Human Reproduction, Vol.30, No.6 pp. 1454–1459, 2015
Advanced Access publication on April 22, 2015 doi:10.1093/humrep/dev085

human reproduction

ORIGINAL ARTICLE ***Reproductive endocrinology***

Han Chinese polycystic ovary syndrome risk variants in women of European ancestry: relationship to FSH levels and glucose tolerance

R. Saxena[1], N.A. Georgopoulos[2], T.J. Braaten[3], A.C. Bjonnes[1], V. Koika[2], D. Panidis[2,4], and C.K. Welt[5,*]

[1]Department of Anaesthesia and Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA [2]Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Patras Medical School, Patras, Greece [3]Department of Internal Medicine, University of Utah, Salt Lake City, UT, USA [4]Division of Endocrinology and Human Reproduction, Second Department of Obstetrics and Gynecology, Aristotle University of Thessaloniki, Thessaloniki, Greece [5]Division of Endocrinology, Metabolism and Diabetes, University of Utah, Salt Lake City, UT, USA

*Correspondence address. Division of Endocrinology, Metabolism and Diabetes, University of Utah, EIHG, 15 N 2030 E, Salt Lake City, UT 84112, USA. Tel: +1-801-585-1875. E-mail: cwelt@genetics.utah.edu

Submitted on December 23, 2014; resubmitted on March 13, 2015; accepted on March 27, 2015

STUDY QUESTION: Are PCOS risk variants identified in women of Han Chinese ethnicity also associated with risk of PCOS or the phenotypic features of PCOS in European women?

SUMMARY ANSWER: One variant, rs2268361-T, in the intron of *FSHR* was associated with PCOS and lower FSH levels, while another variant rs705702-G near the *RAB5B and SUOX* genes was associated with insulin and glucose levels after oral glucose testing in women with PCOS of European ethnicity.

WHAT IS KNOWN ALREADY: Three of the eleven variants associated with PCOS in the Han Chinese genome-wide association studies were also associated with PCOS in at least one European population when corrected for multiple testing (*DENND1A*, *THADA* and *YAP1*). However, additional replication is needed to establish the importance of these variants in European women and to determine the relationship to PCOS phenotypic traits.

STUDY DESIGN, SIZE, DURATION: The study was a case–control examination in a discovery cohort of women with PCOS ($n = 485$) and controls ($n = 407$) from Boston (Boston 1). Replication was performed in women from Greece (cases $n = 884$ and controls $n = 311$) and an additional cohort from Boston (Boston electronic medical record (EMR); $n = 350$ cases and $n = 1258$ controls).

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: Women had PCOS defined by the National Institutes of Health criteria in Boston 1 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. The second cohort from Boston was defined using the EMR and natural language processing. Allele frequencies for variants associated with PCOS in Han Chinese women were examined in PCOS cases and controls, along with the relationship to quantitative traits.

MAIN RESULTS AND THE ROLE OF CHANCE: A variant rs2268361-T in an intron of *FSHR* was associated with PCOS (0.84 [0.76–0.93], OR [95% CI]; $P = 0.002$). The rs2268361-T was associated with lower FSH levels (-0.15 ± 0.05; $P = 0.0029$). A variant rs705702-G near *RAB5B* and *SUOX* was associated with insulin (-0.16 ± 0.05, $P = 0.0029$) and glucose levels (-0.20 ± 0.05, $P = 0.0002$) 120 min after an oral glucose test.

LIMITATIONS, REASONS FOR CAUTION: The study was large and contained replication cohorts, but was limited by a small number of controls in the Greek cohort and a small number of cases in the second Boston cohort. The second Boston group was identified using electronic medical record review, but was validated for the cardinal features of PCOS.

WIDER IMPLICATIONS OF THE FINDINGS: This study demonstrates a cross-ethnic PCOS risk locus in *FSHR* in women of European ancestry with PCOS. The variant may influence FSH receptor responsiveness as suggested by the associated change in FSH levels. The relationship

between a variant near *RAB5B* and *SUOX* and glucose stimulated insulin and glucose levels suggests an influence of one of these genes on glucose tolerance, but the absence of a relationship with PCOS points to potential differences in the international PCOS patient populations.

STUDY FUNDING/COMPETING INTERESTS: The project was supported by Award Number R01HD065029 from the Eunice Kennedy Shriver National Institute Of Child Health & Human Development, Award Number 1 UL1 RR025758, Harvard Clinical and Translational Science Center, from the National Center for Research Resources, award 1-10-CT-57 from the American Diabetes Association and the Partners Healthcare Center for Personalized Genetics Project Grant. C.K.W. is a consultant for Takeda Pharmaceuticals.

TRIAL REGISTRATION NUMBER: NCT00166569.

Key words: genome-wide association / single-nucleotide polymorphism / follicle-stimulating hormone receptor / polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in reproductive age women, affecting 7–10% of this group. Cardinal features include irregular menstrual cycles, hyperandrogenism and polycystic ovarian morphology (Rotterdam, 2004). Obesity and insulin resistance are also common, along with increased risk of diabetes, metabolic syndrome and other cardiovascular diseases (Ehrmann *et al.*, 1995, 1999). Despite the detrimental impact of the disorder on women's health, the etiology remains poorly understood.

Twin studies suggest that genetic influences explain over 70% of PCOS pathogenesis (Vink *et al.*, 2006). A genome-wide association study (GWAS) in Han Chinese women identified eight risk loci for PCOS at 9q22.32, 11q22.1, 12q13.2, 12q14.3, 16q12.1, 19p13.3, 20q13.2 and an independent signal at 2p16.3 (Shi *et al.*, 2012), in addition to three loci identified previously at 2p16.3, 2p21, and 9q33.3 (Chen *et al.*, 2011). Three of the eleven variants associated with PCOS in the Han Chinese GWAS were also associated with PCOS in at least one European population when corrected for multiple testing, including DENN/MADD domain containing 1A (*DENND1A*), thyroid adenoma associated (*THADA*) and yes-associated protein 1(*YAP1*) (Goodarzi *et al.*, 2012; Welt *et al.*, 2012; Louwers *et al.*, 2013; Brower *et al.*, 2014). However, additional replication is needed to establish the importance of these variants in European women and to determine the relationship to PCOS phenotypic traits. We examined the eight new susceptibility variants in a PCOS case–control discovery cohort in Boston (Boston 1) and replicate populations in Greece and a second cohort in Boston identified through the electronic medical record (Boston EMR). We also examined important phenotypic PCOS traits in relation to these variants.

Materials and Methods

Study subjects

Subjects in the discovery cohort from Boston (Boston 1) were of European ethnicity, aged 18–45 years and with PCOS defined by the 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 menses, between 21 and 35 days, and no hyperandrogenism (Welt *et al.*, 2006).

Replication was performed in women from Greece with PCOS defined by the NIH criteria, as above ($n = 783$) and Rotterdam criteria, i.e. clinical or biochemical hyperandrogenism, polycystic ovary morphology and regular menstrual cycles ($n = 101$). Controls 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 ($n = 311$) (Georgopoulos *et al.*, 2013). An additional cohort from Boston was identified using the electronic medical record to identify the term 'polycystic ovary syndrome' in clinical notes and natural language processing to confirm the diagnosis with two out of three Rotterdam criteria (Boston EMR, $n = 350$) (Savova *et al.*, 2010). Controls were also identified through the electronic medical record, age-matched to cases ($n = 1258$).

Ethical approval

The study was approved by the Institutional Review Board 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

All PCOS subjects were studied >10 days after their last menstrual period 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, 5 MHz convex array transducer); and blood samples for lipids, glucose, insulin, gonadotrophin and sex-steroid levels. LH and FSH levels were obtained at 10 min intervals to calculate an average gonadotrophin concentration. Using data from blood samples collected every 10 min over 12 h in women with PCOS and ovulatory controls (Taylor *et al.*, 1997), we documented that the mean LH secretion from 12 h of frequent blood samples correlates well with the value obtained from the mean of three samples collected from 0800–0820 h ($r = 0.92$, $P < 0.01$) (Welt *et al.*, 2006).

SNP selection and genotyping

Ten SNPs from the eight loci reported by Shi *et al.* (2012) and listed in Table I were selected for analysis in all sample sets. Genotyping in the discovery Boston 1 cohort 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 ($n = 15$) (Eigensoft version 6.0.1, github.com/DReichLab/EIG, USA). 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 SNPs (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 (Marchini *et al.*, 2007) using the 1000 genomes phase 1 v2 March 2012 panel. Genotyping in the Greek cohort was performed with single-plex PCR reactions using nanofluidics technology (Fluidigm, San Francisco).

Table I **Odds ratios (OR) and *P*-values for the association of PCOS with ten SNPs (single-nucleotide polymorphism) identified in a genome-wide association study of Han Chinese women with PCOS.**

SNP-allele	Nearest gene	OR Chinese	Frq Chinese	Samples	*P*	Study OR (95% CI)	Frq_{cases}	$Frq_{controls}$	$P_{Combined}$	Combined OR (95% CI)	P_{Het}
rs2268361-T	*FSHR*	0.84	0.496	Boston 1	0.11	0.83 (0.68–1.01)	0.61	0.65	0.002	0.84 (0.76–0.93)	0.9
2p16.3				Greek	0.048	0.79 (0.54–1.00)	0.57	0.62			
				Boston EMR	0.11	0.85 (0.64–1.03)	0.59	0.63			
rs2349415-T	*FSHR*	1.33	0.181	Boston 1	0.61	1.07 (0.87–1.24)	0.35	0.33	0.09	1.14 (0.99–1.31)	0.4
2p16.3				Greek	0.97	1.00 (0.83–1.22)	0.37	0.37			
				Boston EMR	0.04	1.20 (1.01–1.42)	0.38	0.34			
rs4385527-A	*C9orf3*	0.78	0.219	Boston 1	0.12	0.86 (0.71–1.04)	0.4	0.43	0.91	0.99 (0.90–1.10)	0.097
9q22.32				Greek	0.63	0.96 (0.79–1.15)	0.45	0.47			
				Boston EMR	0.13	1.14 (0.96–1.35)	0.46	0.42			
rs3802457-A	*C9orf3*	0.69	0.096	Boston 1	0.36	0.86 (0.49–1.53)	0.026	0.03	0.65	0.93 (0.67–1.30)	0.92
9q22.32				Greek	0.67	0.88 (0.49–1.59)	0.023	0.026			
				Boston EMR	1	1.00 (0.62–1.62)	0.031	0.031			
rs1894116-G	*YAP1*	1.30	0.194	Boston 1	0.37	1.13 (0.83–1.52)	0.11	0.1	0.007	1.25 (1.04–1.49)	0.66
11q22.1				Greek	0.12	1.26 (0.94–1.67)	0.14	0.11			
				Boston EMR	0.03	1.37 (1.04–1.80)	0.11	0.083			
rs705702-G	RAB5B/SUOX	1.32	0.245	Boston 1	0.85	0.95 (0.78–1.16)	0.33	0.34	0.88	1.01 (0.90–1.13)	0.51
12q13.2				Greek	0.38	1.10 (0.89–1.36)	0.27	0.25			
				Boston EMR	0.54	0.95 (0.79–1.13)	0.3	0.32			
rs2272046-C	*HMGA2*	0.67	0.093	Boston 1	0.96	1.00 (0.56–1.80)	0.026	0.026	0.52	1.12 (0.78–1.59)	0.43
12q14.3				Greek	0.15	1.59 (0.84–2.99)	0.031	0.019			
				Boston EMR	0.8	0.93 (0.53–1.63)	0.023	0.025			
rs4784165-G	*TOX3*	1.26	0.325	Boston 1	0.03	1.36 (1.10–1.67)	0.32	0.26	0.02	1.15 (1.02–1.29)	0.16
16q12.1				Greek	0.76	1.03 (0.84–1.26)	0.31	0.31			
				Boston EMR	0.3	1.10 (0.92–1.33)	0.3	0.27			
rs2059807-A	*INSR*	1.24	0.301	Boston 1	0.61	0.91 (0.68–1.10)	0.36	0.39	0.85	1.01 (0.92–1.11)	0.2
19p13.3				Greek	0.16	1.15 (0.94–1.40)	0.36	0.33			
				Boston EMR	0.44	0.93 (0.79–1.11)	0. 37	0.39			
rs6022786-A	*SUMO1P1*	1.24	0.339	Boston 1	0.37	0.95 (0.74–1.13)	0.42	0.43	0.54	1.03 (0.93–1.14)	0.58
20q13.2				Greek	0.16	1.04 (0.86–1.25)	0.47	0.46			
				Boston EMR	0.29	1.10 (0.93–1.30)	0.46	0.44			

For each SNP, the table includes the OR and frequency (Frq) of the risk allele in the Chinese population, the *P*-value, OR, and frequency in cases and controls for the three sample sets, and the *P*-value ($P_{combined}$) and OR ($OR_{combined}$) for the three samples sets combined using a Mantel–Haenszel model (Mantel and Haenszel, 1959), together with the *P*-value, P_{Het}, for the test of heterogeneity in the effect estimates between the sample sets.

Genotyping in the replicate Boston cohort was performed using OpenArray TaqMan assays (Life Technologies, New York, NY, USA).

Statistical analysis

Genetic PCOS case–control association analyses were performed using SNPtest (Marchini *et al.*, 2007) using genotyped and imputed SNPs with observed/expected variance information greater than 0.88. A *P*-value <0.005 was considered significant to account for 10 independent tests. The primary association analysis, but not the replication cohort analyses, included adjustment for four principal components (PCs) calculated by multidimensional scaling (MDS) analysis of identity-by-state distances of ancestry-informative markers. The odds ratios and *P*-values for the combined discovery and replication groups were calculated using a Mantel–Haenszel model (Mantel and Haenszel, 1959). Heterogeneity was tested assuming a log-normal distribution for the effect estimates and using a likelihood ratio χ^2-test with degrees of freedom equal to the number of groups compared minus one. Linear regression using an additive genetic model was used to test for association of Han Chinese PCOS risk variants with 30 log-transformed quantitative traits in the combined sample of PCOS cases and controls in the discovery Boston cohort. A *P*-value <0.0033 was considered significant after Bonferroni correction for 15 independent traits (FSH, LH, prolactin, 17-OH progesterone, testosterone, cholesterol, sex hormone binding globulin (SHBG), estradiol, blood pressure, pulse, thyroid-stimulating hormone (TSH), body mass index (BMI), fasting glucose, fasting insulin and ovarian volume), with other variables highly correlated.

Results

One variant, rs2268361-T, was associated with PCOS in the combined dataset ($P = 0.002$; Table I). The variant was underrepresented in PCOS cases in all three cohorts. The rs2268361 variant was only nominally significant in the subset of subjects in the Boston 1 and Greek cohorts and in the same cohorts diagnosed according to the NIH criteria, likely related to decreased power (Supplementary Tables SI and SII). A second independent SNP in the same region, rs2349415 ($r^2 < 0.1$ in 1000 genomes data from Utah residents with ancestry from northern and western Europe [CEU], Han Chinese in Beijing [CHB] and Japanese in Tokyo [JPT] populations), exhibited a similar directionality as in the Han Chinese population, but was not significant. Two additional variants were nominally significant, but did not remain significant after correction for multiple testing (rs1894116 and rs4784165). The results did not change when only subjects diagnosed according to the NIH criteria in the Boston 1 and Greek cohorts were included (Supplementary Table SII). With the exception of rs705702 and rs2272046, the additional variants also exhibited a similar directionality to the Han Chinese population in two out of three of the cohorts (Table I).

There was an association between rs2268361-T and lower FSH levels (Table II). There was also an association between rs2268361-T and lower FSH (-0.059 ± 0.016, $\beta \pm$ SE; $P < 0.0004$) and LH levels (-0.13 ± 0.03, $\beta \pm$ SE; $P < 0.0004$) in the Greek cohort. There was a relationship between rs705702-G and lower glucose and insulin measurements 120 min after an oral glucose tolerance test (Table II), although the relationship with insulin did not remain significant after correction for BMI. These relationships were not observed ($P = 0.70$ and $P = 0.05$) in previously published meta-analyses of 2 h glucose and 2 h insulin GWAS performed in 15 000 and 7800 non-diabetic subjects without enrichment for PCOS cases (Saxena *et al.*, 2010). It was also not observed in the Greek cohort.

Discussion

The data demonstrate that a PCOS risk variant in Han Chinese women in the region of *FSHR* is also associated with PCOS in women of European ethnicity, including those in the subset diagnosed according to the NIH criteria. The same variant is associated with FSH levels. An additional variant, rs705702, is associated with glucose and insulin levels 120 min after an oral glucose load, although it was not associated with PCOS in a European population. The data point to common PCOS risk variants in women of European and Chinese ethnicity and to potential mechanisms underlying PCOS.

The association between a variant near *FSHR* and PCOS highlights the longstanding interest in the FSH receptor and failure of follicular development in PCOS. While the current variant is an intronic variant in *FSHR*, coding variants in *FSHR* have been examined in association with follicle stimulation and PCOS. Coding sequence changes in exon ten, Ala307Thr and Asn680Ser, appear to influence the ability of exogenous FSH to stimulate the ovary in some studies (Simoni *et al.*, 2002). Since follicle growth is arrested in PCOS, the variants have also been implicated in PCOS pathogenesis. There have been mixed results in association studies of PCOS, with increased frequency of Ala307Thr and Asn680Ser in PCOS individuals in some studies (Du *et al.*, 2010; Dolfin *et al.*, 2011), but not others (Fu *et al.*, 2013, Liaqat *et al.*, 2014, Wu *et al.*, 2014). While there was variability in the number of subjects studied and ethnic background, a recent large study demonstrated no relationship with Ala307Thr in European women (Mutharasan *et al.*, 2013).

The current variant, rs2268361, is located in an intron between exons 8 and 9 in *FSHR*. Therefore, the functional effect is more difficult to ascertain. Nevertheless, the FSH levels are higher in carriers of the ancestral C allele, the alternate to the allele examined, and the risk for PCOS is increased. Therefore, it is possible that the C variant alters FSH receptor expression or action, resulting in a need for higher FSH levels to stimulate the receptor for follicle growth. There is no evidence for change in ovarian expression of *FSHR* in carriers of the variant (GTex), although there are few ovaries in the dataset and these tissues are mainly from

Table II The association of three phenotypic traits with Han Chinese polycystic ovary syndrome risk variants.

SNP-allele	Nearest gene	Measured trait	*P*	β	P_{BMI}	β_{BMI}
rs2268361-T	*FSHR*	FSH	0.0046	-0.15 ± 0.05	0.0029	-0.15 ± 0.05
rs705702-G	*RAB5B/SUOX*	Glucose 120 min post glucose 75 g	0.0002	-0.20 ± 0.05	0.0008	-0.17 ± 0.05
rs705702-G	*RAB5B/SUOX*	Insulin 120 min post glucose 75 g	0.0029	-0.16 ± 0.05	0.0089	-0.12 ± 0.05

The P_{BMI} and β_{BMI} are controlled for body mass index (BMI).

post-menopausal women. Further studies are needed to determine the functional effect of rs2268361 in PCOS.

In addition, rs705702-G was associated with lower glucose and insulin levels 120 min after an oral glucose load, suggesting better insulin sensitivity. rs705702 is found between the *RAB5B* and *SUOX* genes. In previous studies *RAB5B* expression has been found to be 2–3 times greater in skeletal muscle in insulin resistant subjects (Bao *et al.*, 1998). *RAB5B* decreases glucose transporter type 4 (GLUT4) translocation to the plasma membrane, which would be expected to increase insulin resistance (Tessneer *et al.*, 2014). The rs705702-G variant decreases expression of *RAB5B* in skeletal muscle (P-value $= 5.9 \times 10^{-13}$) (Genotype Tissue-Expression Project; www.gtexportal.org), and is associated with lower insulin and glucose associations in the current study. Taken together, the rs705702-G variant would be expected to result in changes that increase insulin sensitivity. rs705702 was not associated with PCOS in the current study and was only nominally significant in previous European studies (Louwers *et al.*, 2013). Therefore, the ultimate role of this variant in PCOS pathogenesis remains to be determined.

The study was large and contained replication cohorts, but was limited by a small number of controls in the Greek cohort and a small number of cases in the Boston EMR cohort. The selection of the second Boston group based on record review may also be contributing to some of the observed differences. However, the cohort was validated for the cardinal features of PCOS (unpublished data) and results without including the cohort demonstrated the same effects with less power. The power to detect a nominal association with PCOS was adequate for all variants examined (Supplementary Table SIII). However, two variants were insufficiently powered to detect an association at the alpha = 0.005 level indicated when a Bonferroni correction is applied (rs3802457 and rs2272046). Finally, the replication Greek and Boston EMR cohorts were not controlled for population stratification. The cases and controls were from the same geographical area in Greece. However, the Boston EMR cohort has only self-reported race. While the failure to control for population stratification theoretically could result in false positive findings, the significant data trended in the same direction as the Boston 1 cohort, which was well defined.

PCOS is a very complex syndrome, with much left to discover in relation to pathogenesis and the relevance of different genetic components. The findings that FSH levels are influenced by the same variant, rs2268361, that is found near the FSHR gene and is significant in Han Chinese and European PCOS populations suggest that disrupting FSH stimulation of follicle development is a key etiologic feature of PCOS. The connection between insulin and glucose levels with rs705702 points to the importance of insulin resistance in women with PCOS, even if it is not associated with pathogenesis in European women. On a clinical level, the presence of the variant may identify patients that are less prone to insulin resistance or type 2 diabetes. These findings provide the foundation to begin to dissect the etiology of PCOS and base treatment recommendations for specific subgroups of women. Thus, the current findings may lead to further understanding of this syndrome and different approaches to therapy.

Supplementary data

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

Acknowledgements

We thank the patients with PCOS who participated in these ongoing studies. We also thank Patrick Sluss, Ph.D., for his assistance in hormone assays over many years.

Authors' roles

R.S. designed the study, analyzed data, wrote and edited the manuscript. A.C.B. analyzed data and edited the manuscript. N.A.G., V.K. and D.P. designed the study, collected data and edited the manuscript. T.J.B. analyzed and interpreted the data, wrote the article and approved the version to be published. 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.

Funding

The project was supported by Award Number R01HD065029 from the Eunice Kennedy Shriver National Institute Of Child Health & Human Development, Award Number 1 UL1 RR025758, Harvard Clinical and Translational Science Center, from the National Center for Research Resources, award 1-10-CT-57 from the American Diabetes Association and the Partners Healthcare Center for Personalized Genetics Project Grant.

Conflict of interest

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

References

Bao S, Zhu J, Garvey WT. Cloning of Rab GTPases expressed in human skeletal muscle: studies in insulin-resistance subjects. *Horm Metab Res* 1998;**30**:656–672.

Brower MA, Jones MR, Rotter JI, Krauss RM, Legro RS, Azziz R, Goodarzi MO. Further investigation in Europeans of susceptibility variants for polycystic ovary syndrome discovered in genome-wide association studies of Chinese individuals. *J Clin Endocrinol Metab* 2015; **100**:E182–186.

Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J *et al.* Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet* 2011;**43**:55–59.

Dolfin E, Guani B, Lussiana C, Mari C, Restagno G, Revelli A. FSH-receptor Ala307Thr polymorphism is associated to polycystic ovary syndrome and to a higher responsiveness to exogenous FSH in Italian women. *J Assist Reprod Genet* 2011;**28**:925–930.

Du J, Zhang W, Guo L, Zhang Z, Shi H, Wang J, Zhang H, Gao L, Feng G, He L. Two FSHR variants, haplotypes and meta-analysis in Chinese women with premature ovarian failure and polycystic ovary syndrome. *Mol Genet Metab* 2010;**100**:292–295.

Ehrmann DA, Barnes RB, Rosenfield RL. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev* 1995;**16**:322–353.

Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care* 1999;**22**:141–146.

Fu L, Zhang Z, Zhang A, Xu J, Huang X, Zheng Q, Cao Y, Wang L, Du J. Association study between FSHR Ala307Thr and Ser680Asn variants and polycystic ovary syndrome (PCOS) in Northern Chinese Han women. *J Assist Reprod Genet* 2013;**30**:717–721.

Georgopoulos NA, Karagiannidou E, Koika V, Roupas ND, Armeni A, Marioli D, Papadakis E, Welt CK, Panidis D. Increased frequency of the anti-mullerian-inhibiting hormone receptor 2 (AMHR2) 482 A>G polymorphism in women with polycystic ovary syndrome: relationship to luteinizing hormone levels. *J Clin Endocrinol Metab* 2013;**98**:E1866–E1870.

Goodarzi MO, Jones MR, Li X, Chua AK, Garcia OA, Chen YD, Krauss RM, Rotter JI, Ankener W, Legro RS *et al.* Replication of association of DENND1A and THADA variants with polycystic ovary syndrome in European cohorts. *J Med Genet* 2012;**49**:90–95.

Liaqat I, Jahan N, Krikun G, Taylor HS. Genetic polymorphisms in Pakistani women with polycystic ovary syndrome. *Reprod Sci* 2015;**22**:347–357.

Louwers YV, Stolk L, Uitterlinden AG, Laven JS. Cross-ethnic meta-analysis of genetic variants for polycystic ovary syndrome. *J Clin Endocrinol Metab* 2013;**98**:E2006–E2012.

Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;**22**:719–748.

Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet* 2007;**39**:906–913.

Mutharasan P, Galdones E, Penalver Bernabe B, Garcia OA, Jafari N, Shea LD, Woodruff TK, Legro RS, Dunaif A, Urbanek M. Evidence for chromosome 2p16.3 polycystic ovary syndrome susceptibility locus in affected women of European ancestry. *J Clin Endocrinol Metab* 2013;**98**:E185–E190.

Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004;**19**:41–47.

Savova GK, Masanz JJ, Ogren PV, Zheng J, Sohn S, Kipper-Schuler KC, Chute CG. Mayo clinical Text Analysis and Knowledge Extraction System (cTAKES): architecture, component evaluation and applications. *J Am Med Inform Assoc* 2010;**17**:507–513.

Saxena R, Hivert MF, Langenberg C, Tanaka T, Pankow JS, Vollenweider P, Lyssenko V, Bouatia-Naji N, Dupuis J, Jackson AU *et al.* Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. *Nat Genet* 2010;**42**:142–148.

Shi Y, Zhao H, Shi Y, Cao Y, Yang D, Li Z, Zhang B, Liang X, Li T, Chen J *et al.* Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. *Nat Genet* 2012;**44**:1020–1025.

Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. *Hum Reprod Update* 2002;**8**:413–421.

Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, Hall JE. Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1997;**82**:2248–2256.

Tessneer KL, Jackson RM, Greisel BA, Olson AL. Rab5 Activity regulates GLUT4 sorting into insulin-responsive and non-insulin-responsive endosomal compartments: a potential mechanism for development of insulin resistance. *Endocrinology* 2014;**155**:3315–3328.

Vink JM, Sadrzadeh S, Lambalk CB, Boomsma DI. Heritability of polycystic ovary syndrome in a Dutch twin-family study. *J Clin Endocrinol Metab* 2006;**91**:2100–2104.

Welt CK, Arason G, Gudmundsson JA, Adams J, Palsdottir H, Gudlaugsdottir G, Ingadottir G, Crowley WF. Defining constant versus variable phenotypic features of women with polycystic ovary syndrome using different ethnic groups and populations. *J Clin Endocrinol Metab* 2006;**91**:4361–4368.

Welt CK, Styrkarsdottir U, Ehrmann DA, Thorleifsson G, Arason G, Gudmundsson JA, Ober C, Rosenfield RL, Saxena R, Thorsteinsdottir U *et al.* Variants in DENND1A are associated with polycystic ovary syndrome in women of European ancestry. *J Clin Endocrinol Metab* 2012;**97**:E1342–E1347.

Wu XQ, Xu SM, Liu JF, Bi XY, Wu YX, Liu J. Association between FSHR polymorphisms and polycystic ovary syndrome among Chinese women in north China. *J Assist Reprod Genet* 2014;**31**:371–377.