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ORIGINAL ARTICLE

Genome-wide association study identifies *GYS2* as a novel genetic factor for polycystic ovary syndrome through obesity-related condition

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To investigate the role of genetic predisposition in the pathogenesis of polycystic ovary syndrome (PCOS) in relation to obesity, we performed a genome-wide association study of PCOS in Koreans ($n = 1741$). PCOS is a heterogeneous endocrinal disorder of uncertain etiology. Obesity is one of the well-known risk factors for PCOS. Genome-wide association study. Women with or without PCOS. A total of 1881 samples were genotyped using Illumina HumanOmni1 Quad v1 and processed by R packages. The PCOS patients were divided into two subgroups according to PCOS diagnostic criteria (Rotterdam and National Institutes of Health (NIH)). For PCOS-associated loci in the two definitions, we successfully confirmed significant associations of *GYS2* for body mass index in the discovery stage. We further replicated pleiotropic associations of *GYS2* in a childhood obesity study ($n = 482$) and in a gestational diabetes study ($n = 1710$), respectively. Our study provides a preliminary framework upon diverse genetic effects underlying PCOS in Korean women. A newly identified *GYS2* gene as a predisposing factor of PCOS might expand understanding of the biological pathways in metabolic and endocrine regulation.
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INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine disorder characterized by its cardinal features; menstrual dysfunction, anovulation, hyperandrogenism and polycystic ovaries.[1] It is also associated with metabolic symptoms; insulin resistance, β-cell dysfunction, impaired glucose tolerance and type 2 diabetes (T2D).[2–4]

Previous studies have established that increasing prevalence of impaired glucose tolerance, insulin resistance and T2D has been associated with an increased risk of PCOS.[5,6] However, because of differential diagnostic criteria and unclear etiology of PCOS, it can be difficult to find reproducible associations from diverse racial and ethnic background.[7–9] Although a recent genome-wide association study (GWAS) has contributed to susceptibility genes or loci discovery for PCOS,[10] identifying a major determinants underlying variation in primary metabolism would be more valuable.[11–13]

PCOS and T2D are obesity-related conditions that share epidemiological and pathophysiological factors.[14–16] A large number of women with PCOS are considered to be overweight or obese, implicating BMI (body mass index) as an important determinant in the manifestation of the syndrome.[17–19] In recent, researchers identified heterogeneous associations between T2D susceptibility genes and risk of PCOS in multiple ethnic populations[20–23] but also demonstrated that its effect was likely through BMI.[24–27]

Given the phenotypic and environmental correlations between PCOS and obesity, it is important to investigate a possible pleiotropic effect for genetic contributions of PCOS through obesity-related conditions. In this study, we therefore hypothesized that genetic variants predisposing to risk of PCOS may contribute to risk of obesity.

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MATERIALS AND METHODS

Subjects

All Korean participants ($n=1881$) in our study were recruited from the Ewha Womans University Medical Center. We selected PCOS patients ($n=774$) and controls ($n=967$) using the Rotterdam criteria announced in 2003 by the ESHRE/ASRM consensus. This proposes a diagnosis of PCOS when a patient meets any two of the following three criteria: (1) oligoovulation/anovulation, (2) hyperandeogenism (clinical or biochemical) and (3) a PCO as determined by ultrasonography. Oligomenorrhoea was defined as <10 periods per year or cycles longer than 35 days, and amenorrhea was defined as the absence of menstruation for >3 months without pregnancy. Clinical hyperandrogenism was defined by a modified Ferriman and Gallwey score of eight or greater in our subjects. Biochemical hyperandrogenism was defined as an elevation of serum androgen levels beyond the 95% confidence limits measured in nonhirsute controls who did not show sonographic evidence of PCO. We also selected 435 women for PCOS based on the revised diagnostic criteria announced in 1990 by the NIH/NICHD consensus, including oligoovulation, clinical androgen excess and ultrasonographic polycystic ovarian morphology. Other causes ($n=61$) of oligomenorrhea or hyperandrogenism such as Cushing's syndrome were excluded by clinical evaluation. All the control subjects ($n=967$) had regular menstrual cycles (21–35 days) non-hyperandrogenism and non-PCO.

The students ($n=482$) aged 9 and 12 years were recruited from Gwacheon city and Kyunggi Province between April and June 2010. This study was conducted as part of the Korean Children–Adolescents Study, which has been monitored yearly as their entry into elementary school at age 7 in Gwacheon City or fourth grade at age 10 in Seoul and Kyunggi Province, Korea. Subjects enrolled in a specific diet program or that were taking any medications known to affect appetite were excluded from the study. The study protocol was approved by the Institutional Review Board of Seoul-Paik Hospital, Inje University and the Korea Center for Disease Control and Prevention. Informed consent was obtained from the children's parents.

The gestational diabetes mellitus (GDM) group ($n=468$) was selected from a hospital-based cohort that recruited GDM women between January 1996 and February 2003 from Cheil General Hospital. For screening, 50 g 1 h oral glucose tolerance test was performed during 24–28 weeks of gestation. Glucose level of 130 mg dl^{-1} or higher was considered positive and warranted diagnostic 100 g oral glucose tolerance test. The glucose and insulin concentrations were measured at 0, 1, 2 and 3 h of glucose challenge. The threshold glucose values for diagnosis of GDM were as follows: fasting $\geqslant$105 mg dl^{-1}, 1 h $\geqslant$190 mg dl^{-1}, 2 h $\geqslant$165 mg dl^{-1} and 3 h $\geqslant$145 mg dl^{-1}. Mean gestational age at diagnosis of GDM was 27.9 ± 2.9 weeks. Nondiabetic control subjects ($n=1242$) were selected from two population-based cohort studies, the rural Ansung and the urban Ansan cohorts. The two cohorts comprised the Korean Genome Epidemiology Study. Only women were eligible as control group according to the following criteria: age$\geqslant$50 years, no previous history of T2D, no first-degree relatives with T2D, fasting plasma glucose level $<$100 mg dl^{-1} and HbA1c $<$6.0%.

Genotyping and quality control

A total of 1881 samples were genotyped using Illumina HumanOmni1 Quad v1 (Illumina Inc., San Diego, CA, USA) and processed by R packages. Samples that exhibited the following properties were excluded: genotyping calls $<$98%, heterozygosity $>$30%, sex inconsistency or identity-by-state (IBS) value $>$0.80. A total of 79 individuals were excluded from sample QC process. Markers with a high missing call rate ($>$1%), minor allele frequency $<$0.05%, and significant deviation from Hardy–Weinberg equilibrium ($P<1\times10^{-6}$) were also excluded. The remaining 619 339 single-nucleotide polymorphisms (SNPs) were used in subsequent analyses for association.[28] Genotyping for the childhood obesity study and the GDM study was conducted using Illumina HumanOmni1 Quad BeadChip (Illumina Inc.) and Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Santa Clara, CA, USA), respectively.

IBS analysis

Pair-wise IBS between individuals was calculated using a subset of pruned markers (73 596 SNPs) that are in approximate linkage equilibrium. IBS analysis was done using the PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) and R statistics packages. The calculated genome-wide average IBS between each pair of individuals was used to estimate individuals who are presumably in first-degree relationships or relationships with more distant relatives whose clusters were tightly linked to the fist-degree relationships.[28]

SNP imputation

Imputation analysis was performed using IMPUTE against all of the HapMap Asian (JPT + CHB) population (release 22/NCBI, build 36 and dbSNP build 126) for a total of 3 091 653 SNPs. Of these, SNPs in each cohort with a posterior probability score $<$0.90, high genotype information content (info $<$0.5), Hardy–Weinberg equilibrium ($P<1\times10^{-7}$), SNP missing rate $>$0.1 or minor allele frequency $<$0.01 were dropped.[28]

Association analyses

Association was analyzed using the PLINK and SAS programs (version 9.1; SAS Institute, Cary, NC, USA). The associations between genotypes and PCOS risk were assessed in an additive model by logistic regression. For BMI validation analysis, quantitative trait loci were tested for association by linear regression analysis in an assistive model.[28]

Quantile–quantile plot

Under the null hypothesis of no association, the genome-wide *P*-values of given SNPs should follow a uniform distribution. Quantile–quantile plots of genome-wide *P*-values were generated to detect SNPs that showed strong deviations from the null distribution because of strong associations with a related trait. The genomic inflation factor was estimated from the median of the χ^2 statistic divided by 0.456.[29]

RESULTS

To detect the genetic susceptibility to PCOS, we first conducted a GWAS screen in Korean women ($n=1741$; Figure 1 and Supplementary Table 1). The quantile–quantile plot of the observed *P*-values derived from the trend test showed significant deviation from the null distribution only in the tail, suggesting truly associated variants. The estimated value of the genomic control inflation factor ($\lambda=0.995$) indicated limited evidence of population stratification and supported the validity of ignoring stratification in our study samples (Supplementary Figure 1). The clinical and endocrine characteristics of the PCOS cases and the controls are described in Table 1. After excluding imputed SNPs, we identified three PCOS-associated loci (rs10841843, rs6487237 and rs7485509) at 12p12.2 from our GWAS in the Rotterdam consensus on diagnostic criteria of PCOS (Table 2). In addition, we further confirmed reproducible associations of *GYS2* with more stringent NIH definition (data not shown).

In an attempt to determine diverse effects of the identified *GYS2* as a predisposing factor for PCOS, we performed a linear regression analysis for BMI. In addition to genotyped three SNPs (rs10841843, rs6487237 and rs7485509), we also found 14 additional imputed associations between variants in *GYS2* and BMI (Table 2). To clarify whether our PCOS signals were driven by BMI, we performed the association analysis with adjustment for BMI. The result showed that all association signals of *GYS2* remained significant after the adjustment with BMI. This result indicates that PCOS signals that we identified in our study were not driven as the secondary association result for BMI.

To validate the possible relationship between PCOS and obesity, we next investigated an association of *GYS2* in an independent childhood obesity study ($n=482$). *GYS2* variants were associated with BMI ($P=0.051$). We further investigated pleiotropic associations in a GD study ($n=1710$). Our lookup study revealed moderate associations

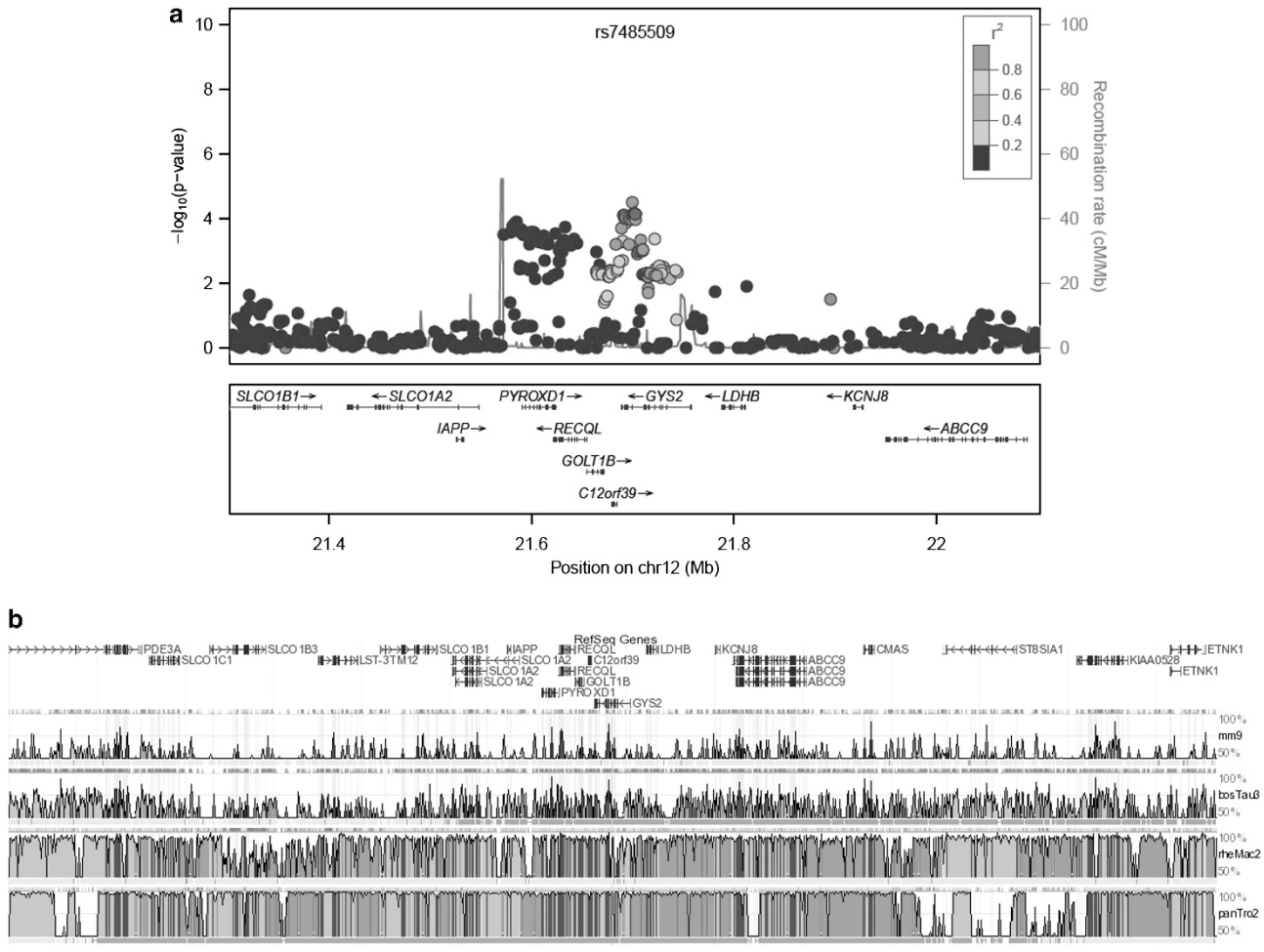

Figure 1 Result of the GWAS. (**a**) The blue-to-red color gradient reflects lower-to-higher LD values (r^2). The scatter plot and peak indicate the negative logarithm of *P*-values for each SNP. Overall and discovery *P*-values are represented by red and blue diamonds, respectively. Imputed SNPs are represented by gray circles. (**b**) Sequence conservation in four mammalian species (Chimpanzee (pan), monkey (rhe), cow (bos) and mouse (mm9)) relative to the human sequence (http://ecrbrowser.dcode.org/). Blue and yellow correspond to coding exons and untranslated regions, respectively. Peaks within the conserved profile that do not correspond to transcribed sequences are highlighted in red (intergenic) or salmon (intronic). Regions colored in green correspond to transposable elements and simple repeats. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Table 1 Clinical and biochemical characteristics of PCOS cases and controls

	Cases (774)	*Controls (967)*
AGE	23.76 ± 4.72	26.55 ± 4.54
BMI	22.57 ± 4.17	21.21 ± 2.74
WAIST	76.02 ± 10.31	72.72 ± ± 7.30
FG_SCORE	1.65 ± 2.82	0.81 ± 1.52
FREE_T	0.90 ± 0.48	0.41 ± 0.20
TOTAL_T	67.06 ± 20.30	46.03 ± 15.52

Abbreviations: BMI, body mass index; FG, Ferriman–Gallwey; PCOS, polycystic ovary syndrome; T, testosterone (ng dl^{-1}).

for gestational diabetogenic ($P = 0.015$) and obesogenic ($P = 0.059$) effects, respectively.

DISCUSSION

PCOS is a heterogeneous endocrinopathy with a prevalence of ~5–10% in women of reproductive age. Women with PCOS predominantly suffer from metabolic disturbances, in particular from insulin resistance. What are dependent or independent variables in metabolic traits associated with PCOS? Experts still are not certain whether women with PCOS tend to make it easier to gain weight, or obese women may be more prone to develop PCOS. Preliminary association studies are necessary to elucidate possible relationships. In this study, we investigated whether PCOS-associated genes or variants contribute to obesity as a risk factor of metabolic and endocrine derangements.

Obesity has a negative effect on the clinical manifestation of PCOS, affecting 20–50% of white European and up to 50% of women in the United Kingdom form South Asia.[26,30] Obese PCOS women have more severe hyperandrogenism and related clinical features such as hirsutism, irregular menstrual cycle and anovulation than non-obese PCOS women. A further clinical and endocrinological improvement in obese women with PCOS can be reversed by weight loss.[31] These facts obviously emphasize that obesity may have an important pathogenic role in the development of the syndrome in susceptible individuals.

Recent studies suggested that endoplasmic reticulum stress, leading to accumulation of misfolded/unfolded proteins in endoplasmic

Table 2 Association statistics of *GYS2* in the GWAS of PCOS and BMI

CHR	SNP	Position (bp)	Major allele	Minor allele	MAF	PCOS		BMI	
						OR (95% CI)	P	$\beta \pm$ s.e.m.	P
12	rs2417995	21581644	C	A	0.2182	0.70 (0.59–0.83)	7.60×10^{-5}	-0.35 ± 0.15	1.83×10^{-2}
12	rs10841842	21582041	T	A	0.2182	0.70 (0.59–0.83)	7.60×10^{-5}	-0.35 ± 0.15	1.83×10^{-2}
12	**rs10841843***	21583158	C	T	0.2184	0.70 (0.59–0.84)	8.02×10^{-5}	-0.35 ± 0.15	1.80×10^{-2}
12	rs10492118	21583225	C	T	0.2027	0.69 (0.58–0.83)	9.71×10^{-5}	-0.42 ± 0.15	6.19×10^{-3}
12	rs1871130	21584216	G	A	0.2029	0.69 (0.58–0.83)	9.32×10^{-5}	-0.42 ± 0.15	6.48×10^{-3}
12	rs1871129	21584510	A	T	0.2029	0.69 (0.58–0.83)	9.32×10^{-5}	-0.42 ± 0.15	6.48×10^{-3}
12	rs1871128	21584511	T	A	0.2029	0.69 (0.58–0.83)	9.32×10^{-5}	-0.42 ± 0.15	6.48×10^{-3}
12	rs1904121	21590775	C	T	0.2032	0.68 (0.57–0.82)	3.08×10^{-5}	-0.32 ± 0.15	3.01×10^{-2}
12	rs6487236	21591183	A	G	0.2241	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}
12	**rs6487237***	21591195	A	C	0.2187	0.70 (0.59–0.84)	8.14×10^{-5}	-0.34 ± 0.15	2.10×10^{-2}
12	rs6487238	21591206	A	G	0.2189	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}
12	rs7962754	21591762	G	T	0.2187	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}
12	rs1580300	21592417	A	T	0.2187	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}
12	rs2126885	21592704	T	C	0.2187	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}
12	**rs7485509***	21593272	T	C	0.2187	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}
12	rs7485489	21593287	T	A	0.2187	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}
12	rs2169745	21593770	C	G	0.2187	0.70 (0.58–0.83)	6.97×10^{-5}	-0.34 ± 0.15	2.07×10^{-2}

Abbreviations: BMI, body mass index; bp, base pair; CHR, chromosome; CI, confidence interval; MAF, minor allele frequency; OR, odd ratio; PCOS, polycystic ovary syndrome; SNP, single-nucleotide polymorphism.
The genotyped SNPs (rsID*) are represented in bold. The others refer to imputed SNPs. Position in bp is from the NCBI build 36. Effect sizes are listed as $\beta \pm$ s.e.m.

reticulum tubules and impaired endoplasmic reticulum function, was associated with obesity and insulin resistance.[32,33] Moreover, the intrinsic follicular dysplasia associated with the ovarian granulosa cell apoptosis was observed with significantly higher apoptotic rates in granulosa cells of patients with PCOS. It was suggested that endoplasmic reticulum stress might be involved in the pathogenesis of anovulation in patients with PCOS. Obesity impairs insulin resistance and exacerbates reproductive and metabolic features of PCOS. It is also associated with obstetric and pregnancy complications such as premature delivery, miscarriage and GD.[18,34] Some investigators reported that women with PCOS are at increased risk for GD.[35–37]

In our study, we performed a GWAS of PCOS as a first step and followed up with multiple association studies of obesity, and identified *GYS2* gene as a new susceptibility gene that significantly impacts the risk for PCOS through obesity-related conditions. The human *GYS2* at 12p12.2, which encodes for a rate-limiting liver glycogen synthase, is an enzyme responsible for the synthesis of 1, 4-linked glucose chains in glycogen and its activity is highly regulated through phosphorylation at multiple sites and by allosteric effectors, mainly glucose 6-phosphate. Previous studies reported that defects in *GYS2* gene cause the inherited monogenic disease glycogen storage disease 0.[38,39] In addition, *GYS2* gene was identified as one of the adipose tissue-enriched genes contributing to obesity from a stratified transcriptomics analysis.[40]

A recent GWAS and replication study identified three new susceptibility loci on chromosome 2p16.3, 2p21 and 9q33.3 and a novel *YAP1* gene for PCOS in Han Chinese.[10,41] We also evaluated the significance in the previously reported 7 genome-wide significant loci for PCOS (rs13405728 at 2p16.3; rs12468394, rs13429458 and rs12478601 at 2p21; rs10818854, rs2479106 and rs10986105 at 9q33.3). Except for rs2479106 in *DENND1A*, the six of seven SNPs had a significant *P*-value between 2×10^{-2} and 8×10^{-4}. In addition, *follicle stimulating hormone receptor*, a candidate gene for PCOS, was shown to be associated with PCOS in Korean women ($2.2 \times 10^{-3} < P < 5.9 \times 10^{-4}$), compared with Chinese ($2 \times 10^{-3} < P < 4 \times 10^{-4}$). In candidate gene-based approaches, well-known diabetogenic and metabolic insulin signaling-mediated genes were confirmed with risk of PCOS.[20,21,42] Given the correlation among these phenotypes, environment-wide association studies might have to be considered to identify potential pleiotropic variants and major pathophysiological factors.

In conclusion, a *GYS2* gene on chromosome 12p12.2 was identified in a PCOS GWAS through obesity-related condition, and confirmed further associations in an independent childhood obesity study and a GD study. Our data demonstrate a pleiotropic association between obesity and PCOS, the possible relationship remains to be clarified with combined analyses in a well-established international consortium of large cohorts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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