ from other studies among PCOS cohorts that only included first-degree relatives. Further, the increased risk of metabolic disturbances might be even more pronounced when only first-degree relatives with a positive PCOS FHx are included. As PCOS was diagnosed according to the Rotterdam criteria, our findings might differ from other PCOS cohorts diagnosed by different criteria (AES or NIH) that might present with a more severe metabolic phenotype (38, 39). Of note, a more favourable metabolic profile has been suggested in non-hyperandrogenic PCOS women (23). Thus, our results are restricted to PCOS women fulfilling the Rotterdam criteria. Nevertheless, previous results from our PCOS cohort have demonstrated that the prevalence of metabolic disturbances including prediabetes and T2DM is similar in PCOS women with and without hyperandrogenism (32). Likewise, the prevalence of the MS and its components has been shown to be similar in PCOS women fulfilling Rotterdam criteria and AES criteria (1). Further, the Endocrine Society has recently suggested in their clinical practice guidelines to use the Rotterdam criteria for diagnosing PCOS (33). Another limitation is the fact that we have no information on PCOS FHx in control women. Moreover, we did not use the hyperinsulinaemic, euglycaemic glucose clamp technique, which is considered gold standard when assessing insulin resistance and sensitivity *in vivo* (40). It is, however, difficult to perform this time-consuming expensive clamp technique in large studies. Strengths of our study include the large sample size and the performance of an OGTT with measurements of glucose and insulin concentrations in all women. We were therefore able to calculate ISI (Matsuda) that correlates reasonably well with the estimates of whole-body insulin sensitivity determined by the glucose clamp and has recently been validated using glucose clamp studies (20).

In summary, we present evidence that PCOS women with a positive T2DM FHx have an adverse metabolic profile, whereas PCOS women with a positive PCOS FHx have increased prevalence of clinical and biochemical hyperandrogenism. PCOS women with a positive FHx of both T2DM and PCOS had the highest prevalence of metabolic and endocrine disturbances. Our findings suggest that the assessment of FHx might allow risk stratification of PCOS women, which is important considering the high prevalence of PCOS. Future studies on the genetic background of PCOS might focus on FHx of PCOS and T2DM.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

E Lerchbaum contributed to study conception and design, acquisition and analysis of data, and drafting of article; V Schwetz contributed to acquisition of data and revising the article for important intellectual content; A Giuliani contributed to acquisition of data and revising the article for important intellectual content; B Obermayer-Pietsch contributed to conception, design, acquisition of data and drafting the article. All authors approved the final version of the manuscript.

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