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ORIGINAL RESEARCH

Identification of Genetic Variants for Female Obesity and Evaluation of the Causal Role of Genetically Defined Obesity in Polycystic Ovarian Syndrome

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Purpose: Observational studies have demonstrated an increased risk of polycystic ovarian syndrome (PCOS) in obese women. This study aimed to identify genetic variants influencing obesity in females and to evaluate the causal association between genetically defined obesity and PCOS in Korean women.

Methods: Two-stage GWAS was conducted to identify genetic variants influencing obesity traits (such as body mass index [BMI], waist–hip ratio [WHR], and waist circumference [WC]) in Korean women. Two-sample Mendelian randomization (MR) analysis was employed to evaluate the causal effect of variants as genetic instruments for female obesity on PCOS.

Results: Meta-analysis of 9953 females combining discovery (N = 4658) and replication (N = 5295) stages detected four (rs11162584, rs6760543, rs828104, rs56137030), six (rs139702234, rs2341967, rs73059848, rs5020945, rs550532151, rs61971548), and two genetic variants (rs7722169, rs7206790) suggesting a highly significant association ($P < 1\times10^{-6}$) with BMI, WHR, and WC, respectively. Of these, an intron variant rs56137030 in *FTO* achieved genome-wide significant association ($P = 3.39\times10^{-8}$) with BMI in females. Using variants for female obesity, their effect on PCOS in 946 cases and 976 controls was evaluated by MR analysis. MR results indicated no significant association between genetically defined obesity and PCOS in Korean women.

Conclusion: This study, for the first time, revealed genetic variants for female obesity in the Korean population and reported no causal association between genetically defined obesity and PCOS in Korean women.

Keywords: female obesity, polycystic ovarian syndrome, causal relation, genome-wide association study, Mendelian randomization

Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and/or increased health problems. Obesity acts as an important risk factor for various diseases, increasing the risk of high blood pressure, type 2 diabetes, and dyslipidemia in obese people more than in those with adequate weight.[1] Body mass index (BMI) and the waist–hip ratio (WHR) or waist circumference (WC) are the commonly used anthropometric measurements to measure general obesity and

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abdominal (central) obesity, respectively. Environmental and genetic factors influence obesity. Numerous genetic studies have been conducted to gain insight into the genetic basis of obesity.[2–7] Genome-wide association studies (GWAS) have identified a large number of loci for BMI,[4] WHR,[6,8] and WC.[6]

Obesity in women is associated with alterations in the reproductive cycle, including reduced fertility, as well as an increased risk of polycystic ovarian syndrome (PCOS) and oligo-ovulation or anovulation.[9–13] Furthermore, the tendency toward menstrual and ovarian disturbances associated with obesity may predispose women to an increased risk of ovarian, breast, and endometrial cancer.[14]

PCOS is a common endocrine disorder among women of reproductive age. PCOS is characterized by a variety of phenotypes, such as menstrual dysfunction, hyperandrogenism, and metabolic abnormalities with ethnic differences.[15] Accurate diagnosis is difficult because the diagnostic criteria are variable. Three diagnostic criteria have been proposed: the 1990 National Institutes of Health criteria, the 2003 European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine criteria, and the 2006 Androgen Excess Society criteria. The exact pathogenesis of PCOS has not been fully elucidated. PCOS is a complex disorder that is influenced by environmental and genetic factors.[16] Early diagnosis and treatment along with weight loss may reduce the risk of long-term complications, such as type 2 diabetes and heart disease.

Mendelian randomization uses genetic variants to determine whether an observational association between a risk factor and an outcome is consistent with a causal effect. Mendelian randomization relies on the natural, random assortment of genetic variants during meiosis yielding a random distribution of genetic variants in a population. Individuals are naturally assigned at birth to inherit a genetic variant that affects or does not affect a risk factor. The Mendelian randomization approach exploits the fact that genotype precedes life events and is therefore not affected by lifestyle, socioeconomic variables, or any factor that follows conception.[17] The basic principles of Mendelian randomization can be understood through comparison with randomized clinical trials (RCTs). RCTs are costly and time-consuming and may be impractical to carry out, or there may not be an intervention to test a certain hypothesis, limiting the number of clinical questions that can be answered by an RCT.

In this study, we conducted a meta-analysis of GWASs to identify female-specific genetic variants associated with obesity traits, such as BMI, WHR, and WC, in Korean females. The identified variants for each obesity trait were used as genetic instruments for the subsequent Mendelian randomization analysis to assess the causal relationship between obesity and PCOS.

Methods

Subjects

Subjects for the discovery stage were recruited from the Korean Association Resource study (KARE) cohort, a population-based cohort of 8842 participants. Details of the participant recruitment criteria and the study design have been provided elsewhere.[18–20] A total of 4658 females were present in the KARE study cohort (Supplementary Figure 1 and Table 1). Participants included in the studies used for the replication stage included unrelated Korean participants from the Health Examinee shared control study (HEXA) cohort,[21] as well as the Cardiovascular disease association study (CAVAS)

Table 1 Clinical Characteristics of Female Subjects in Study Cohorts

Variable	KARE	HEXA	CAVAS1816	CAVAS3667
N (female/male)	4658/4182	2048/1647	957/859	2290/1375
Age (year)	52.6(9.02)	51.6(7.66)	60.85(6.42)	58.87(10.00)
BMI	24.9(3.26)	23.7(3.0)	25.24(3.42)	23.96(3.09)
WHR	0.87(0.086)	0.84(0.06)	0.89(0.06)	0.89(0.07)
WC (cm)	81.7(9.62)	79.2(8.36)	85.1(8.50)	82.14(8.91)
FPG (mg/dL)	85.23(19.85)	91.29(26.08)	114.80(43.32)	93.05(9.33)
HbA1C (%)	5.79(0.93)	na	na	na
T2D (case/control)	563/2059	81/1709	306/410	na

Note: Numbers indicate average and standard deviation for each variable.
Abbreviations: BMI, body mass index; WHR, waist–hip ratio; WC, waist circumference; FPG, fasting plasma glucose; HbA1C, hemoglobin A1c; T2D, type 2 diabetes; na, not available.

cohort (formerly RURAL cohort).[19] The HEXA cohort consisted of 3695 subjects of which 2048 were females. The CAVAS cohort included two sub-studies of CAVAS1816 and CAVAS3665. The CAVAS1816 study included 1816 subjects, of which 957 were females. A total of 2290 female subjects were selected from the 3665 subjects in the CAVAS3667 study (Supplementary Figure 1 and Table 1). The study design and cohort characteristics of the CAVAS cohort have been described previously.[19]

Subjects for the PCOS GWAS consisting of 1000 PCOS cases and 1000 controls were recruited from the endocrinology and gynecology clinics at Ewha Woman's University Hospital for the Mendelian randomization analysis between obesity traits and PCOS. Details of the participant recruitment criteria and the study design have been described previously.[22]

Genotyping, Imputation, and Quality Control

Single nucleotide polymorphism (SNP) genotyping was carried out for the KARE study subjects using an Affymetrix Genome-wide Human SNP array 5.0. Genotyping using an Affymetrix Genome-Wide Human SNP array 6.0 was carried out for the HEXA and CAVAS1816 study subjects. The CAVAS3667 study subjects were genotyped using the Illumina HumanOmni I-Quad vI array. Samples with a genotyping missing call rate >1% and heterozygosity >30% were excluded from the sample pool. Markers with a missing SNP call rate >5%, with a minor allele frequency (MAF) <0.01, and a Hardy–Weinberg equilibrium (HWE) test *P*-value $<1 \times 10^{-6}$ were eliminated. Details on genotyping quality control for the genotype data have been described previously.[18–21]

Subjects from the Ewha Woman's University Hospital PCOS GWAS were genotyped using the HumanOmni-Quad v I array (Illumina). Samples with a high missing genotype call rate (>2%) or high heterozygosity (>30%) were excluded. Also, subjects whose computed average pairwise identity-by-state value were higher than that estimated from first-degree relatives of Korean sib-pair samples (>0.8) were excluded from analyses. Markers with a high missing gene call rate (>1%), low MAF (<0.05), and a significant deviation from HWE *P*-value $<1 \times 10^{-6}$ were excluded. The remaining 636,870 SNPs in 1922 samples (976 cases and 946 controls) were used for association in subsequent analyses.[22]

For the genotype data, including the KARE, HEXA, CAVAS1816, and CAVAS3667 studies, SNP imputation was performed to increase the coverage of common variants employing the minimac3 program with 1000 Genome Phase3 individuals as the imputation reference panel.[23] For the genotype data of the Ewha Woman's University Hospital PCOS study, SNP imputation was performed using the IMPUTE2 program with 1000 Genome phase3 individuals as the imputation reference panel.[24] Imputed SNPs of poor imputation quality (Rsq <0.3 for minimac3 imputation and info score <0.5 for IMPUTE2 imputation) were excluded. In addition, imputed SNPs with missing gene call rates >1% (MAF < 0.01, and HWE test *P*-value $<1 \times 10^{-7}$) were omitted for subsequent analyses.

Phenotype Measurements

Based on the World Health Organization Asia-Pacific Perspective 2000, subjects with BMI $\geq$ 25 kg/m^2 were grouped as obese while those with 18.5 $\leq$ BMI $\leq$ 22.9 kg/m^2 were considered normal. The WHR diagnostic standard for obesity is 0.85 for women and 0.95 for men (Korean Society for the Study of Obesity). The WC diagnostic standard for obesity is 85 cm (34 inches) for women and 90 cm (36 inches) for men (Korean Society for the Study of Obesity).[25]

In this study, PCOS patients were diagnosed according to the Rotterdam criteria, which have been described elsewhere.[22] Individuals with specific disorders, such as adult-onset congenital adrenal hyperplasia, hyperprolactinemia, and androgen-secreting neoplasia, were excluded from the study. Patients taking medications (eg, steroids, oral contraceptives, metformin, or thiazide diuretics) before starting the study were excluded. Among the regular-cycling volunteers, 1000 women were recruited to serve as the control group. None of the controls had a family history of diabetes or PCOS. Subjects were excluded if they had been on hormonal medication within 3 months of the evaluation or had used other drugs that could affect basal parameter status.

Statistical Analyses

The associations between the genetic variants and each obesity trait (BMI, WHR, and WC) were tested after adjusting for age and participant area. Single variant tests for the imputed genotype data of the four GWAS datasets (KARE, HEXA,

CAVAS1816, and CAVAS3667 studies) were performed using the Score test in the rvtests software package.[26] A meta-analysis using the METAL program[27] was performed combining the four GWAS results based on the inverse-variance weighting method assuming fixed effects with Cochran's Q test used to assess between-study heterogeneity.[28]

Genetic variants for the obesity traits identified from the meta-analysis were tested for their association with PCOS from the Ewha Woman's University Hospital PCOS imputation dataset. Frequentist association tests were conducted for the PCOS case-normal control analysis in an additive model using the SNPTEST program.[29]

Functional Annotation of Associated Loci

For the functional annotation, obesity trait-associated variants were investigated for their overlap with Expression Quantitative Trait Loci (eQTLs) from the GTEx portal (https://www.gtexportal.org/home/).[30] Expressed genes (eGenes) showing nominal association (P-value $<10^{-4}$) between obesity GWAS variants and their expression in each tissue were identified in the GTEx dataset and assigned as the candidate genes. If a variant is not an eQTL, the gene in which a variant is located or closest to a variant is assigned as the candidate gene.

Mendelian Randomization Analysis

A two-sample Mendelian randomization (MR) analysis was performed to investigate the existence of a causal relationship between obesity and PCOS. Summary association results from the non-overlapping obesity and PCOS sets of individuals were used for the two-sample MR in this study. The odds of PCOS risk were divided by the β coefficients of the levels of obesity traits (BMI, WHR, or WC) to determine ratio estimates for each instrumental variable (IV) (here, genetic variants associated with obesity traits). The effects of the individual genetic instruments were combined using inverse-variance weighted (IVW) analysis, resulting in a weighted mean estimate of the risk of PCOS per 1-standard deviation increase in the levels of obesity traits (BMI, WHR, or WC). The MendelianRandomization package in R statistical software (https://CRAN.R-project.org/package=MendelianRandomization) was used to perform the two-sample MR analysis.[31]

Results

Identification of Genetic Variants for Obesity Traits in Females

We conducted a two-stage sex-stratified GWAS to discover the genetic loci for obesity traits in females. In stage 1, the discovery stage, SNPs across the whole genome were tested for their association with one of the obesity traits (BMI, WHR, or WC) in 4658 female subjects from the KARE study. SNPs selected from the stage 1 linear analysis (P-value <0.05) after adjusting for area and age were taken forward to stage 2, the replication stage. A total of 5295 subjects were included for the replication analysis of female subjects from the HEXA, CAVAS1816, and CAVAS3667 studies. A meta-analysis was conducted for SNPs that were validated for their association with obesity traits in the replication stage by combining the association results from the discovery and replication stages (Supplementary Figure 1).

Our meta-analysis in female subjects identified four SNPs showing evidence of a suggestive association (P-value $<10^{-6}$) with BMI. Of those, three SNPs (rs11162584, rs6760543, and rs828104) were female-specific without showing an association with BMI in male subjects. One SNP rs56137030 in *FTO* showed a genome-wide significant association with BMI in females (P-value = 3.39 X 10^{-8}) but it also showed a nominal association with BMI in males (P-value = 2.00 X 10^{-2}). The meta-analysis for WHR and WC identified six and two suggestively associated SNPs (P-value $<10^{-6}$), respectively. Of those, four WHR (rs139702234, rs73059848, rs550532151, and rs61971548) and two WC (rs7722169 and rs7206790) associated SNPs were female-specific (Table 2, Figure 1, and Supplementary Figure 2).

Investigation of Functional Relevance of GWAS Loci to Obesity Traits

If a variant responsible for a GWAS locus also affects gene expression, it is known that the relevant gene could be involved in the biological mechanism of disease pathogenesis.[32] In this context, to gain insight into the functional relevance of genetic variants identified from our GWA meta-analysis to female obesity, we investigated their effects on gene expression from the GTEx portal.

SNP rs6760543 showing association for BMI locates between *LOC105373352* and *TMEM18*. The GTEx

Table 2 Obesity Traits (BMI, WHR, and WC) Associated Loci in Females. Discovery Stage Was GWAS for Obesity Traits in Each Sex-Stratified Group of KARE Cohort. Overall Association Results (P_{meta}) Were Obtained from Meta-Analyses Combining Discovery (KARE) and Replication (HEXA, CAVAS1816, CAVAS3667) Stages

Obesity Trait	CHR	SNP	BP (GRCh37)	EA	EAF	Female										Male (N = Up to 8063)		Females + Males (N = Up to 18,016)	
						Discovery		Replication						Overall (N = Up to 18,016)					
						KARE		HEXA		CAVAS1816		CAVAS3667							
						Beta (se)	*P*	Beta (se)	*P*	Beta (se)	*P*	Beta (se)	*P*	Beta (se)	P_{meta}	Beta (se)	P_{meta}	Beta (se)	P_{meta}
BMI	1	rs11162584	79,444,706	A	0.19	0.335 (0.087)	1.15E-05	0.330 (0.113)	3.60E-03	0.093 (0.510)	8.60E-01	0.304 (0.162)	6.20E-02	0.325 (0.063)	2.43E-07	0.105 (0.061)	8.70E-02	0.216 (0.045)	1.37E-06
	2	rs6760543	622,388	G	0.09	−0.399 (0.116)	6.10E-04	−0.558 (0.159)	4.50E-05	−0.766 (0.368)	3.70E-02	−0.109 (0.255)	6.70E-01	−0.432 (0.086)	4.50E-07	−0.113 (0.085)	1.80E-01	−0.279 (0.062)	5.96E-06
	9	rs828104	128,014,635	G	0.41	0.229 (0.068)	7.30E-04	0.096 (0.093)	3.00E-01	0.155 (0.157)	3.20E-01	0.363 (0.091)	6.78E-05	0.225 (0.045)	5.96E-07	0.021 (0.046)	6.50E-01	0.133 (0.033)	4.66E-05
	16	rs56137030§	53,825,905	A	0.13	0.426 (0.102)	2.65E-05	0.317 (0.143)	2.70E-02	0.453 (0.228)	4.60E-02	0.324 (0.151)	3.00E-02	0.382 (0.069)	3.39E-08	0.157 (0.068)	2.00E-02	0.274 (0.049)	2.58E-08
WHR	3	rs139702234	12,310,506	A	0.02	0.016 (0.007)	1.20E-02	0.030 (0.006)	4.66E-06	−0.026 (0.042)	5.30E-01	0.024 (0.023)	3.10E-01	0.023 (0.005)	5.20E-07	0.003 (0.005)	5.70E-01	0.010 (0.004)	4.16E-03
	3	rs2341967§	14,467,263	A	0.27	−0.008 (0.002)	9.83E-07	−0.003 (0.002)	2.10E-01	−0.003 (0.004)	4.60E-01	−0.005 (0.003)	6.60E-02	−0.006 (0.001)	5.49E-07	−0.003 (0.001)	1.90E-02	−0.002 (0.001)	5.91E-02
	3	rs73059848	187,100,086	T	0.02	−0.022 (0.005)	4.56E-06	na	na	na	na	−0.081 (0.022)	2.20E-04	−0.024 (0.005)	1.51E-07	−0.003 (0.004)	5.50E-01	−0.016 (0.003)	2.96E-06
	6	rs5020945§	32,450,134	C	0.49	0.004 (0.001)	9.30E-04	0.006 (0.002)	6.80E-04	0.005 (0.003)	6.50E-02	0.003 (0.002)	1.80E-01	0.005 (0.001)	3.43E-07	0.002 (0.001)	3.00E-02	0.004 (0.001)	1.49E-07
	13	rs550532151	75,487,171	C	0.01	0.035 (0.008)	1.45E-05	0.031 (0.017)	6.20E-02	na	na	0.248 (0.066)	1.70E-04	0.037 (0.007)	3.48E-07	0.000 (0.006)	9.70E-01	0.021 (0.005)	6.14E-05
	14	rs61971548	52,476,539	T	0.20	−0.004 (0.002)	2.50E-02	−0.009 (0.002)	5.32E-05	−0.010 (0.004)	2.10E-02	−0.007 (0.003)	1.50E-02	−0.006 (0.001)	1.42E-07	0.001 (0.001)	6.20E-01	−0.004 (0.001)	4.54E-05
WC	5	rs7722169	3,141,415	T	0.09	−1.579 (0.320)	7.93E-07	−0.421 (0.425)	3.20E-01	−1.508 (1.356)	2.70E-01	−1.806 (0.769)	1.90E-02	−1.233 (0.239)	2.38E-07	0.029 (0.227)	8.99E-01	−0.641 (0.175)	2.25E-04
	16	rs7206790	53,797,908	G	0.15	0.973 (0.257)	1.52E-04	1.225 (0.371)	9.72E-04	1.019 (0.502)	4.20E-02	0.199 (0.400)	6.20E-01	0.887 (0.175)	4.14E-07	0.079 (0.174)	6.50E-01	0.425 (0.130)	1.03E-03

Notes: Information for the SNP ID and chromosomal position is based on NCBI genome build 37/hg19. SNPs marked with § are not female-specific.

Abbreviations: CHR, chromosome; BP, physical position (base-pair); EA, effect allele; EAF, effect allele frequency; se, standard error; BMI, body mass index; WHR, waist–hip ratio; WC, waist circumference; na, not available.

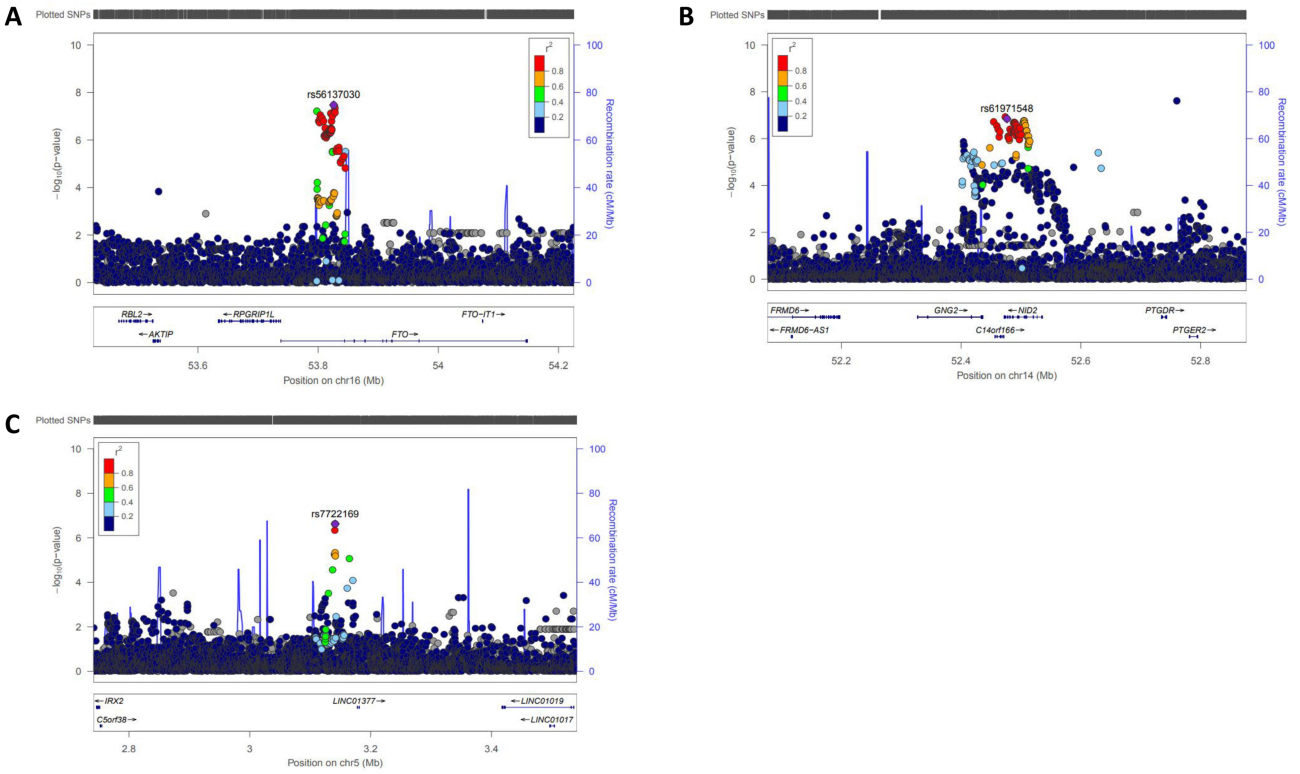

Figure 1 Regional association plots of rs56137030 (**A**), rs61971548 (**B**), and rs7722169 (**C**), showing the evidence of association in females for BMI, WHR, and WC, respectively. In the top panel of each, each SNP is plotted as a circle along the chromosomal position. Association analysis results represented as $-\log_{10}P$ for SNPs (y-axis on left) are shown in a genomic region 400 kb to either side of the lead SNP (shown as a purple diamond). Recombination rates (cM/Mb) within loci are estimated from 1000 Genomes Phase 3 ASN and indicated as blue lines (y-axis on right). The magnitude of pair-wise linkage disequilibrium (LD) between the lead SNP and other SNPs is demonstrated by color, ranging from high (red) to low (blue). In the bottom panels, the locations of known genes are indicated in the region. Genomic positions are based on GRCh37/hg19.

Abbreviations: BMI, body mass index; WHR, waist–hip ratio; WC, waist circumference.

eQTL dataset indicated that this variant was also associated with *ALKAL2* expression in subcutaneous adipose tissue (P-value = 3.50 X 10^{-5}). Another BMI associated variant rs828104 showed association with the expression of *GAPVD1* in subcutaneous adipose tissue (P-value = 2.10 X 10^{-8}) and *PRPS1P2* in visceral adipose tissue (P-value = 8.50 X 10^{-5}). SNP rs56137030 for BMI also was associated with the gene expression of *FTO* in skeletal muscle (P-value = 4.10 X 10^{-7}) as well as *IRX3* in pancreas (P-value = 4.30 X 10^{-6}) (Table 3).

Among variants for WHR or WC, only eQTL information of rs61971548 was available in the GTEx dataset. This variant showed an association with *NID* expression in thyroid (P-value = 4.70 X 10^{-6}). For WHR or WC variants that had no significant eQTLs or no available eQTL information in the GTEx dataset, the candidate gene was assigned to be the one where a variant is located or closest to a variant (Table 3).

Evaluation of the Causal Role of Genetically Defined Obesity in PCOS

We conducted two-sample Mendelian randomization (MR) to assess the causal correlation between genetically defined obesity traits and PCOS. The key assumptions of two-sample MR are (1) the IV is causally related to the risk factor; (2) confounding factors of the association between risk factors and outcome should not be related to the IV; and (3) the IV only affects the outcome through its effect on the risk factors. In this study, IVs were genetic variants associated with a risk factor; the risk factor is one of the obesity traits (BMI, WHR, or WC), and the outcome is PCOS. Type 2 diabetes and related traits, such as fasting plasma glucose (FPG) and glycated hemoglobin (HbA1C), were considered confounding factors (Figure 2).

Association analyses of individual genetic instruments (SNPs) for the risk factors (obesity traits) with

Table 3 eQTLs of GWAS Variants for Obesity Traits

Trait	CHR	rsID	BP (GRCh37)	EA	GWAS				eQTL				
					P_{meta}	Beta	Near Gene	Functional Consequence	P_{eQTL}	NES	eGene	Gencode ID	Tissue
BMI	1	rs11162584	79,444,706	A	2.43E-07	0.325	*ADGRL4*	intron variant	na	na	na	No significant eQTLs	na
	2	rs6760543	622,388	G	4.50E-07	−0.432	*LOC105373352/ TMEM18*	intergenic	3.50E-05	−0.16	*ALKAL2*	ENSG00000189292.15	Adipose - Subcutaneous
	9	rs828104	128,014,635	G	5.96E-07	0.225	*LOC107987127*	intron variant	2.10E-08	0.12	*GAPVD1*	ENSG00000165219.21	Adipose - Subcutaneous
									8.50E-05	−0.16	*PRPS1P2*	ENSG00000232630.1	Adipose - Visceral (Omentum)
	16	rs56137030[§]	53,825,905	A	3.39E-08	0.382	*FTO*	intron variant	4.10E-07	0.13	*FTO*	ENSG00000140718.20	Muscle - Skeletal
									4.30E-06	0.38	*IRX3*	ENSG00000177508.11	Pancreas
WHR	3	rs139702234	12,310,506	A	5.20E-07	0.023	*PPARG*	up stream	na	na	na	na	na
	3	rs2341967[§]	14,467,263	A	5.49E-07	−0.006	*SLC6A6*	intron variant	na	na	na	No significant eQTLs	na
	3	rs73059848	187,100,086	T	1.51E-07	−0.024	*RTP4*	down stream	na	na	na	No significant eQTLs	na
	6	rs5020945[§]	32,450,134	C	3.43E-07	0.005	*HLA-DRB9/HLA-DRB5*	intergenic	na	na	na	na	na
	13	rs550532151	75,487,171	C	3.48E-07	0.037	*LOC107984620*	down stream	na	na	na	na	na
	14	rs61971548	52,476,539	T	1.42E-07	−0.006	*NID2, RTRAF*	intron variant and 3′-utr variant	4.70E-06	0.19	*NID2*	ENSG00000087303.17	Thyroid
WC	5	rs7722169	3,141,415	T	2.38E-07	−1.233	*LINC01377*	long intergenic non-protein coding RNA 1377	na	na	na	No significant eQTLs	na
	16	rs7206790	53,797,908	G	4.14E-07	0.887	*FTO*	intron variant	na	na	na	No significant eQTLs	na

Notes: Information for the SNP ID and chromosomal position is based on NCBI genome build 37/hg19. SNPs marked with § are not female-specific. eQTL data were available from the GTEx portal.
Abbreviations: eQTL, expression quantitative trait loci; CHR, chromosome; BP, physical position (base-pair); NES, normalized effect size; BMI, body mass index; WHR, waist–hip ratio; WC, waist circumference; na, not available.

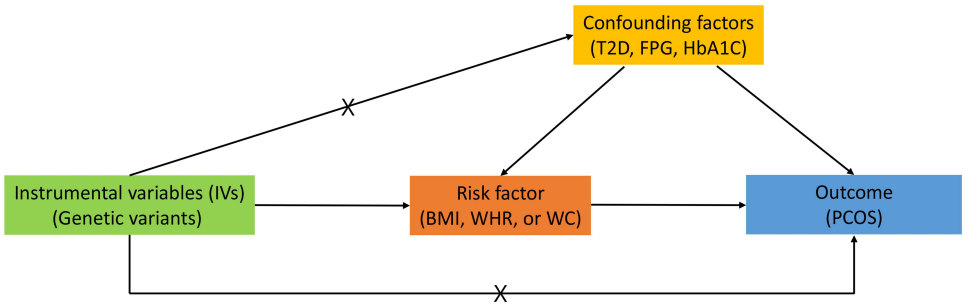

Figure 2 Diagram displaying the components of the Mendelian randomization. Genetic variants as the instrumental variables are associated with biomarker (or exposure), but not with confounding factors as well as with outcome disease. Biomarker is a modifiable risk factor for outcome disease.

the outcome (PCOS) as well as confounding factors (type 2 diabetes and related traits) demonstrated that 2 of 12 SNPs were associated with confounding factors (rs6760543, $P_{T2D} = 3.2 \times 10^{-3}$, $P_{FPG} = 1.7 \times 10^{-2}$, $P_{HbA1C} = 1.4 \times 10^{-2}$; rs2341967, $P_{T2D} = 2.8 \times 10^{-2}$) (Table 4). To fulfill the key assumptions, we conducted a two-sample MR analysis using nine SNPs after excluding two SNPs showing an association with confounding factors and one SNP (rs550532151) that was not available in the PCOS dataset (Table 4). Our MR results indicated no association between genetically defined obesity and PCOS in Korean women (Table 5 and Figure 3).

Discussion

Abnormalities in women's health are caused by a variety of factors. An individual's lifestyle, genetics, hormonal imbalances, and ethnicity all play a role in women's health. Obesity in women is associated with alterations in the reproductive cycle with a reduction in fertility, as well as an increased risk of PCOS and oligo-ovulation or anovulation. As obesity is strongly influenced by genetic and environmental factors (heritability 40–70%), numerous genetic studies have been conducted in diverse ethnic groups. However, a sex-stratified genetic study for obesity has never been carried out specifically in an East Asian

Table 4 Association of Individual Genetic Instruments for Obesity Traits with PCOS Risk

CHR	SNP	BP (GRCh37)	EA	Risk Factors (Obesity Traits)			Outcome (PCOS)			§Confounding Factors (T2D Traits)		
				Beta	se	$P_{obesity}$	Beta	se	P_{PCOS}	P_{T2D}	P_{FPG}	P_{HbA1C}
BMI-associated SNPs												
1	rs11162584	79,444,706	A	0.325	0.063	2.43E-07	−0.055	0.082	4.98E-01	2.33E-01	9.62E-01	1.15E-01
2	rs6760543	622,388	G	−0.432	0.086	4.50E-07	−0.118	0.109	2.78E-01	3.19E-03	1.69E-02	1.40E-02
9	rs828104	128,014,635	G	0.225	0.045	5.96E-07	0.086	0.066	1.95E-01	5.67E-01	8.12E-01	7.36E-01
16	rs56137030	53,825,905	A	0.382	0.069	3.39E-08	−0.177	0.098	6.98E-02	7.92E-01	1.32E-01	9.05E-01
WHR-associated SNPs												
3	rs139702234	12,310,506	A	0.023	0.005	5.20E-07	0.253	0.279	3.62E-01	3.57E-01	6.31E-01	8.68E-01
3	rs2341967§	14,467,263	A	−0.006	0.001	5.49E-07	−0.025	0.084	7.64E-01	2.78E-02	3.01E-01	4.46E-01
3	rs73059848	187,100,086	T	−0.024	0.005	1.51E-07	0.079	0.226	7.28E-01	1.27E-01	1.77E-01	4.44E-01
6	rs5020945	32,450,134	C	0.005	0.001	3.43E-07	0.019	0.071	7.84E-01	7.19E-02	1.92E-01	6.10E-02
13	rs550532151	75,487,171	C	0.037	0.007	3.48E-07	na	na	na	2.86E-01	8.36E-01	6.79E-01
14	rs61971548	52,476,539	T	−0.006	0.001	1.42E-07	−0.071	0.078	3.65E-01	3.22E-01	9.35E-01	7.69E-01
WC-associated SNPs												
5	rs7722169	3,141,415	T	−1.233	0.239	2.38E-07	−0.133	0.111	2.28E-01	1.79E-01	7.40E-01	3.66E-01
16	rs7206790	53,797,908	G	0.887	0.175	4.14E-07	−0.027	0.098	7.79E-01	6.43E-01	1.71E-01	9.30E-01

Notes: Information for the SNP ID and chromosomal position is based on NCBI genome build 37/hg19. §Association results were obtained from the KARE study.
Abbreviations: CHR, chromosome; BP, physical position (base-pair); EA, effect allele; se, standard error; BMI, body mass index; WHR, waist–hip ratio; WC, waist circumference; T2D, type 2 diabetes; FPG, fasting plasma glucose; HbA1C, hemoglobin A1c; na, not available.

Table 5 Mendelian Randomization Results for Obesity Traits on PCOS Risk (Inverse-Variance Weighted)

Obesity Trait	Beta	se	*P*-value	[§]Number of SNPs
All	0.023	0.066	0.728	9
BMI	−0.124	0.153	0.420	3
WHR	4.370	5.877	0.457	4
WC	0.053	0.070	0.450	2
[†]VF	0.053	0.070	0.445	6

Notes: [§]Number of SNPs included in the calculation of MR analysis. All combining BMI, WHR, and WC. [†]VF combining WHR and WC.
Abbreviations: BMI, body mass index; WHR, waist–hip ratio; WC, waist circumference; VF, visceral fat.

population. In this regard, we conducted a genome-wide association meta-analysis for obesity traits (such as BMI, WHR, and WC) in about 18,000 Korean female subjects.

The meta-analyses identified genetic variants suggesting associations ($P_{\text{female-meta}} < 10^{-6}$) with BMI (four SNPs), WHR (six SNPs), and WC (two SNPs) (Table 2) in female subjects. Of the 12 associated SNPs with one of the obesity traits, two SNPs (rs56137030 and rs7206790) were located in the intron region of the known obesity gene, *FTO*.[33] An association between SNP rs56137030 and BMI was also detected in males ($P_{\text{male-meta}} = 0.02$) as well as in all subjects ($P_{\text{all-meta}} = 2.58 \times 10^{-8}$). The other *FTO* SNP, rs7206790, was female-specific showing an association with WC only in females ($P_{\text{female-meta}} = 4.14 \times 10^{-7}$) but not in males ($P_{\text{male-meta}} = 0.65$). SNP rs56137030 further showed association with the *FTO* gene expression ($P_{\text{eQTL}} = 4.10 \times 10^{-7}$) in subcutaneous adipose tissue from the GTEx dataset, implying that *FTO* is the causal gene for BMI (Table 3).

SNPs rs6760543 and rs828104 associated with BMI are located between *LOC105373352* and *TMEM18*, and in the intron region of *LOC107987127*, respectively. The eQTL information available from the GTEx dataset demonstrated that rs6760543 and rs828104 showed association with the gene expression of *ALKAL2* ($P_{\text{eQTL}} = 3.50 \times 10^{-5}$) and *GAPVD1* ($P_{\text{eQTL}} = 2.10 \times 10^{-8}$) in subcutaneous adipose tissue, respectively (Table 3). The coding product of *ALKAL2* (*ALK And LTK Ligand 2*) is a ligand for receptor tyrosine kinases LTK and ALK. It has been known that the stimulation of ALK signaling may be involved in regulation of cell proliferation and transformation.[34] The encoded protein of *GAPVD1* (GTPase Activating Protein And VPS9 Domains 1) has been known to act as a GTPase-activating protein (GAP) and a guanine nucleotide exchange factor (GEF) and participates in various processes such as endocytosis, insulin receptor internalization or LC2A4/GLUT4 trafficking.[35] Based on this notion, it is postulated that *ALKAL2* and *GAPVD1* are likely functional genes for BMI.

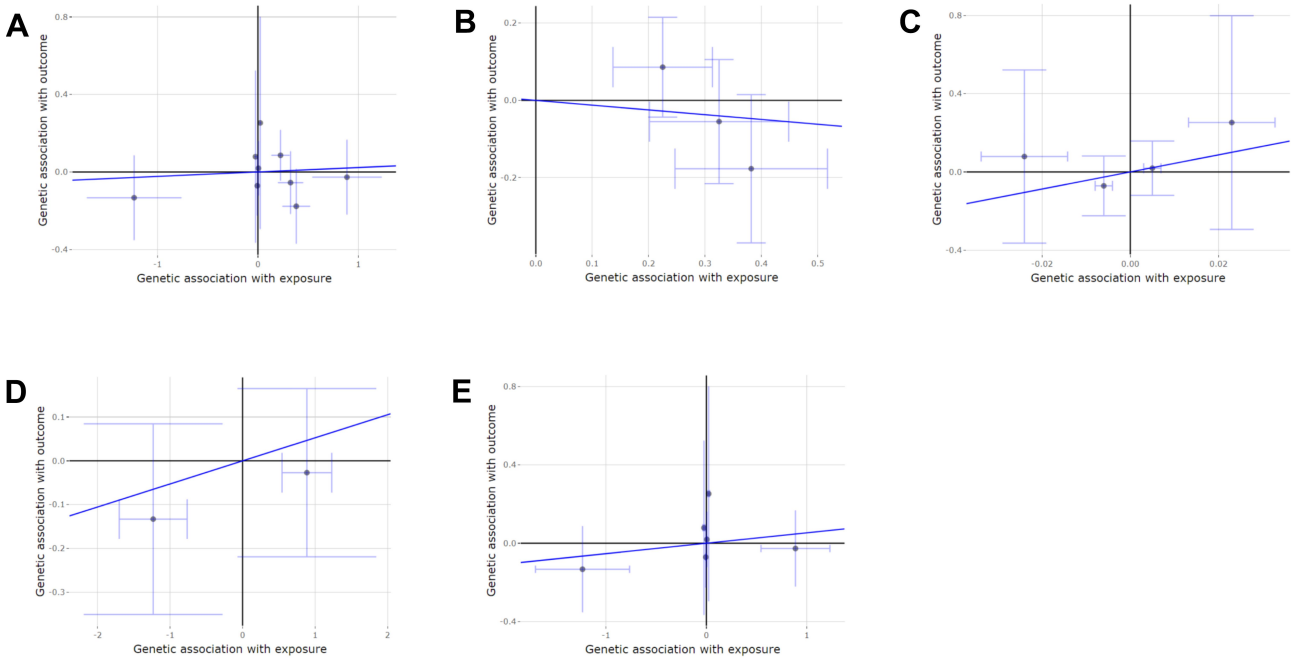

Figure 3 The results of the Mendelian randomization (MR) analyses between obesity traits (all (**A**), BMI (**B**), WHR (**C**), WC (**D**), or visceral fat (**E**)) and PCOS. The x-axis shows the genetic association with exposure (obesity traits). The y-axis shows the genetic association with outcome (PCOS).

SNP rs139702234 was associated with WHR and is located upstream of *PPARG* (Peroxisome Proliferator Activated Receptor Gamma). The eQTL information of this SNP was not available in the GTEx dataset. The encoded *PPARG* protein forms heterodimers with retinoid X receptors, and these heterodimers regulate transcription of various genes. *PPARG* is known to be involved in adipogenesis and has been implicated in the pathology of numerous diseases, including obesity, diabetes, atherosclerosis, and cancer.[36] The association between SNP rs139702234 and WHR was only detected in females in this study. The biological functions of candidate genes of the remaining nine SNPs were not clear with regard to obesity traits (Table 3).

Reproductive disturbances are more common in obese women regardless of the PCOS diagnosis. Obesity is a common finding in women with PCOS and aggravates many of its reproductive and metabolic features. The relationship between PCOS and obesity is complex and not well understood.[37] PCOS is the most common endocrine cause of infertility, but the risk of type 2 diabetes, hypertension, dyslipidemia, and cardiovascular disease is much higher than that in control women.[38] PCOS is a complex disease for women, ranging from metabolic syndromes to reproductive abnormalities. To gain insight into the causal relationship between obesity and PCOS, we performed an MR analysis using SNPs detected in this study for their association with obesity traits in females.

MR is an analytical method that uses genetic variants as a tool to identify modifiable risk factors affecting individual health. In particular, two-sample MR is a method of estimating the causal association between risk factors and outcomes using two different research samples. Integrating multiple data provides an opportunity to significantly improve statistical power.[39] Of the 12 SNPs detected in this study in female subjects, two SNPs (rs6760543 and rs2341967) were removed due to their association with type 2 diabetes, FPG, or HbA1C, which were considered confounding factors for obesity and PCOS (Table 4). As the effect of a genetic variant on the results is through a different pathway than through a risk factor, it is problematic for MR studies in violation of instrumental variables (IVs). SNP rs550532151 was also removed because this SNP was not available in the PCOS dataset. The results of the MR with nine SNPs are shown in Table 5. The MR results revealed no significant association between PCOS and any of the obesity traits (BMI, WHR, or WC). The combined MR results for all nine SNPs were also unrelated.

The obesity traits and PCOS were highly correlated when logistic regression was performed using the epidemiological data from the PCOS study. However, the results of the MR analysis using only genetic factors in this study did not reach significance. This result may be due to reverse causality, a form of confounding that is difficult to account for. It arises if the outcome or preclinical aspects of the disease that lead to the outcome affect the risk factor. People with symptoms of PCOS may participate in more exercise and have a better diet than those without symptoms. This would lead to a negative association between obesity (risk factor) and PCOS (outcome). Another interpretation of the negative association in our MR analyses is vertical pleiotropy in which one factor affects downstream outcomes.[40] As one of the downstream consequences of insulin resistance is obesity, vertical pleiotropy may have confounded the causal relationship between obesity traits and PCOS. An MR analysis between genetically defined insulin dysregulation and PCOS would be valuable to pursue in the future. In addition, it may be assumed that the racial differences have been reflected in our negative MR results because Korean PCOS patients are less obese than Caucasian patients.

Ethics Approval and Consent to Participate

This study was approved by the Institutional Review Board (HIRB-2017-030) of Hallym University. Informed consent was obtained from all subjects of the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest.

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