

A Genome-Wide Association Study on Abdominal Adiposity-Related Traits in Adult Korean Men

Hyun-Jin Kim^a Ho-young Son^b Joohon Sung^c Jae Moon Yun^d
Hyuktae Kwon^d Belong Cho^{d,e} Jong-Il Kim^{b,f,g} Jin-Ho Park^{d,e}

^aNational Cancer Control Institute, National Cancer Center, Goyang, South Korea; ^bDepartment of Biomedical Sciences, Department of Biochemistry & Molecular Biology, Seoul National University College of Medicine, Seoul, South Korea; ^cInstitute of Health and Environment, Seoul National University, Seoul, South Korea; ^dDepartment of Family Medicine, Seoul National University Hospital, Seoul, South Korea; ^eDepartment of Family Medicine, Seoul National University College of Medicine, Seoul, South Korea; ^fGenomic Medicine Institute, Medical Research Center, Seoul National University, Seoul, South Korea; ^gCancer Research Institute, Seoul National University College of Medicine, Seoul, South Korea

Keywords

Genome-wide association study · Abdominal fat · Visceral fat · Korean population

Abstract

Introduction: Although previous genome-wide association studies (GWASs) have identified genetic susceptibility loci for abdominal adiposity, GWASs on Asian samples remain scarce. Therefore, we performed a GWAS for abdominal adipose tissue depots in a Korean population. **Methods:** A total of 1,937 Korean men were included in the study. Areas of abdominal fat were quantified by computed tomography. We performed a GWAS analysis under an additive model, and a replication study was conducted on 480 additional Korean adult men. **Results:** In the discovery step, we identified a total of 10 single-nucleotide polymorphisms (SNPs) associated with adiposity indicators ($p < 1 \times 10^{-5}$). The top SNP, rs1028014, for visceral adipose tissue (VAT) was located in the *ZMAT4* gene and remained significant after adjustment for body mass index (BMI). Three additional SNPs were also

associated with VAT-adj-BMI and located within the *SLC26A10*, *FAM155A*, and *COL4A1-COL4A2* genes, respectively. In addition, we identified a SNP (rs4668224) of the *MYO3B* gene for visceral-to-subcutaneous fat ratio. For subcutaneous adipose tissue and total adipose tissue, two (rs6585735 and rs363527) and three SNPs (rs1487892, rs9357565, and rs1985358) were found, respectively. Overall, eight SNPs were used in the replication study; however, none of the SNPs reached our level of significance for replication ($p < 0.0063$). Nevertheless, rs4773144 of *COL4A1-COL4A2* for VAT-adj-BMI was the most interesting SNP identified in previous GWASs for coronary artery disease (based on the same risk allele “G”), along with functional effects. **Conclusion:** This study suggests for the first time that an SNP (rs4773144) of *COL4A1-COL4A2* may contribute to the increase in VAT level, especially in adult Korean men.

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Jong-Il Kim and Jin-Ho Park contributed equally to this work.

Introduction

Obesity, which is defined as an excess of body fat, has been recognized as an important determinant of cardiovascular disease (CVD), including hypertension, heart failure, and coronary artery disease (CAD) [1, 2]. However, obesity is a highly heterogeneous condition with differences observed among the individuals who are at risk for these diseases, which can be explained by the different regional deposition pattern of adipose tissue [3, 4]. Specifically, visceral fat accumulation appears to be more closely related to adverse health outcomes [5, 6], whereas the role of subcutaneous fat remains controversial [7]. Therefore, in obesity-related studies, it may be important to use computed tomography (CT)-measured indicators that can accurately evaluate adipose tissue compartments.

The development of obesity has a strong genetic component, and its heritability is estimated to range between 40% and 70% [8]. Similarly, abdominal adiposity, including visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), has genetic components [9, 10]. Previously, genome-wide association studies (GWASs) were performed to identify novel genetic susceptibility for CT-derived adipose fat measures and found several candidate loci, such as *RGS6*, *NGEF*, *THESL2*, *BBS9*, *ADCY8*, *KCNK9*, *GLIS3*, and *SRFBP1* [11–14]. Although new genetic susceptibility loci for regional fat deposition have been discovered by these GWASs, the majority of them focused on subjects with European ancestry. To date, no GWAS has been performed to identify genetic loci for CT-measured adipose tissue in Asian populations.

Regional fat deposition, especially visceral fat, varies across different ethnic groups [15–17]. Previous studies have shown that South Asian or Chinese individuals have a relatively greater amount of VAT compared with Europeans [15, 17]. In 2018, Kadowaki et al. [16] compared the distribution of abdominal fat in four populations with different ethnic backgrounds (i.e., Caucasians in Allegheny County, Pennsylvania, in the United States, Japanese Americans in Honolulu, Hawaii, Japanese, and Koreans). The proportion of VAT area to total adipose tissue (TAT) area (VAT%) was higher in Koreans or Japanese compared with Caucasians. In addition, abdominal fat-related studies conducted in the Korean population have demonstrated that the relative distributions of visceral and subcutaneous fat depots in Korean adults, especially men, were different from those of the other populations above [18, 19]. The area of visceral fat and subcutaneous fat in Korean men was similar, whereas the area of visceral fat in other populations was relatively smaller than that of

subcutaneous fat. Such a high VAT/SAT ratio in Korean men has been reported to contribute to the development of various diseases such as metabolic syndrome and hypertension [6, 19]. The cause of the ethnic differences in abdominal fat deposition is not yet fully understood, but genetic factors may be important. Therefore, it is necessary to perform an independent GWAS for abdominal adipose tissue depots in Korean men.

This study aimed to understand the genetic background of abdominal adipose tissue in Korean men. We carried out a GWAS for abdominal adiposity measured directly by CT in Korean adult men and report the first GWAS results in an Asian population in this setting.

Materials and Methods

Participants

The study samples were collected from 2009 to 2013 at two health screening centers run by the Seoul National University Hospital (the Seoul National University Hospital Health Promotion Center and the Seoul National University Hospital Healthcare System Gangnam Center). The study included diverse subjects who live in all 16 Korean administrative districts, including Seoul. Briefly, in South Korea, adults in the general population usually undergo regular comprehensive health checkups for early detection or prevention of various diseases, and many people across the country prefer to visit large hospitals, such as Seoul National University Hospital, for a more accurate comprehensive examination. For this reason, a national sample from the general population was included in our study. In addition, the abdominal CT scan is an option, not a mandatory test, when the participants receive regular comprehensive health checkups. These data were originally collected to identify genetic background of abdominal adiposity traits, especially visceral fat, in Korean population. Abdominal fat distribution is very different according to sex, and Korean males are known to have a larger distribution of visceral fat than females. For this reason, only adult men were considered for initial data collection. During this period, we collected samples from a total of 2,102 individuals who met the inclusion criteria of our study: (1) adults aged ≥ 20 years; (2) those who did not take any medications, such as weight-reduction drugs, antidiabetics, and thyroid drugs; and (3) those who had not undergone surgery or procedures related to obesity. Of these, we excluded 165 participants for whom DNA samples or phenotypic information, including abdominal adiposity traits, were not available. Furthermore, to identify duplicate samples with high PIHAT > 0.8 [20] among these samples, we computed the PIHAT values for each pair of individuals using PLINK tool (version 1.9). No duplicate samples with high PIHAT > 0.8 were observed, and therefore a total of 1,937 adult men were finally included for the GWAS analysis. In addition to duplicate samples, we assessed the relatedness using a threshold of $PI_HAT > 0.25$, and a total of 18 samples pairs were removed. This study was approved by the Institutional Review Board of the Seoul National University Hospital Biomedical Research Institute and the National Cancer Center. Informed consent was waived by the Institutional Review Board because this was a retrospective study that used de-identified data.

Assessment of Abdominal Adiposity

Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). We have described the details of abdominal adiposity measurement in our previous studies [6, 18, 21]. Briefly, the cross-sectional areas of each fat compartment in abdominal adiposity, measured using a CT scanner (Somatom Sensation 16 CT scanner, Siemens AG, Erlangen, Germany), were estimated by the Rapidia 2.8 CT software (Infinit, Seoul, South Korea). The VAT area was estimated by delineating the intra-abdominal fat bound by the parietal peritoneum or transversalis fascia. The SAT area was calculated by subtracting the VAT area from the total fat area excluding the vertebrae and spinal muscles. Using these fat areas, the visceral-to-subcutaneous fat ratio (VSR) was also calculated.

SNP Genotyping

We extracted genomic DNA from whole-blood samples using a QuickGene-610L device (Fujifilm, Tokyo, Japan) according to the manufacturer's protocol. Genome-wide single-nucleotide polymorphisms (SNPs) were genotyped using the HumanCore Bead-Chip kit (Illumina, San Diego, CA, USA). Before analysis, we checked the quality of all genotyped SNPs using SNP call rate ($>99\%$) and Hardy-Weinberg equilibrium (HWE) ($p \geq 0.0005$). The common SNPs with minor allele frequencies (MAFs) $\geq 5\%$ were included in the analysis, to minimize the false-positive association results afforded by rare and low-frequency genetic variants. Therefore, after quality evaluation, a total of 213,837 SNPs were finally used in our GWAS analysis.

Statistical Analysis

We checked the distribution of these traits before analyses to meet the normality assumptions. As a result, the best transformation approach for normality needed to be considered, since all adiposity traits followed a non-normal distribution (all Shapiro-Wilk p value < 0.05). First of all, we transformed with natural log and square root approaches; however, the transformed traits still did not follow a normal distribution (all Shapiro-Wilk p value < 0.05). Therefore, we applied a rank-based inverse normal transformation to these traits, which is robust against deviation from outliers or normality. The MAFs and HWE p for all SNPs were calculated using the PLINK tool (version 1.9). We drew Q-Q plots using the "qqman" package of R software (version 4.1.2) and estimated the genomic inflation factor (λ) using the "median" method. We performed a multiple linear regression analysis under an additive genetic model to test the associations between genome-wide SNPs and adiposity traits using the PLINK software. The association results were adjusted for site of recruitment and age. Most of the tested SNPs were strongly correlated with each other and were not independent. Considering this setting, a Bonferroni correction approach based on the number of tested SNPs may result in overly strict or conservative adjustments. Therefore, we screened the candidate genetic variants using a relaxed genome-wide significance threshold of $p < 1 \times 10^{-5}$ in the discovery step. The regional association plot for a new adiposity locus was also visualized using the LocusZoom software. In addition, we identified the tissue-specific expression level of genes of interest from GTEx Portal.

Replication

The validation of genetic loci identified in the discovery step is an effective gold-standard approach to identify real associations. The replication cohort was sampled from the same health screen-

ing center (Seoul National University Hospital Health Promotion Center) as the discovery cohort but at a later time point (recruited from 2014 to 2015). Unlike the discovery cohort where only men were recruited, a total of 481 men and 327 women samples with abdominal CT information were collected during this period. Of these, only 481 adult men were included in the replication step, as in the discovery cohort. We assessed duplicates (PIHAT > 0.8) and related samples (PIHAT > 0.25), and 1 duplicate and 50 related samples were removed, respectively. Phenotypic information was obtained in the same manner as described for the initial discovery sample. Similar to discovery cohort, the distribution of all adiposity traits did not follow a normal distribution (all Shapiro-Wilk p value < 0.05). All traits were applied a rank-based inverse normal transformation to approximate the normal distribution (all Shapiro-Wilk p value ≥ 0.05). We also extracted genomic DNA from the validation samples via the same process as described for the initial sample using a QuickGene-610L apparatus (Fujifilm, Tokyo, Japan). Among the SNPs identified in the GWAS discovery phase, we finally selected eight candidate SNPs (rs1028014, rs9357565, rs1871417, rs9555336, rs4773144, rs6585735, rs363527, and rs4668224) because the PCR primers of two SNPs (rs1487892 and rs1985358) were not available. We also checked the proxy SNPs (within 100 kb) which are in a high LD (r^2 of greater than 0.8) with the two SNPs in our GWAS array data (HumanCore Bead-Chip kit). As a result, no proxy SNP for rs1985358 was observed ($0 < r^2 < 0.34$), but a proxy SNP (rs10789547) for rs1487892 was identified ($r^2 = 0.91$). However, the proxy SNP (rs10789547) in the replication was not additionally included in the replication due to budget and time issues. All samples were genotyped using the TaqMan SNP Genotyping Assay and a ViiA7 genotyping system (Applied Biosystems, Inc., Carlsbad, CA, USA) at the Genomic Medicine Institute Research Service Center. Before analysis, we identified the QC for validation SNPs using MAFs $\geq 5\%$ and HWE ($p \geq 0.0005$). All eight candidate SNPs met this criterion. Similar to the discovery step, linear regression analyses were performed to confirm associations between candidate SNPs and adiposity traits in the additive genetic model. To evaluate the statistical significance of the replication study, we used a strict Bonferroni correction based on the number of tested SNPs ($p < 0.05/8$ [number of SNPs tested = 8] = 0.0063).

Results

Table 1 lists the basic characteristics of the subjects included in the discovery and replication phases. In both discovery and replication phases, all subjects were adult men over 20 years of age ($n = 1,937$ for discovery and $n = 480$ for replication). The mean age of the replication sample (55.5 years) was higher compared with that of the discovery set (48.9 years) ($p < 0.0001$). The mean (standard deviation) value of BMI in discovery set ($24.7 \text{ kg}/m^2$) was similar to that in replication set ($24.3 \text{ kg}/m^2$) ($p = 0.0524$).

Moreover, the distributions of VAT (cm^2) and SAT (cm^2) were completely different between the discovery and replication samples. The mean value of VAT (130.0

Table 1. Characteristics of study participants

Characteristics	Discovery step (n = 1,937) n (%) or mean (SD)	Replication step (n = 480) n (%) or mean (SD)	p value
Sex	Men	Men	–
Year of recruitment	2009–2013	2014–2015	–
Recruitment site			
Site A	1,437	480	–
Site B	500	–	–
Age, years	48.9 (7.0)	55.5 (9.8)	<0.0001
BMI, kg/m ²	24.7 (2.8)	24.3 (3.0)	0.0524
WC, cm	88.4 (7.7)	87.6 (8.5)	0.2680
VAT, cm ²	130.0 (53.0)	153.2 (72.2)	<0.0001
SAT, cm ²	141.4 (55.4)	131.5 (54.6)	0.0005
VSR	0.98 (0.90)	1.22 (0.55)	<0.0001
TAT, cm ²	271.4 (95.8)	284.7 (107.4)	0.0135

BMI, body mass index; WC, waist circumference; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; VSR, visceral-to-subcutaneous ratio; TAT, total adipose tissue. Data are provided as means (standard deviations) for continuous variables such as age, BMI, WC, VAT, SAT, VSR, and TAT.

Table 2. Genome-wide association results for abdominal adipose tissue in a discovery sample ($p < 1 \times 10^{-5}$)

Trait	Chr	SNP	Position ^a	Major/minor allele	MAF	Function	Nearest gene (distance)	β (SE)	p value ^b	p value ^c
VAT	8	rs1028014	40698232	C/T	0.25	Intron	<i>ZMAT4</i> (–)	–0.19 (0.04)	2.42×10^{-7}	3.03×10^{-7}
	19	rs1985358	3729042	A/C	0.30	Intron	<i>TJP3</i> (–)	–0.16 (0.03)	2.16×10^{-6}	9.25×10^{-7}
	6	rs9357565	47881668	G/A	0.20	Intron	<i>PTCHD4</i> (–)	0.18 (0.04)	5.87×10^{-6}	9.09×10^{-6}
VAT-adj-BMI	12	rs1871417	58018979	T/C	0.29	Intron	<i>SLC26A10</i> (–)	–0.11 (0.03)	5.95×10^{-6}	1.44×10^{-5}
	13	rs9555336	107904621	G/A	0.25	Intron	<i>FAM155A</i> (–)	0.12 (0.03)	7.62×10^{-6}	4.90×10^{-6}
	8	rs1028014	40698232	C/T	0.25	Intron	<i>ZMAT4</i> (–)	–0.12 (0.03)	8.66×10^{-6}	1.04×10^{-5}
	13	rs4773144	110960712	A/G	0.37	Intron	<i>COL4A1-COL4A2</i> (–)	0.11 (0.02)	9.25×10^{-6}	9.66×10^{-6}
SAT	10	rs6585735	123203921	T/C	0.47	Intergenic	<i>FGFR2</i> (33.9 kb)	–0.15 (0.03)	1.89×10^{-6}	3.22×10^{-6}
	21	rs363527	31014373	C/T	0.18	Intron	<i>GRIK1</i> (–)	0.18 (0.04)	8.57×10^{-6}	8.95×10^{-6}
VSR	2	rs4668224	171082383	T/G	0.45	Intron	<i>MYO3B</i> (–)	0.15 (0.03)	1.29×10^{-6}	8.12×10^{-7}
TAT	11	rs1487892	106692416	G/A	0.33	Intron	<i>GUCY1A2</i> (–)	–0.15 (0.03)	5.60×10^{-6}	7.76×10^{-6}
	6	rs9357565	47881668	G/A	0.20	Intron	<i>PTCHD4</i> (–)	0.18 (0.04)	6.22×10^{-6}	1.40×10^{-5}
	19	rs1985358	3729042	A/C	0.30	Intron	<i>TJP3</i> (–)	–0.15 (0.03)	7.46×10^{-6}	3.64×10^{-6}

Chr, chromosome; SNP, single-nucleotide polymorphism; MAF, minor allele frequency; SE, standard error; VAT, visceral adipose tissue; BMI, body mass index; SAT, subcutaneous adipose tissue; VSR, visceral-to-subcutaneous ratio; TAT, total adipose tissue. ^aSNP positions are based on Human GRCh37/hg19 from UCSC Genome Browser. ^bp value was calculated from discovery cohort after removing duplicate samples (PIHAT > 0.8). ^cp value was calculated from discovery cohort after removing related samples (PIHAT > 0.25).

cm²) in discovery set was slightly lower than that of SAT (141.4 cm²), and the mean VSR was 0.98, whereas the mean VAT (153.2 cm²) in replication set was higher than the SAT (131.5 cm²).

The results of the genome-wide association analysis for adiposity traits are shown in Figure 1 and Table 2. Fig-

ure 1 depicts quantile-quantile and Manhattan plots of p values from the GWAS. The genomic inflation factor is approximately 1, indicating no population stratification (Fig. 1). More detailed association results for adiposity traits are summarized in Table 2. Three SNPs for VAT reached our significance level of $p < 1 \times 10^{-5}$. The top

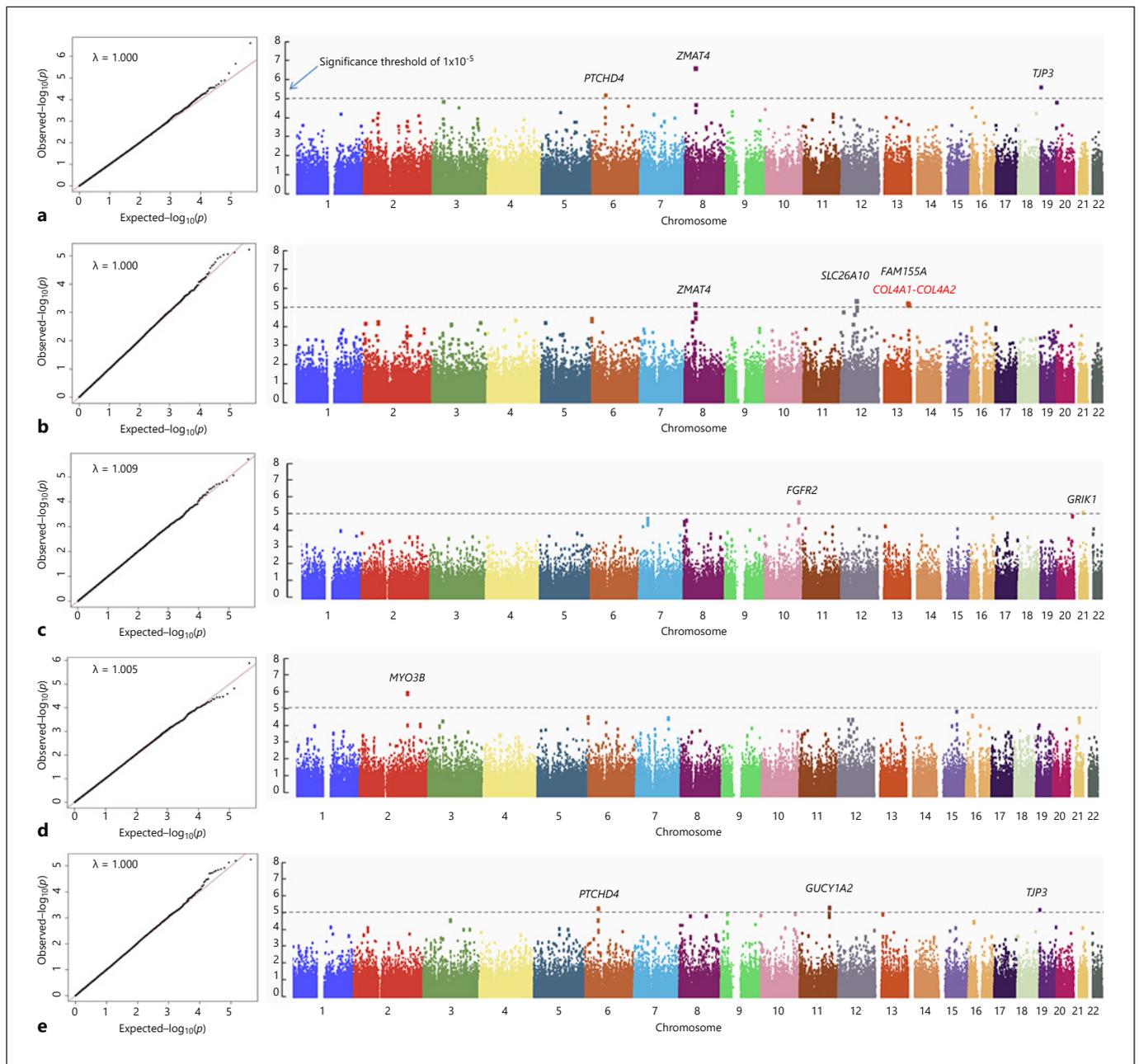


Fig. 1. Q-Q and Manhattan plots for (a) VAT, (b) VAT-adj-BMI, (c) SAT, (d) VSR, and (e) TAT in discovery step.

SNP, rs1028014 ($p = 2.42 \times 10^{-7}$), for VAT was located in an intron of the *ZMAT4* gene. Two other SNPs (rs1985358 and rs9357565) were located in the introns of the *TJP3* and *PTCHD4* genes, respectively. For VAT-adj-BMI, a total of four SNPs passed our GWAS threshold. The top SNP, rs1028014, identified in VAT remained significant after adjustment for BMI. The two SNPs (rs9555336 and

rs4773144) associated with VAT-adj-BMI were located within introns of the *FAM155A* and *COL4A1-COL4A2* genes on chromosome 13, respectively. In addition, SNP rs1871417 located in the introns of the *SLC26A10* on chromosome 12 was observed for VAT-adj-BMI. In addition, we identified two significant loci on chromosomes 10 and 21 for SAT. rs6585735, which is located 33.9 kb

Table 3. Replication results for abdominal adipose tissue from validation cohort

Trait	Chr	SNP	Position ^a	Major/minor allele	MAF	Nearest gene (distance)	β (SE)	p value ^b	p value ^c
VAT	8	rs1028014	40698232	C/T	0.25	<i>ZMAT4</i> (–)	–0.03 (0.08)	0.7334	0.5916
	6	rs9357565	47881668	G/A	0.22	<i>PTCHD4</i> (–)	0.12 (0.09)	0.1702	0.2010
VAT-adj-BMI	12	rs1871417	58018979	T/C	0.31	<i>SLC26A10</i> (–)	–0.03 (0.05)	0.4463	0.3907
	13	rs9555336	107904621	G/A	0.22	<i>FAM155A</i> (–)	0.07 (0.05)	0.2186	0.3932
	8	rs1028014	40698232	C/T	0.25	<i>ZMAT4</i> (–)	–0.04 (0.05)	0.3884	0.4567
	13	rs4773144	110960712	A/G	0.36	<i>COL4A1-COL4A2</i> (–)	0.02 (0.05)	0.6946	0.7737
SAT	10	rs6585735	123203921	T/C	0.43	<i>FGFR2</i> (33.9 kb)	–0.10 (0.07)	0.1551	0.1098
	21	rs363527	31014373	C/T	0.15	<i>GRIK1</i> (–)	0.07 (0.09)	0.4270	0.5613
VSR	2	rs4668224	171082383	T/G	0.48	<i>MYO3B</i> (–)	0.02 (0.02)	0.2522	0.2198
TAT	6	rs9357565	47881668	G/A	0.22	<i>PTCHD4</i> (–)	0.08 (0.06)	0.2016	0.2128

Chr, chromosome; SNP, single-nucleotide polymorphism; SE, standard error; VAT, visceral adipose tissue; BMI, body mass index; SAT, subcutaneous adipose tissue; VSR, visceral-to-subcutaneous ratio; TAT, total adipose tissue. ^aSNP positions are based on Human GRCh37/hg19 from UCSC Genome Browser. ^b p value was calculated from discovery cohort after removing duplicate samples (PIHAT > 0.8). ^c p value was calculated from discovery cohort after removing related samples (PIHAT > 0.25).

away from the *FGFR2* gene, was the SNP with the strongest p (1.89×10^{-6}) for SAT, whereas rs4668224, which is located in the intron of the *MYO3B* gene on chromosome 2, was identified for VSR. We also identified three candidate SNPs related to TAT that passed our screening criteria. Among them, rs1487892, which is located in the intron of the *GUCY1A2* gene on chromosome 11, was the SNP with the strongest signal ($p = 5.60 \times 10^{-6}$) for TAT. In addition, the summary statistics of all SNPs of the top hits (e.g., with $1 \times 10^{-5} \leq p$ value $< 1 \times 10^{-3}$) were presented in online supplementary Tables S1–S5 (see www.karger.com/doi/10.1159/000524670 for all online suppl. material). The statistic power of our sample was calculated with the genetic power calculator [22]. The sample had approximately 75% power, assuming a total QTL variance of 60%, and additive QTL effects of 10%, MAF of 30%, and a marker-allele D' value of 0.2. We also calculated statistical power for candidate SNPs based on effect estimates and allele frequencies from our discovery findings. The powers were estimated to range from 24% to 99%, and the power for three SNPs was higher than 90%, including *COL4A1-COL4A2* rs4773144 (95%), *FGFR2* rs6585735 (99%), and *MYO3B* rs4668224 (99%) (data not shown).

To verify the GWAS findings obtained in the discovery phase, a replication study was conducted in an additional cohort of Korean adult men, and the results are summarized in Table 3. A total of eight SNPs were test-

ed for association with each adiposity trait in 480 adult men. Unfortunately, we observed that none of the SNPs in the total samples reached our significance level ($p < 0.0063$). Nevertheless, we focused on one genetic locus (*COL4A1-COL4A2*) that is well known to be associated with obesity and CAD in other previous studies. Detailed information on the genomic location near the *COL4A1-COL4A2* gene is shown in Figure 2. In fact, gene expression data from GTEx showed that the expression level of the *COL4A1* gene was relatively high in VAT compared with various other tissues (online suppl. Fig. S1). Moreover, the level of expression of the *COL4A1* gene in VAT was slightly higher than that detected in SAT.

Furthermore, we assessed the associations of SNPs from previously published loci of abdominal adiposity [11–14] (online suppl. Table S6). At the locus level, we identified the nine loci (*NGEF*, *RGS6*, *LYPLAL1*, *FTO*, *BBS9*, *KCNK9*, *MLLT10*, *CYCSP30*, and *GLIS3*) with a nominal association with abdominal fat. Of these known SNPs, three variants (rs12374818, rs10506943, and rs7919823) were absent in East Asian. We confirmed the frequency of these variants from NCBI dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>), and the all frequencies of alternative alleles in East Asian population were zero.

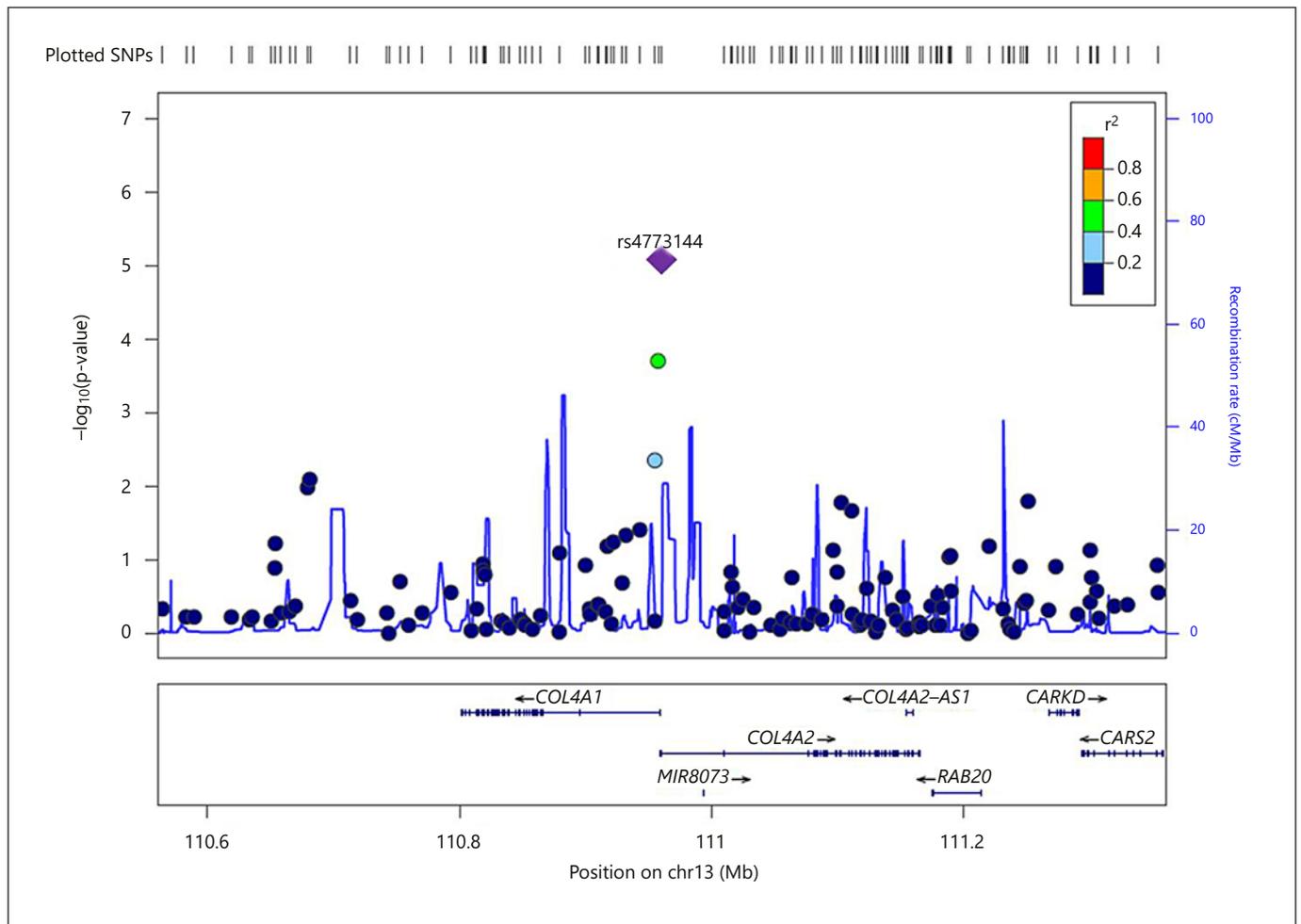


Fig. 2. Regional association plot for *COL4A1-COL4A2* on chromosome 13. The purple diamond indicates the strongest SNP (rs4773144) in each gene region. The colors of the circles represent LD structures with the strongest SNP (blue, $r^2 \leq 0.2$; sky, $0.2 < r^2 \leq 0.4$; green, $0.4 < r^2 \leq 0.6$; orange, $0.6 < r^2 \leq 0.8$; red, $r^2 > 0.8$). SNP locations are based on Human GRCh37/hg19 from UCSC Genome Browse.

Discussion

The purpose of this study was to discover new genetic loci for abdominal fat traits measured by CT in a Korean population. We performed a GWAS for adiposity traits, such as VAT, VAT-adj-BMI, SAT, TAT, and VSR, in adult Korean men. A total of 10 SNPs for these adiposity traits reached the genome-wide threshold of $p < 1 \times 10^{-5}$, eight of which were tested in the replication step. None of the SNPs in the total cohort of adult men achieved our significance level for a replication ($p < 0.0063$).

Although there were no significantly validated SNPs, we specifically focused on a genetic locus of *COL4A1-COL4A2*, which is well known to be associated with obesity and CAD [23–26]. Collagen type IV alpha 1 (*COL4A1*)

and 2 (*COL4A2*) are extracellular matrix proteins that constitute a major structural component of basement membranes. They are widely expressed in multiple tissues, including the adipose tissue, lung, and heart. This locus has been previously associated with childhood obesity in Hispanics. In 2012, Comuzzie et al. [23] reported that weight z-score change showed the significant association with an intronic variant (rs494558) in *COL4A1* on chromosome 13. In addition, in previous large-scale association studies, genetic variants of *COL4A1-COL4A2* were found to be associated with CAD [24–26]. In 2011, Schunkert and colleagues [26] carried out a large-scale GWAS meta-analysis for CAD in populations with European ancestry. They found 13 new susceptibility loci for CAD, one of which was the rs4773144 SNP of *COL4A1-COL4A2* that

was identified in our study. This genetic locus was confirmed by the CARDIoGRAM Consortium study, which is based on individuals of European and South Asian ancestry [25], and the risk allele (G) was also the same as that of our result. Notably, Yang and colleagues [27] investigated the functional effects of rs4773144, an intronic SNP known as a major risk variant of CAD, and found that this variant regulates the level of expression of *COL4A1-COL4A2*, vascular cell survival, and atherosclerotic plaque stability. This finding may be useful for explaining the link between this genetic variant and CAD risk.

In addition, several studies have reported significant associations between *COL4A1-COL4A2* variants and cardiovascular events, such as hemorrhagic stroke and sporadic cerebral small vessel disease [28, 29]. Obesity is one of the major risk factors for these diseases, especially abdominal visceral fat [30, 31]. Abdominal fat accumulation is associated with an increased risk of CVD, and this negative effect is stronger for VAT compared with SAT [31]. The ratio between VAT and SAT is also an independent predictor of cardiovascular events and mortality [30]. Given that visceral fat has a negative effect on CVD, the link between *COL4A1-COL4A2* variants and CVD may be explained by VAT. Interestingly, an analysis of the obese secretome in VAT and SAT showed that *COL4A2* was only detected in the VAT obese secretome and not in the SAT secretome [32]. This supports our finding that a genetic variant of *COL4A1-COL4A2* is associated with abdominal visceral fat, independently of BMI.

To date, genome-wide associations regarding abdominal adiposity have been primarily studied in specific populations, such as those with European ancestry [11–14]. In 2009, Norris et al. [13] performed a GWAS for CT-derived measures of adiposity for the first time in a Hispanic sample and found novel loci, such as *RGS6* and *NGEF*, related to adipose fat measures. Our research team discovered that an intronic SNP (rs11678490) of *NGEF* was associated with VAT, independently of BMI, in Korean men [18]. Another GWAS on body fat distribution performed sex-specific analyses and discovered the association of a genetic variant (rs1659258) located near *THESL2* with VAT in women alone [11]. In 2016, Sung et al. [14] replicated this variant in European American women and identified additional loci that affected VAT (*BBS9*, *ADCY8*, and *KCNK9*) and SAT (*MLLT10/DNAJC1/EBLN1*). In addition, a recent family-based genome-wide study performed on Mexican Americans revealed several loci for regional fat deposition (*GLIS3* and *SRFBP1*). Those authors also identified interactive effects with sex at the *PAPPA2* and *TBX15* loci via a genome-

wide SNP-sex interaction analysis [12]. In our sample, five loci (*LYPLAL1*, *FTO*, *BBS9*, *ADCY8*, and *GLIS3*) showed the nominal associations at the gene level.

This was the first GWAS to identify candidate SNPs for abdominal fat traits measured by CT in an Asian population. Although several GWASs have reported new genetic loci associated with VAT or SAT in specific populations, GWASs on Asian populations remain scarce. Therefore, we provided important evidence of the association between the *COL4A1-COL4A2* variant (i.e., rs4773144) and VAT in Korean men. Nevertheless, our study has several limitations. First, none of the SNPs reached a genome-wide significance level of $p < 5 \times 10^{-8}$ for $\alpha = 0.05$. Thus, we considered the relaxed criterion of $p < 1 \times 10^{-5}$ to select top-ranking SNPs. Second, the sample size used in the replication analysis was not sufficient to verify the top-ranking SNPs. This was because an abdominal CT scan was an optional, rather than mandatory, test at the time at which the participants received regular comprehensive health checkups. This may also lead to a bias if more healthy or more sick people have the CT scan performed. Third, our study failed to replicate the candidate SNPs. Lastly, all SNPs identified by GWAS in the discovery step lay within the single intron of a gene or in the intergenic region. An additional imputation research is needed to enhance coverage across the genome.

Conclusion

This study suggests a susceptibility locus (i.e., *COL4A1-COL4A2*) that contributes to abdominal visceral fat among adult Korean men, despite failing to replicate the results of the discovery phase. Considering that visceral fat closely affects CVD, this finding suggests that the previously known associations between the *COL4A1-COL4A2* variants and CVD in other populations may be more clearly interpreted as a mediator, VAT. However, additional studies are needed to establish the causality between *COL4A1-COL4A2* variants, visceral abdominal fat, and CVD.

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Statement of Ethics

This study was approved by the Institutional Review Board of the Seoul National University Hospital Biomedical Research Institute (H-1901-096-1004) and the National Cancer Center

(NCC2019-0106). Informed consent was waived by the Institutional Review Board because this was a retrospective study that used de-identified data.

Conflict of Interest Statement

The authors declare no conflict of interests.

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Author Contributions

H.-J.K., J.-I.K., and J.-H.P. conceived and designed the study. H.-Y.S. and J.S. performed the experiments. H.-J.K., J.M.Y., H.K., and B.C. analyzed and interpreted the data. H.-J.K. wrote this paper and J.-I.K., and J.-H.P. reviewed the paper.

Data Availability Statement

Data that support the findings of this study are available on reasonable request from the corresponding author at pjhn@snu.ac.kr.

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