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ORIGINAL ARTICLE ***Reproductive endocrinology***

Gene variants associated with age at menopause are also associated with polycystic ovary syndrome, gonadotrophins and ovarian volume

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STUDY QUESTION: Is there a relationship between the genetic risk for polycystic ovary syndrome (PCOS) and genetic variants that influence timing of menopause?

SUMMARY ANSWER: The genetic risk score, which sums the contribution of variants at all menopause loci, was associated with PCOS.

WHAT IS ALREADY KNOWN: Ovarian parameters and anti-Mullerian hormone levels suggest that women with PCOS should have a later age at menopause.

STUDY DESIGN, SIZE, DURATION: The study was a case–control examination of genetic variants associated with age at menopause in a discovery cohort of women with PCOS ($n = 485$) and controls ($n = 407$) from Boston recruited from 2003 to 2012. Replication was performed in women from Greece (cases, $n = 884$ and controls, $n = 311$).

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: PCOS was defined by the National Institutes of Health criteria in Boston and Greece ($n = 783$), with additional subjects fulfilling the Rotterdam criteria (hyperandrogenism, polycystic ovary morphology and regular menses) in Greece ($n = 101$). Controls in Boston and Greece had regular menstrual cycles and no hyperandrogenism. Allele frequencies for variants previously associated with age at menopause were examined in PCOS cases and controls, along with the relationship to quantitative traits.

MAIN RESULTS AND ROLE OF CHANCE: The variant rs11668344-G was associated with decreased risk of PCOS (odds ratio: 0.77 [0.59–0.93]; $P = 0.004$). There was a strong relationship between the late menopause allele rs12294104-T and increased LH levels ($\beta \pm$ SE; 0.26 ± 0.06; $P = 5.2 \times 10^{-5}$) and the LH:FSH ratio (0.28 ± 0.06; $P = 2.7 \times 10^{-6}$). The minor allele at rs10852344-T was associated with smaller ovarian volume (-0.16 ± 0.05; $P = 0.0012$). A genetic risk score calculated from 16 independent variants associated with age at menopause was also associated with PCOS ($P < 0.02$), LH and the LH:FSH ratio (both $P < 0.05$).

LIMITATIONS, REASONS FOR CAUTION: The variant rs11668344 was not associated with PCOS in the Greek cohort, but results exhibited the same direction of effect as the Boston cohort. However, it is possible that the individual association was a false positive in the Boston cohort.

WIDER IMPLICATIONS OF THE FINDINGS: The study demonstrates that gene variants known to influence age at menopause are also associated with risk for PCOS. Further, our data suggest that the relationship between age at menopause and PCOS may be explained, at least in part, by effects on LH levels and follicle number. The data point to opposing influences of the genetic variants on both menopausal age and PCOS.

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Key words: ultrasound / ovarian volume / LH / genetic variant

Introduction

Polycystic ovary syndrome (PCOS) is a common disorder, present in up to 10% of women of reproductive age, despite its adverse impact on health. It is possible that PCOS has a high prevalence in the population based on a selective advantage conferred by a longer reproductive lifespan. In women with PCOS, the greater number of follicles and the attenuated fall in ovarian volume across reproductive aging in both cross-sectional and longitudinal studies (Alsamarai *et al.*, 2009) suggest a prolonged reproductive lifespan. Similarly, anti-Mullerian hormone levels, a marker of antral follicle number (de Vet *et al.*, 2002; Pigny *et al.*, 2003), exhibit a less pronounced longitudinal decrease across aging in women with PCOS (Mulders *et al.*, 2004). One small longitudinal study suggests that women with PCOS have a later menopause (Schmidt *et al.*, 2011). Taken together, the data suggest that ovarian aging in women with PCOS is delayed compared with that in control women.

Recent genome-wide association studies (GWAS) in large populations have identified common genetic variants associated with age at menopause (He *et al.*, 2009; Stolk *et al.*, 2009; Stolk *et al.*, 2012). A large meta-analysis identified 13 loci and confirmed 4 previous loci associated with age at menopause that are located in regions encompassing genes associated with DNA repair and immune function, and may therefore be related to germline and somatic cell aging (Stolk *et al.*, 2012). Additional variants are located in or near genes expressed in the hypothalamus and ovary (He *et al.*, 2009), organs that are implicated in the etiology of PCOS (Rebar *et al.*, 1976; Waldstreicher *et al.*, 1988; Nelson *et al.*, 1999; Maciel *et al.*, 2004). Taken together, the possibility that women with PCOS have a later age at menopause and a greater number of ovarian follicles at all ages, and that variants associated with age at menopause have physiological roles in the target organs involved in the etiology of PCOS, raises the hypothesis that genetic variants associated with age at menopause are also associated with risk for PCOS. In addition, we hypothesized that variants associated with menopause would also be associated with ovarian parameters and gonadotrophin levels.

Methods

Subjects

Subjects in the discovery cohort from Boston were recruited from 2003 to 2012, were of European ethnicity, aged 18–45 years and diagnosed with PCOS according to the National Institutes of Health (NIH) criteria, i.e. irregular menses and clinical or biochemical hyperandrogenism ($n = 527$). Subjects with non-classic congenital adrenal hyperplasia, hypothyroidism, elevated prolactin levels, Cushing syndrome and primary ovarian insufficiency were excluded (Welt *et al.*, 2006). Control subjects ($n = 426$) consisted of women aged 18–45 years with regular menstrual cycles of between 21 and 35 days and no hyperandrogenism (Welt *et al.*, 2006).

Replication was performed in women from Greece with PCOS, defined by the Rotterdam criteria ($n = 884$). Of these subjects, 783 fulfilled the NIH criteria with irregular menses and clinical or biochemical hyperandrogenism (Georgopoulos *et al.*, 2013). The remaining 101 subjects fulfilled the Rotterdam criteria with clinical or biochemical hyperandrogenism, polycystic ovarian morphology and regular menstrual cycles. Controls ($n = 311$) had regular ovulation, serum progesterone levels >10 ng/ml in the luteal phase of the menstrual cycle, and no evidence of clinical or biochemical hyperandrogenism (Georgopoulos *et al.*, 2013).

The study was approved by the Institutional Review Boards at Partners Healthcare, the School of Medicine at the University of Patras and the Aristotle University of Thessaloniki. All subjects gave written informed consent.

Protocol

Boston PCOS subjects and Greek PCOS subjects underwent extensive phenotyping to examine the relationship between gene variants and quantitative traits, as previously described (Supplementary data, Table SI) (Welt *et al.*, 2012; Georgopoulos *et al.*, 2013). All Boston discovery PCOS subjects were studied ≥ 10 days after their last menstrual period to avoid the time period in which LH is suppressed after a progesterone rise (Taylor *et al.*, 1997) and after a 12 h fast (Welt *et al.*, 2006). Subjects underwent a detailed history; physical exam including measurement of waist circumference at the umbilicus and hip circumference at the widest diameter; a pelvic ultrasound (Phillips, 8 MHz convex array transducer); and blood samples were taken for measuring LH and FSH levels (Taylor *et al.*, 1997) and sex-steroid levels, as described previously (Welt *et al.*, 2006). Three blood samples were obtained at 10 min intervals and LH and FSH levels in these were averaged. Using data from blood samples collected every 10 min over 12 h in women with PCOS and ovulatory controls (Taylor *et al.*, 1997), the mean LH secretion from 12 h of frequent blood samples correlated well with the value obtained from the mean of three samples collected from 0800 to 0820 h ($r = 0.92$, $P < 0.01$) (Welt *et al.*, 2006). The volume of the ovary by ultrasound was calculated as length $\times$ width $\times$ height in centimeters multiplied by 0.5233. The maximum ovarian volume was analyzed in the absence of a dominant follicle >10 mm, a corpus luteum or a cyst (Rotterdam, 2004). Follicle number was counted in a single plane with the greatest number of follicles visible.

Genotyping

Variants associated with age at menopause were selected from previous GWASs of age at menopause, with an initial analysis performed in 2011 and new variants added in 2013 (Table I) (He *et al.*, 2009; Stolk *et al.*, 2009; Stolk *et al.*, 2012). In 2013, variants previously replicated in the Boston cohort were genotyped in the Greek cohort to determine whether genetic risk variants were similar to those in the original Boston cohort (Supplementary data, Table SII) (Welt *et al.*, 2012). Patient DNA was isolated from whole blood according to manufacturer's specifications (Qiagen, USA). Genotyping in Boston was performed using the OmniHumanExpress Bead Chip (Illumina, San Diego, CA, USA). Subjects were removed for inbreeding ($n = 16$) and for population stratification after analysis using Eigenstrat ($n = 60$), with some samples overlapping ($n = 15$) (Patterson *et al.*, 2006; Price *et al.*, 2006). Therefore, a total of 93.6% of the samples passed the quality control review. Only single nucleotide polymorphisms (SNPs) present at a frequency of $\geq 1\%$ in the population were included. Of these 951 117 SNPs, 940 474 (98.9%) passed quality control. SNPs removed included 3851 for a call rate of $<95\%$ or unmatched call rates in cases and controls, 6123 which demonstrated a batch effect ($P < 1 \times 10^{-6}$) and 1624 that departed from Hardy–Weinberg equilibrium ($P < 1 \times 10^{-6}$). Imputation was performed in the software package Impute 2

Table I **Odds ratios (OR) and *P*-values for polycystic ovary syndrome (PCOS) risk in women from Boston and Greece at 17 loci identified in genome-wide association studies for age at menopause.**

SNP	Chr	Location	Nearest gene	Anc/Der	Effect of Der allele on menopause	Cohort	freq-case	freq-ctls	OR (95% CI)	*P*-value	P-value$_{com}$	P-value$_{het}$	Imputation quality
rs1635501	1	242 040 775	*EXO1*	T/C	Early	Boston	0.515	0.515	1.00 (0.83–1.20)	0.79			0.894
rs2303369	2	27 715 416	*FNDC4*	C/T	Early	Boston	0.32	0.361	0.83 (0.68–1.01)	0.14			G
rs10183486	2	171 699 097	*TKL1*	C/T	Early	Boston	0.334	0.337	0.99 (0.81–1.20)	0.97			0.955
rs4693089	4	84 373 622	*HELQ*	G/A	Early	Boston	0.495	0.512	0.93 (0.78–1.12)	0.8			0.993
rs890835[a]	5	175 956 271	*RNF44*	C/A	Early	Boston	0.124	0.1	1.28 (0.94–1.72)	0.29	0.99	0.04	G
						Greek	0.105	0.127	0.81 (0.61–1.08)	0.15			
rs365132	5	176 378 574	*UIMC1*	T/G	Early	Boston	0.476	0.467	1.04 (0.86–1.25)	0.65			G
rs2153157	6	10 897 488	*SYCP2L*	A/G	Early	Boston	0.458	0.463	0.98 (0.81–1.18)	0.76			0.997
rs805303	6	31 616 366	*BAG6*	G/A	Early	Boston	0.335	0.363	0.88 (0.73–1.07)	0.17			G
rs2517388	8	37 977 732	*ASH2L*	G/T	Early	Boston	0.84	0.819	1.16 (0.91–1.49)	0.25			G
rs3736830[a]	13	50 306 221	*KPNA3*	C/G	Early	Boston	0.182	0.151	0.79 (0.62–1.03)	0.18			
rs4886238	13	61 113 739	*TDRD3*	A/G	Early	Boston	0.68	0.661	1.08 (0.89–1.33)	0.52			0.981
rs10852344	16	12 016 919	*GSPT1*	C/T	Early	Boston	0.565	0.559	1.02 (0.85–1.23)	0.75			G
rs11668344	19a[b]	55 833 664	*TMEM150B*	A/G	Early	Boston	0.324	0.386	0.76 (0.63–0.92)	0.003	0.004	0.44	0.992
						Greek	0.321	0.355	0.86 (0.71–1.04)	0.13			
rs4806660	19a[b]	55 824 634	*BRSK1*	T/C		Boston	0.338	0.395	0.78 (0.65–0.95)	0.02			0.978
rs1172822	19a[b]	55 819 845	*TMEM105B*	T/C		Boston	0.334	0.39	0.78 (0.64–0.95)	0.02			G
rs12461110	19b	56 320 663	*NLRP11*	G/A	Early	Boston	0.321	0.345	0.90 (0.74–1.09)	0.06	0.08	0.89	0.988
						Greek	0.29	0.319	0.90 (0.74–1.09)	0.22			
rs4246511	1	39 380 385	*RHBDL2*	C/T	Late	Boston	0.338	0.308	1.15 (0.93–1.41)	0.24			0.874
rs12294104	11	30 382 899	*FSHβ*	C/T	Late	Boston	0.196	0.168	1.21 (0.95–1.54)	0.09	0.11	0.86	0.979
						Greek	0.161	0.144	1.13 (0.87–1.47)	0.34			
rs2307449	15	89 863 928	*POLG*	G/T	Late	Boston	0.597	0.591	1.02 (0.85–1.23)	0.72			G
rs16991615	20	5 948 227	*MCM8*	G/A	Late	Boston	0.086	0.066	1.46 (0.98–2.17)	0.06	0.62	0.34	G
						Greek	0.063	0.057	1.11 (0.75–1.64)	0.62			

For each single nucleotide polymorphism (SNP) the table includes the ancestral (Anc) allele, the derived (Der) allele, the effect of derived allele on age at menopause, frequency of the derived allele in cases (freq-case) and controls (freq-ctls) for the three sample sets, the odds ratio (OR) and the *P*-value; the combined *P*-value of the cohorts when available using a Mantel–Haenszel model (P-value$_{com}$) (Mantel and Haenszel, 1959); together with a *P*-value for the test of heterogeneity in the effect estimates between the sample sets (P_{het}).

[a]Variant did not reach significance in previous meta-analysis (Stolk *et al.*, 2012).

[b]Variants are in linkage disequilibrium.

CI, confidence interval.

(Marchini et al., 2007) using the 1000 genomes phase 1 v2 March 2012 panel. Data from eight independent directly genotyped menopause SNPs, and from eight independent high quality imputed menopause SNPs with observed over expected variance >0.88 were used for association analyses. No data were available for the chromosome 12 menopause locus represented by the SNP rs2277339. Genotyping in the Greek cohort was performed using single-plex PCR reactions employing commercially available nanofluidics technology, with assays designed by Fluidigm (San Francisco, CA, USA).

Statistical analysis

Genetic PCOS case-control association analyses were performed using the SNP test (Marchini et al., 2007). A *P*-value of <0.0031 was considered significant after Bonferroni correction to account for 16 independent genetic markers tested. Our primary association analysis included adjustment for four principal components (PCs) calculated by multidimensional scaling analysis of identity-by-state distances of ancestry-informative markers. Linear regression using an additive genetic model was used to test for association of menopause risk variants with 16 log-transformed quantitative traits in the combined sample of PCOS cases and controls (LH, FSH, LH:FSH ratio, testosterone, dehydroepiandrosterone sulphate, androstenedione, sex hormone-binding globulin, 17OH progesterone, progesterone, estradiol [E_2], Ferriman Gallwey score, thyroid-stimulating hormone, prolactin, acne, ovarian volume and follicle number). A *P*-value of <0.0033 was considered significant after Bonferroni correction for 15 independent traits (LH:FSH ratio excluded, as it was derived). Five variants with the lowest *P*-values or surrogate markers were genotyped in the replication cohort from Greece. The odds ratios (ORs) and *P*-values for the combined group were calculated using a Mantel–Haenszel model (Mantel and Haenszel, 1959). A genetic risk score of menopause variants weighted by the log of the OR from meta-analyses of the menopause GWAS was generated as previously described (Cornelis et al., 2009). We evaluated the contribution of the weighted genetic risk score to PCOS in logistic regression models adjusting for five PCs, as well as age and BMI. Genetic risk scores were also calculated separately for variants with derived alleles associated with earlier and later age at menopause.

Results

Validation of the Greek cohort was performed using a marker previously identified in the Boston cohort (Supplementary data, Table SII) (Welt et al., 2012). The rs10986105 variant was associated with PCOS in the entire Greek cohort (OR: 1.99 [1.21–3.21, 5–95% confidence intervals]; $P = 0.006$). The other variants were not significant (Supplementary data, Table SII). The *P*-values were also nominally significant for two variants when using only the subset of Greek subjects who fulfilled the NIH criteria ($n = 734$); with rs10986105 (1.87 [1.13–3.11]; $P = 0.01$) and rs12478601 (0.82 [0.68–0.99]; $P = 0.04$) associated with PCOS.

The frequency of the derived minor allele at rs11668344-G on chromosome 19q13.4 was lower in both the Boston and Greek PCOS subjects (Table I). The result was significant in the Boston discovery cohort ($P < 0.0033$), and the Greek replication cohort exhibited the same direction of effect, although the combined *P*-value did not reach significance when corrected for multiple testing.

The derived minor allele at rs12294104-T on chromosome 11 was significantly associated with higher average LH levels and a higher LH:FSH ratio (Table II). There was also an association between rs12294104-T and higher LH levels (0.16 ± 0.04, $\beta \pm$ SE; $P < 0.0004$) and LH:FSH ratio (0.17 ± 0.04, $\beta \pm$ SE; $P < 0.0001$) in the Greek cohort. There was no relationship between the variant and FSH or thyroid-stimulating hormone (TSH) levels. The derived minor allele rs10852344-T on chromosome 16 was associated with smaller ovarian volume.

Genetic risk scores calculated from the variants associated with both earlier and later menopause were also associated with PCOS (Table III). The data were also significant for all variants combined $P < 0.05$). The risk scores indicated an inverse relationship with earlier menopause variants and a positive relationship with later menopause variants. The genetic risk score calculated from earlier menopause variants was also inversely associated with LH and the LH:FSH ratio (both $P < 0.05$). The relationship was not entirely accounted for by variant rs12294104. There was no relationship between the genetic risk score and ovarian volume or follicle number.

Discussion

These data suggest that common DNA variants exert an opposing effect on age at menopause and risk for PCOS, suggesting PCOS may have genetic influences that extend the age at menopause. The minor allele at chromosome 19q13.4 rs11668344-G, a variant associated with earlier age at menopause (Murray et al., 2011; Stolk et al., 2012), was less common in women with PCOS. In addition, the genetic risk score indicates overrepresentation of variants associated with later age at menopause and underrepresentation of variants associated with earlier age at menopause in relation to PCOS. The minor allele at chromosome 11 rs12294104 was associated with higher LH levels and a higher LH:FSH ratio while the minor allele at chromosome 16 rs10852344, associated with earlier age at menopause (Stolk et al., 2012), was also associated with smaller ovarian volume. Expression of genes close to these variants associated with age at menopause,

Table II Quantitative traits associated with menopause variants in women with PCOS and controls (Boston cohort).

Chr	SNP-derived allele	Effect of derived allele on menopause	Trait	Derived allele frequency	$\beta \pm$ SE	*P*-value
11	rs12294104-T	Late	LH:FSH ratio	0.174	0.28 ± 0.06	2.70×10^{-6}
			Average LH level	0.174	0.26 ± 0.06	5.22×10^{-5}
			Average FSH level	0.174	-0.01 ± 0.04	0.78
			TSH level	0.168	0.007 ± 0.039	0.86
16	rs10852344-T	Early	Maximum ovarian volume	0.552	-0.16 ± 0.05	0.0012

TSH, thyroid-stimulating hormone.

Table III Genetic risk scores calculated from 16 menopause risk variants and the association with PCOS traits.

	Earlier menopause risk variants ($n = 12$)			Later menopause risk variants ($n = 4$)		
	OR (95% CI)	***P*-value**	**P_{het}**	**OR (95% CI)**	***P*-value**	**P_{het}**
PCOS	0.95 (0.90–1.00)	0.073	0.17	1.14 (1.04–1.26)	0.0078	0.86
PCOS/BMI[a]	0.93 (0.87–0.99)	0.027	0.32	1.14 (1.03–1.26)	0.0099	0.85
	$\beta \pm$ SE	*P*-value	P_{het}	$\beta \pm$ SE	*P*-value	P_{het}
LH/FSH ratio	-0.03 ± 0.01	0.016	0.85	0.03 ± 0.03	0.23	3×10^{-6}
LH/FSH ratio without rs12294104[b]				-0.04 ± 0.03	0.19	0.06
LH	-0.03 ± 0.01	0.027	0.70	0.09 ± 0.05	0.055	2×10^{-4}
LH without rs12294104[b]				-0.17 ± 0.02	0.03	0.70
FSH	-0.005 ± 0.01	0.71	0.60	0.009 ± 0.04	0.84	0.68
Ovarian volume	-0.02 ± 0.01	0.25	0.05	-0.04 ± 0.05	0.42	0.85
Maximum number of follicles	-0.01 ± 0.02	0.38	0.59	0.06 ± 0.04	0.14	0.32

The $\beta \pm$ SE indicates the direction of the association of the quantitative trait in the combined case and control sample, and the OR and 95% CI, the *P*-value and the *P*-value for the test of heterogeneity in the effect estimates between the sample sets (P_{het}) are presented.
[a]Data controlled for BMI.
[b]Genetic risk score without variant rs12294104.

BRSK1, *TMEM150B* and *BCAR4*, has been identified in the ovary (He *et al.*, 2009; Stolk *et al.*, 2009; Stolk *et al.*, 2012; Angulo *et al.*, 2013) and *FSHβ* is a subunit in the critical polypeptide for stimulating follicle growth. Therefore, the effect of these variants on reproductive capacity may occur through the ovarian oocyte complement or central control of reproduction.

The derived minor allele at rs11668344 on 19q13.4, associated with earlier menopause and a lower risk for PCOS in our study, lies in an intron of *TMEM150B*. The gene is a member of the damage-related autophagy modulator family of transmembrane proteins that contain a conserved Fragl/DRAM/Sfk1sequence (ncbi.nlm.nih.gov/Structure) (Stolk *et al.*, 2012). The family is ubiquitously expressed in all tissues, suggesting an important role in cellular function. While there are no data to implicate *TMEM150B* in ovarian or neuronal processes that might regulate reproduction, other variants in the region were also associated with age at menopause and were nominally associated with PCOS in the current data (He *et al.*, 2009; Stolk *et al.*, 2009).

Variants in the same region with similar effects on age at menopause in earlier studies were also nominally significant in the Boston discovery cohort and support the finding (rs1172822-C and rs4806660-T; $P = 0.02$) (He *et al.*, 2009; Stolk *et al.*, 2009). The rs1172822 variant in the region sits in an intron of *BRSK1*. *BRSK1* is expressed in the ovary (genatlas.medecine.univ-paris5.fr). Although there was no relationship between rs1172822 or rs11668344 and follicle number or ovarian volume in the current study, previous studies suggest that rs1172822 is associated with lower AMH levels in cancer survivors (van Dorp *et al.*, 2013). Further studies will be needed to determine whether one of these genes, or others, explain the possible relationship to PCOS in the region.

Importantly, the genetic risk score also supports the hypothesis that genetic variants associated with age at menopause are also associated with risk for PCOS. The genetic risk score addresses the hypothesis by summing the risk at all variants associated with age at menopause to determine whether there is an overall relationship with risk for PCOS (Cornelis *et al.*, 2009). The genetic risk score improves the ability to detect a relationship by aggregating information from all variants because each variant has only a small effect on age at menopause. Taken together with data from single loci, the genetic risk score highlights the impact of age at menopause variants on PCOS risk.

The minor allele at chr11 rs12294104-T was associated with later menopause and with gonadotrophin levels. Although the variant associated with later menopause does not sit within the FSHβ gene, it is close, at 126 kb downstream, and variants found in GWASs have been demonstrated to affect transcription of the gene in close proximity (McCarroll *et al.*, 2008). However, the variant was not directly associated with serum FSH levels, but rather with LH levels. FSH levels are tightly regulated by E_2 and inhibin feedback in humans, to control monofollicular development (Gougeon, 2010). Therefore, it is possible that any effect of the variant on expression of FSHβ would result in only a small change in serum FSH levels. Supporting this concept, the higher LH:FSH ratio was more powerfully associated with the variant than LH levels alone, suggesting that FSH levels are relatively lower in women who carry the variant. Larger sample sizes may be needed to demonstrate a relationship between the variant and FSH levels directly.

The mechanism for the increase in LH levels is not clear. FSHβ pairs with the alpha subunit that it shares in common with LH, hCG and TSH to regulate folliculogenesis. A decrease in FSHβ could result in increased free alpha subunit, which could then combine with LHβ to increase levels. There is evidence from mouse models that decreased *Fshb* expression or *Fshb* deletion, in the absence of changes in *CGA* expression, result in increased LH levels in females (Abel *et al.*, 2014; Fortin *et al.*, 2014), consistent with the hypothesis. Similarly, in women with a FSHβ mutation, LH is elevated (Kottler *et al.*, 2010). Alternatively, small changes in FSH levels could affect E_2 secretion and feedback on GnRH and LH, although we were unable to detect differences in E_2 levels in the current study, likely related to the high levels of E_2 across

the cycle in women. There was no evidence of expression differences in the pituitary or hypothalamus for any gene in the region (GTex) (Consortium, 2013). Further studies will be needed to determine whether other factors in the region of the variant act in a trans manner to change *LHβ* expression on chromosome 19.

The rs10852344-T allele, associated with earlier age at menopause, was also shown to be associated with smaller ovarian volume (He *et al.*, 2009; Stolk *et al.*, 2012). The variant is located near the gene *GSPT1*, G1 to S phase transition 1, encoding a protein that regulates mRNA decay (Delage *et al.*, 2011); *TNFRSF17*, a tumor necrosis factor receptor, associated with the NFKB inflammatory pathway linked to aging (Coquery and Erickson, 2012) and *BCAR4,* the breast cancer anti-estrogen resistance 4 protein, expressed in mature oocytes and in early embryo development (Angulo *et al.*, 2013). All are interesting candidate genes based on their function or location.

Our data were limited by the small size of the control group in the Greek replication cohort. The Greek replication cohort trended in the same direction as the discovery set for the variants of interest, but did not increase the significance of the discovery findings. Larger replicate groups will be important to map the associated regions for determination of the causal risk variants for PCOS (McCarthy and Hirschhorn, 2008).

Women with PCOS have a decreased frequency of variants associated with earlier age at menopause and increased frequency of variants associated with later age at menopause, compared with controls. The variants associated with menopause are also associated with ovarian parameters on ultrasound and gonadotrophin levels. These data point to the potential genetic relationship between PCOS and age at menopause, suggesting a longer reproductive lifespan in women with PCOS. These data also highlight the possible importance of the ovary, along with the hypothalamus/pituitary, in the etiology of reproductive aging and in the pathophysiology of PCOS.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

R.S. designed the study, analyzed data, wrote and edited the manuscript. A.C.B. analyzed data and edited the manuscript. N.G., V.K. and D.P. designed the study, recruited subjects, collected data and edited the manuscript. C.K.W. designed the study, recruited subjects, collected samples, assisted with data analysis and wrote and edited the manuscript. All authors approved the final manuscript.

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Conflict of interest

C.K.W. is a consultant for Takeda Pharmaceuticals.

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