

Prevalence of Polycystic Ovary Syndrome in Women from Opposite-Sex Twin Pairs

Esther A. M. Kuijper, Jacqueline M. Vink, Cornelis B. Lambalk, and Dorret I. Boomsma

Division of Reproductive Medicine (E.A.M.K., C.B.L.), Department of Obstetrics and Gynaecology, VU University Medical Center, 1007 MB Amsterdam, The Netherlands; and Department of Biological Psychology (J.M.V., D.I.B.), VU University, 1081 BT Amsterdam, The Netherlands

Introduction: Intrauterine androgens of a male fetus may influence the female fetus in opposite-sex twin pairs. Because female intrauterine overexposure to androgens could lead to polycystic ovary syndrome (PCOS), the prevalence of PCOS should be higher in women from opposite-sex twin pairs. Therefore, the aim of the current study was to evaluate the prevalence of PCOS in women from opposite-sex twin pairs compared to women from same-sex twin pairs, sisters, and female spouses of twins.

Subjects and Methods: Data from 1325 monozygotic twins, 1191 dizygotic twins (711 women from same-sex twin pairs and 480 women from opposite-sex twin pairs), 745 sisters of twins, and 218 spouses of male twins were evaluated. PCOS was defined as less than nine natural menstrual cycles a year combined with either hirsutism or acne. The prevalence of PCOS was compared using a χ^2 test. Binary logistic regression analyses were conducted to test for confounding effects of smoking, age, and body mass index.

Results: No significant differences in PCOS prevalence were found between women from same-sex twin pairs (either monozygotic or dizygotic), opposite-sex twin pairs, sisters, and spouses.

Conclusion: The prevalence of PCOS is not different in women from opposite-sex and same-sex twin pairs, singleton sisters, or spouses. This indicates that possible androgen exposure of the female fetus, caused by a shared intrauterine environment with a male fetus, does not result in PCOS-like traits. (*J Clin Endocrinol Metab* 94: 1987–1990, 2009)

There are some indicators that intrauterine androgens of a male fetus may influence females of opposite-sex twin pairs. Animal studies show that sex hormones can diffuse through the fetal membranes and the amniotic fluid and therefore are able to influence the developing fetus. Studies in mammals show even permanently altered hormone levels, reproductive organs, aggressive behavior, and susceptibility to endocrine disruption within females who were positioned between males *in utero*. This intrauterine effect is attributable to the transfer of testosterone from male fetuses to adjacent female fetuses (1–3). It has been shown that intrauterine overexposure to androgens in the nonhuman primate leads to female offspring with polycystic ovary syndrome (PCOS)-like traits (2, 4).

In humans, females of an opposite-sex twin pair had fewer offspring and therefore a reduced lifetime fecundity in a histor-

ical data set collected from twins born in Finland between 1734 and 1888. This study suggested that females ($n = 31$) born as part of an opposite-sex twin pair were 25% less likely to reproduce than female twins ($n = 35$) born as part of a same-sex twin pair (5). Acquisition of testosterone from the male co-twin was suggested as a possible cause. However, Medland *et al.* (6) showed that in modern populations from Australia, The Netherlands, and the United States there were no reproduction differences among female twins from same-sex ($n = 1979$) and opposite-sex ($n = 913$) dizygotic (DZ) pairs. In all three samples, there were no differences in the number of children, age of first pregnancies, or psychological femininity between women from same-sex or opposite-sex twin pairs (6).

If women of opposite-sex twin pairs have reduced fecundity through androgen excess produced by the male co-twin and such

overexposure causes PCOS-like symptoms, then a higher prevalence of PCOS among the females of opposite-sex twin pairs is expected.

PCOS is defined, according to the Rotterdam criteria, by at least two of the following criteria: oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism (defined as acne/hirsutism or elevated serum androgen levels), or polycystic ovaries on ultrasound (defined as a volume >10 cc or >12 follicles smaller than 10 mm in both ovaries) (7). For this study, data on oligo- or anovulation, acne, and hirsutism were obtained by self-report.

The aim of the current study was to evaluate the prevalence of PCOS in women of an opposite-sex twin cohort compared with females from same-sex twin pairs, singleton sisters, and biologically unrelated women (spouses of male twins).

Subjects and Methods

Subjects

This study is part of an ongoing twin family study on health-related behavior in participants of The Netherlands Twin Register (8, 9). For this study, data of the 2000 survey were used ($n = 4228$). Subjects were excluded when sufficient data on menstrual cycle, acne, or hirsutism were missing ($n = 749$). A total of 1325 female monozygotic (MZ) twins, 1191 DZ twins (711 women from same-sex twin pairs and 480 women from opposite-sex twin pairs), 745 sisters of twins, and 218 spouses of male twins were included in the study. Twin zygosity was based on (longitudinal) questionnaire data or, when available, on DNA typing. Agreement between zygosity based on questionnaire data and zygosity based on DNA results is 96%. PCOS was defined as oligomenorrhea combined with hyperandrogenism (10). Information about cycle irregularity, excessive hair growth, and acne was obtained from the following questions: 1) What was the number of menstrual cycles per year when not using contraception? (Answer categories: nine or more, less than nine, less than six, two or less); 2) Do you suffer from increased hair growth? (Answer categories: yes, no); and 3) Do you suffer from acne/pimples? (Answer categories: yes, no).

Furthermore, the survey provided information on date of birth, height, weight, and smoking habits. Group characteristics are listed in Table 1.

Statistical analyses

We used χ^2 tests to compare the prevalence of PCOS between female MZ twins, female DZ twins, females from opposite-sex twin pairs, sisters

TABLE 1. The characteristics of the total and random study population

	PCOS	Non-PCOS	P^a
Total population (n)	98	3381	
Age (yr)	27.1 (9.1)	30.0 (9.8)	0.005
BMI (kg/m ²)	23.0 (4.8)	22.8 (3.7)	0.645
Smoking status			
Yes	38	1435	
No	60	1944	0.466
Random selection (n)	65	2278	
Age (yr)	25.7 (8.5)	29.7 (9.6)	0.001
BMI (kg/m ²)	22.1 (4.2)	22.8 (3.7)	0.119
Smoking status			
Yes	21	972	
No	44	1305	0.095

^a Student's t test for age and BMI or χ^2 test for smoking status.

TABLE 2. The prevalence of PCOS within the different twins, their sisters and spouses

	PCOS	Non-PCOS	% PCOS
MZ twins	36	1289	2.7
Female DZ twins	21	690	3.0
Females of an opposite sex twin	13	467	2.7
Sisters of a twin	23	722	3.1
Spouses of a twin	5	213	2.3
Total	98	3381	2.9

PCOS is defined as less than nine menstruations per year and acne or hirsutism.

of twins, and spouses of male twins. In addition, binary logistic regression analyses were used to test for confounding effects of body mass index (BMI), age, and smoking initiation. BMI data were divided into three categories: less than 20, 20–25, and more than 25 kg/m². Age was put into the model as a categorical variable, with two groups: less than 30 yr and 30–45 yr. Women over 45 yr of age were excluded from the analyses ($n = 324$). From the total sample of 3479, this resulted in a sample used in these analyses of 3155 [MZ = 1176, DZ = 647, females of a dizygotic opposite-sex twin pair (DOS) = 454, sisters of a twin = 664, and spouses = 214]. Smoking initiation was a dichotomous variable with two categories: yes, 1; or no, 0. Because data from twins and singleton sisters are not independent, all analyses have also been done using a random selection of just one person per family. This resulted in a total sample of 2343 subjects (MZ females = 783, DZ females = 449, DOS females = 427, sisters of a twin = 466, spouses = 218); after age selection, a sample of 2151 remained for these analyses (MZ = 686, DZ = 406, DOS = 425, sisters of a twin = 420, spouses = 214).

Results

Characteristics of the study population are described in Table 1. Mean age in the PCOS group was 2 yr lower than in the non-PCOS group. BMI and smoking status did not differ between groups.

The prevalence of PCOS in twins (MZ, DZ, and DOS), their sisters, and spouses of twins is reported in Table 2. A χ^2 test ($P = 0.970$; $df = 4$) showed that the PCOS prevalence between the groups did not differ significantly. When comparing only women from MZ, DZ, and DOS twin pairs, these results are virtually the same (χ^2 : $P = 0.948$; $df = 2$), indicating that there are no significant differences in PCOS prevalence between these groups. Analyses done with one random person per family showed almost the same results (χ^2 : $P = 0.943$, $df = 4$). A χ^2 test for six or less or two or less menstruations per year in DOS, MZ, DZ female twins, spouses of twins, and sisters of twins showed no significant differences between these groups ($P = 0.278$; $df = 4$), indicating no differences in the prevalence of severe oligomenorrhea or amenorrhea between DOS twins and the other groups.

The association between PCOS and being a female from an opposite-sex twin pair was tested in a binary logistic regression model with and without BMI, age, and smoking initiation as possible confounders (Table 3). Results, both in the total and in the random sample, indicate that being a female of an opposite-sex twin pair is not associated with PCOS. These results are not altered by correcting for BMI, age, or smoking status.

TABLE 3. Results of the binary logistic regression analyses

	Total population (n = 3479)			Random selection (n = 2343)		
	β	OR	CI	β	OR	CI
Model 1						
Females from an opposite-sex twin pair	-0.047	0.954	0.528–1.725	0.016	1.016	0.538–1.919
Model 2						
Females from an opposite-sex twin pair	-0.037	0.963	0.531–1.750	0.014	1.014	0.534–1.926
BMI group 1	-0.056	0.946	0.557–1.605	0.027	1.027	0.549–1.926
BMI group 2	0.120	1.128	0.595–2.140	-0.201	0.818	0.353–1.892
Age <30 yr	0.486	1.626	0.683–3.873	1.050	2.858	0.677–12.068
Age 30–45 yr	0.200	1.221	0.489–3.052	0.710	2.034	0.453–9.133
Smoking initiation	-0.046	0.955	0.621–1.467	-0.278	0.757	0.442–1.297

Table 3 shows the results of the binary logistic regression analyses with PCOS as the dependant variable and being a female from an opposite-sex twin pair as the primary determinant. Model 1 indicates the crude model, model 2 is after correction for possible confounders (BMI, age, and smoking initiation). BMI is divided into three groups (<20, 20–25, and >25 kg/m²). Group 1 is used as reference. Age groups are: 1) <30 yr, and 2) between 30 and 45 yr. Smoking initiation: yes, 1; no, 0. Results are shown for both the total population and a random selection of one person per family. OR, Odds ratio; CI, confidence interval.

Discussion

According to our observations, the prevalence of PCOS does not differ significantly between women from opposite-sex or same-sex twin pairs, singleton sisters of twins, and biologically unrelated women (spouses of male twins). This indicates that intrauterine hormonal environmental conditions probably do not strongly contribute to the development of PCOS, provided that there are differences between women from opposite-sex and dizygotic same-sex twin pairs in their uterine environment. With regard to the latter, solid human data are not available except for some demographic findings that suggest differences in androgen exposure (11).

If intrauterine exposure to androgens contributes to the development of PCOS, these androgens are more likely to come from the female fetus itself rather than from the male co-twin. This is compatible with the finding that PCOS is highly heritable as shown in Dutch twin families (10). Furthermore, there is accumulating evidence for a genetic basis of PCOS (12–19). Because offspring of PCOS mothers share some of the maternal traits, another possible source of androgens is the mother, but maternal androgen excess is unlikely to affect the fetus because excessive placental aromatase activity presents as an effective barrier (20). Recent studies show that hyperandrogenism in women who develop PCOS may be due to polymorphisms in the SHBG and androgen receptor genes (21). This underlines again the genetic component that seems most important in developing PCOS.

The prevalence of PCOS in the present study is rather low compared with other countries where 6–10% is reported (22–27). Nevertheless, it is in accordance with another study reporting a PCOS prevalence in The Netherlands of around 3–4% for oligomenorrhea combined with hyperandrogenism (28).

This difference in prevalence between studies may be due to characterizing the PCOS phenotype. According to the Rotterdam criteria, there are three possible polycystic ovary phenotypes: 1) oligomenorrhea and hyperandrogenism; 2) oligomenorrhea and PCOS ovaries; and 3) hyperandrogenism and PCOS ovaries (7). For this study, we have chosen the first phenotype to represent a PCOS female because ultrasound data have been shown earlier to be nonspecific, with PCOS ovaries present in as many as 20–25% of the general population (29). Furthermore,

this phenotype is shown to be highly heritable (10), and in our sample reliable ultrasound data were not available because data were collected with a mailed questionnaire. One could argue that PCOS might be difficult to diagnose with self-report data on acne, hirsutism, and oligo- or amenorrhea, but studies by Taponen *et al.* (30, 31) showed that the prevalence of PCOS was as high as 70% in women reporting both oligo- or amenorrhea and hirsutism. Furthermore, the combination of these symptoms could reliably identify women with typical PCOS endocrine profiles (30, 31).

Age might be a possible confounder in this study because PCOS women were significantly younger than non-PCOS women. However, the absolute difference was less than 3 yr. Regression analyses showed no difference in PCOS prevalence between the different types of twins, their sisters, and spouses, before and after correction for age. Although the mean BMI was normal in both groups, there were obese subjects in the study population, and therefore analyses were done with and without correction for BMI. We have shown that the PCOS prevalence in our population is independent of BMI.

We conclude that there are no indications that the prevalence of PCOS is higher in females from opposite-sex twin pairs compared with women from same-sex twin pairs, a singleton sister of a twin, and biologically unrelated women (spouses of male twins). This may indicate that possible androgen overexposure of the female fetus, caused by a shared intrauterine environment with a male fetus, does not result in PCOS-like traits.

Acknowledgments

Address all correspondence and requests for reprints to: C. B. Lambalk, Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, VU University Medical Center, 1007 MB Amsterdam, The Netherlands. E-mail: cb.lambalk@vumc.nl.

This work was supported by The Netherlands Organization for Scientific Research (NWO) Grant 985-10-002). J.M.V. is financially supported by NWO (VENI 451-06-004). E.A.M.K. was supported by the Stichting Wetenschappelijk Onderzoek Gynaecologie.

Disclosure Summary: E.A.M.K. and C.B.L. have nothing to disclose. J.M.V. and D.I.B. received a NWO research grant for their project (985-10-002).

References

- Ryan BC, Vandenberg JG 2002 Intrauterine position effects. *Neurosci Biobehav Rev* 26:665–678
- Abbott DH, Padmanabhan V, Dumesic DA 2006 Contributions of androgen and estrogen to fetal programming of ovarian dysfunction. *Reprod Biol Endocrinol* 4:17
- Cohen-Bendahan CC, van de Beek C, Berenbaum SA 2005 Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. *Neurosci Biobehav Rev* 29:353–384
- Abbott DH, Barnett DK, Levine JE, Padmanabhan V, Dumesic DA, Jacoris S, Tarantal AF 2008 Endocrine antecedents of polycystic ovary syndrome in fetal and infant prenatally androgenized female rhesus monkeys. *Biol Reprod* 79:154–163
- Lummaa V, Pettay JE, Russell AF 2007 Male twins reduce fitness of female co-twins in humans. *Proc Natl Acad Sci USA* 104:10915–10920
- Medland SE, Loehlin JC, Willemsen G, Hatemi PK, Keller MC, Boomsma DI, Eaves LJ, Martin NG 2008 Males do not reduce the fitness of their female co-twins in contemporary samples. *Twin Res Hum Genet* 11:481–487
- 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 81:19–25
- Boomsma DI, Vink JM, van Beijsterveldt TC, de Geus EJ, Beem AL, Mulder EJ, Derks EM, Riese H, Willemsen GA, Bartels M, van den Berg M, Kupper NH, Polderman TJ, Posthuma D, Rietveld MJ, Stubbe JH, Knol LI, Stroet T, van Baal GC 2002 Netherlands Twin Register: a focus on longitudinal research. *Twin Res* 5:401–406
- Boomsma DI, de Geus EJ, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TC, Hudziak JJ, Bartels M, Willemsen G 2006 Netherlands Twin Register: from twins to twin families. *Twin Res Hum Genet* 9:849–857
- Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI 2006 Heritability of polycystic ovary syndrome in a Dutch twin-family study. *J Clin Endocrinol Metab* 91:2100–2104
- Cohen-Bendahan CC, van Goozen SH, Buitelaar JK, Cohen-Kettenis PT 2005 Maternal serum steroid levels are unrelated to fetal sex: a study in twin pregnancies. *Twin Res Hum Genet* 8:173–177
- Recabarren SE, Smith R, Rios R, Maliqueo M, Echiburú B, Codner E, Cassorla F, Rojas P, Sir-Petermann T 2008 Metabolic profile in sons of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 93:1820–1826
- Legro RS, Spielman R, Urbanek M, Driscoll D, Strauss 3rd JF, Dunaif A 1998 Phenotype and genotype in polycystic ovary syndrome. *Recent Prog Horm Res* 53:217–256
- Legro RS, Driscoll D, Strauss 3rd JF, Fox J, Dunaif A 1998 Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *Proc Natl Acad Sci USA* 95:14956–14960
- Franks S, McCarthy MI, Hardy K 2006 Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl* 29:278–285; discussion, 286–290
- Xita N, Tsatsoulis A 2006 Review: fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metab* 91:1660–1666
- Jahanfar S, Eden JA, Warren P, Seppälä M, Nguyen TV 1995 A twin study of polycystic ovary syndrome. *Fertil Steril* 63:478–486
- Amato P, Simpson JL 2004 The genetics of polycystic ovary syndrome. *Best Pract Res Clin Obstet Gynaecol* 18:707–718
- Franks S, Webber LJ, Goh M, Valentine A, White DM, Conway GS, Wiltshire S, McCarthy MI 2008 Ovarian morphology is a marker of heritable biochemical traits in sisters with polycystic ovaries. *J Clin Endocrinol Metab* 93:3396–3402
- Hensleigh PA, Carter RP, Grotjan Jr HE 1975 Fetal protection against masculinization with hyperreactio luteinalis and virilization. *J Clin Endocrinol Metab* 40:816–823
- Xita N, Georgiou I, Lazaros L, Psofaki V, Kolios G, Tsatsoulis A 2008 The role of sex hormone-binding globulin and androgen receptor gene variants in the development of polycystic ovary syndrome. *Hum Reprod* 23:693–698
- Asunción M, Calvo RM, San Millán JL, Sancho J, Avila S, Escobar-Morreale HF 2000 A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J Clin Endocrinol Metab* 85:2434–2438
- Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO 2004 The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab* 89:2745–2749
- Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zappanti ED, Bartzis MI 1999 A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *J Clin Endocrinol Metab* 84:4006–4011
- Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R 1998 Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *J Clin Endocrinol Metab* 83:3078–3082
- Kumarapeli V, Seneviratne Rde A, Wijayaratne CN, Yapa RM, Dodampahala SH 2008 A simple screening approach for assessing community prevalence and phenotype of polycystic ovary syndrome in a semi-urban population in Sri Lanka. *Am J Epidemiol* 168:321–328
- Archer JS, Chang RJ 2004 Hirsutism and acne in polycystic ovary syndrome. *Best Pract Res Clin Obstet Gynaecol* 18:737–754
- van Hooff MH, Voorhorst FJ, Kaptein MB, Hirasings RA, Koppelaar C, Schoemaker J 1998 Relationship of the menstrual cycle pattern in 14–17 year old adolescents with gynaecological age, body mass index, and historical parameters. *Hum Reprod* 13:2252–2260
- Carmina E, Azziz R 2006 Diagnosis, phenotype, and prevalence of polycystic ovary syndrome. *Fertil Steril* 86(Suppl 1):S7–S8
- Taponen S, Ahonkallio S, Martikainen H, Koivunen R, Ruokonen A, Sovio U, Hartikainen AL, Pouta A, Laitinen J, King V, Franks S, McCarthy MI, Järvelin MR 2004 Prevalence of polycystic ovaries in women with self-reported symptoms of oligomenorrhoea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. *Hum Reprod* 19:1083–1088
- Taponen S, Martikainen H, Järvelin MR, Sovio U, Laitinen J, Pouta A, Hartikainen AL, McCarthy MI, Franks S, Paldanius M, Ruokonen A 2004 Metabolic cardiovascular disease risk factors in women with self-reported symptoms of oligomenorrhoea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. *J Clin Endocrinol Metab* 89:2114–2118