Twin Research and Human Genetics (2022), **25**, 234–244
doi:10.1017/thg.2022.39

CAMBRIDGE UNIVERSITY PRESS

Article

Exploring the Genetic Association between Obesity and Serum Lipid Levels Using Bivariate Methods

Ji Ke[1], Wenjing Gao[1], Biqi Wang[1], Weihua Cao[1], Jun Lv[1], Canqing Yu[1], Tao Huang[1], Dianjianyi Sun[1], Chunxiao Liao[1], Yuanjie Pang[1], Zengchang Pang[2], Liming Cong[3], Hua Wang[4], Xianping Wu[5], Yu Liu[6] and Liming Li[1]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China, [2]Qingdao Municipal Center for Disease Control and Prevention, Qingdao, China, [3]Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China, [4]Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China, [5]Sichuan Center for Disease Control and Prevention, Chengdu, China and [6]Heilongjiang Provincial Center for Disease Control and Prevention, Harbin, China

Abstract

It is crucial to understand the genetic mechanisms and biological pathways underlying the relationship between obesity and serum lipid levels. Structural equation models (SEMs) were constructed to calculate heritability for body mass index (BMI), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and the genetic connections between BMI and the four classes of lipids using 1197 pairs of twins from the Chinese National Twin Registry (CNTR). Bivariate genomewide association studies (GWAS) were performed to identify genetic variants associated with BMI and lipids using the records of 457 individuals, and the results were further validated in 289 individuals. The genetic background affecting BMI may differ by gender, and the heritability of males and females was 71% (95% CI [.66, .75]) and 39% (95% CI [.15, .71]) respectively. BMI was positively correlated with TC, TG and LDL-C in phenotypic and genetic correlation, while negatively correlated with HDL-C. There were gender differences in the correlation between BMI and lipids. Bivariate GWAS analysis and validation stage found 7 genes (*LOC105378740*, *LINC02506*, *CSMD1*, *MELK*, *FAM81A*, *ERAL1* and *MIR144*) that were possibly related to BMI and lipid levels. The significant biological pathways were the regulation of cholesterol reverse transport and the regulation of high-density lipoprotein particle clearance ($p < .001$). BMI and blood lipid levels were affected by genetic factors, and they were genetically correlated. There might be gender differences in their genetic correlation. Bivariate GWAS analysis found MIR144 gene and its related biological pathways may influence obesity and lipid levels.

Keywords: Twins; BMI; lipids; structural equation model; genome-wide association study

(Received 22 June 2022; revise received 11 November 2022; accepted 15 November 2022; First Published online 6 January 2023)

Obesity is a worldwide epidemic with a high prevalence in both developed and developing countries, causing a global disease burden and socioeconomic challenges (Rohde et al., 2019). Obesity contributes to multiple chronic disease states, such as type 2 diabetes (Bragg et al., 2018), nonalcoholic steatohepatitis (Pang et al., 2019), coronary artery disease (Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration et al., 2014), cancer (Parr et al., 2010), and even causes death (Flegal et al., 2007). Serum lipids, such as total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) can reflect metabolic processes. Dyslipidemia, a characteristic of metabolic syndrome (MetS), is also a risk factor for insulin resistance, type 2 diabetes and cardiovascular disease (Alshehry et al., 2016; Cadby et al., 2020; Rankinen et al., 2015).

Consistent conclusions on the association between obesity and serum lipid levels have been drawn. Obesity-related phenotypes such as body mass index (BMI) are positively correlated with TC, TG and LDL-C levels, but negatively associated with HDL-C levels. However, most of these studies only used Pearson correlation or multiple stepwise regression methods (Abbasi et al., 2013; Tao et al., 1992; Zhu et al., 2005), and only a few have constructed a genetic model or performed variance decomposition utilizing familial or twin samples to assess the correlation between the two phenotypes (Cadby et al., 2018; Pang et al., 2010; Tang et al., 2006). Monozygotic twins (MZ) are considered 100% genetically similar, whereas dizygotic twins (DZ) share an average of 50% genetic materials. The heritability of a phenotype can be evaluated by constructing a structural equation model (SEM) using twin samples, and the correlation between two phenotypes and the proportion of genetic and environmental contributions can be assessed by applying bivariate SEM (Liao et al., 2015).

Since obesity and serum lipid levels are correlated and even genetically related, many studies have explored genes or single-nucleotide polymorphisms (SNPs) that are related to both phenotypes simultaneously to identify their common underlying biological pathway. Genomewide association studies (GWAS) have recognized numerous variants in recent years. The simplest method for identifying pleiotropic variants is to find the overlap between the two phenotype GWAS results. Alternatively,

Authors for correspondence: Wenjing Gao, Email: pkuepigwj@126.com; Liming Li, Email: lmleeph@vip.163.com

Cite this article: Ke J, Gao W, Wang B, Cao W, Lv J, Yu C, Huang T, Sun D, Liao C, Pang Y, Pang Z, Cong L, Wang H, Wu X, Liu Y, and Li L. (2022) Exploring the Genetic Association between Obesity and Serum Lipid Levels Using Bivariate Methods. *Twin Research and Human Genetics* **25**: 234–244, https://doi.org/10.1017/thg.2022.39

candidate SNPs or genes that are significantly associated with one trait can be tested for their relationship with another. For example, the FTO gene has been widely confirmed to be related to obesity, and researchers have explored whether SNPs in this gene are related to serum lipids, with inconsistent conclusions (Al-Attar et al., 2008; Gao et al., 2018; Kring et al., 2008). Other studies constructed genetic risk score based on significant SNPs of one phenotype in GWAS studies to test for association with another phenotype, or conducted Mendelian randomization analysis (Emdin et al., 2017; Kim et al., 2016; Lotta et al., 2018). However, these methods mentioned above are likely to have conservative results because they have low detection power and may neglect some true associations (Solovieff et al., 2013).

Cross-phenotype (CP) associations indicate that certain genetic variants are related to multiple phenotypes, which is linked to pleiotropy in complex diseases (Solovieff et al., 2013). The cross-phenotype association (CPASSOC) method is a meta-analysis that incorporates results from the summary statistics of several phenotypes from one or from various studies. This approach has higher statistical power than single-trait analysis and can be used to describe population structure and relatedness (Zhu et al., 2015). SNPs associated with obesity and serum lipid levels can be explored using this program.

This study aimed to: (1) calculate the heritability of BMI, TC, TG, HDL-C and LDL-C and assess the correlation between BMI and the four serum lipid phenotypes; (2) identify genetic variants jointly associated with BMI and lipid levels; and (3) explore biological pathways related to BMI and lipid metabolism.

Materials and Methods

Study Sample

All participants were recruited from the Chinese National Twin Registry (CNTR), the first established, largest population-based twin registry in China. The CNTR has enrolled 61,566 twin pairs from 11 regions since 2001 (Gao et al., 2019). Details of this twin registry have been previously reported (Gao et al., 2019; Li et al., 2013). The data for this study was based on follow-up surveys in Shandong, Jiangsu, Zhejiang, Sichuan and Heilongjiang provinces in 2013 and 2017−2018, and contains questionnaire response information, anthropometric assessments and fasting blood biochemical tests.

In the construction of the SEM in this study, participants who were aged over 18 and willing to cooperate with physical examination and blood collection were included, and those who were pregnant or being treated with weight- or lipid-lowering medicine were excluded. Only twins reared together were included in the study. If any one of the twin pairs was excluded, the other one was also excluded. Zygosity was determined based on age and gender, and whether strangers were confused about the appearance of twins during the baseline investigation, with an accuracy of 86.98% (Wang et al., 2015). SEM analysis was conducted on data from the 1197 twin pairs who remained (475 MZ male pairs, 252 MZ female pairs, 207 DZ male pairs, 79 DZ female pairs, 184 DZ opposite-sex pairs); 805 people had genetic information from either genotyping or whole-genome sequencing.

All participants provided written informed consent, and the Biomedical Ethics Committee at Peking University approved this study (IRB00001052-13022/14021).

Measurements

Height and weight were measured using a portable stadiometer and digital balance (Body Composition Analyzer/Scale, TANITA, Tokyo, Japan; Liao et al., 2015). If repeated measurements were available, the average of this phenotype was calculated. BMI was defined as weight (kg)/height^2 (m^2).

Covariates, including age, gender, smoking, alcohol consumption and physical activity were obtained from questionnaires. Smoking status and alcohol consumption were divided into three categories (never, former, and current). Physical activity was calculated based on the participants' responses to the time spent on work, transportation, daily life and rest time. The metabolic equivalent task (MET) value was calculated, and the results were coded into three categories (low, medium, high; Ainsworth et al., 2000; Fan et al., 2014).

The collection, storage and testing of blood samples have been described previously (Liao et al., 2015). DNA was extracted for genotyping or sequencing.

Genotyping

Genomic DNA was extracted from the whole peripheral blood using BioTeke whole blood DNA extraction Kit. DNA quantification and quality were measured by electrophoresis and UV spectrophotometer. Genotyping was performed using the Human Omni ZhongHua-8 BeadChip (Illumina Inc, San Diego, USA). DNA genotyping was performed on 480 twins, including 125 MZ twin pairs, 112 DZ twin pairs and 6 unrelated individuals. Duplicated samples ($n = 1$) and samples with SNP call rate <95% ($n = 16$) were excluded. There were 867,807 SNPs in autosomes initially. We excluded single SNPs based on the following criteria: SNP genotype missingness rate > 5% ($n = 7651$), minor allele frequency (MAF) < 0.05 ($n = 160{,}312$), Hardy-Weinberg equilibrium (HWE) significance < 10^{-6} ($n = 185$). Finally, the genetic data of 457 subjects and 697,297 SNPs were imputed with reference to 1000 Genomes (Phase 3) using IMPUTE2 (Howie et al., 2009). Imputed SNPs were examined based on the following criteria: if MAF > 3%, imputation INFO should be > 0.3; or if 1% < MAF ≤ 3%, INFO should be > 0.6; or if 0.5% < MAF ≤ 1%, INFO should be > 0.8; or if 0.1% < MAF ≤ 0.5%, INFO should be > 0.9. Finally, 9,395,080 SNPs passed quality control.

Sequencing

After random interruption, end repair and PCR, the DNA extracted from the whole peripheral blood became sequencing raw reads. After data filtering, data quality control, mapping to reference, marking duplicates, indel realignment and base recalibration, the raw reads were transformed into variant SNPs. The whole sequencing procedures were performed by BGI Genomics Co., Ltd, China with qualified control.

Statistical Analysis

Structural equation modeling. For classic SEM in a twin study, phenotypic variation can be divided into additive genetic effects (A), dominance genetic effects (D), shared environmental effects (C) and nonshared environmental effects (E). The proportion of variance of A and D within the overall variation is defined as heritability. Sex-specific differences in genetic effects can be examined by including DZ opposite-sex twins, with different parameters for

https://doi.org/10.1017/thg.2022.39 Published online by Cambridge University Press

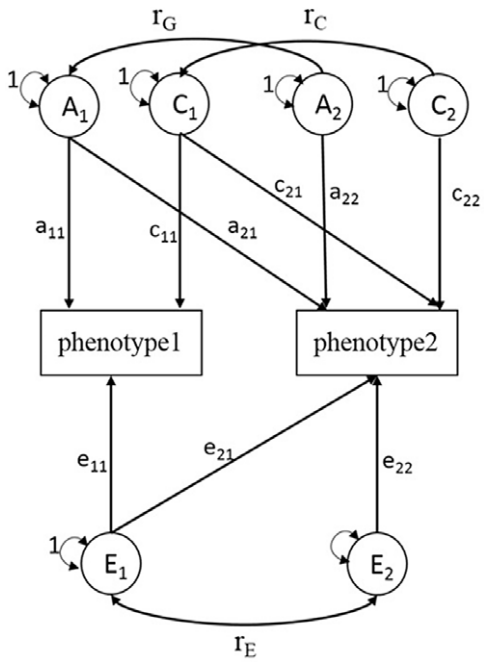

Fig. 1. Choleskey decomposition of bivariate structural equation model (ACE model). A1, A2 = additive genetic variances; C1, C2 = shared environmental variances; E1, E2 = nonshared environmental variances; a_{11}, a_{22} = additive genetic path coefficients; c_{11}, c_{22} = shared environmental path coefficients; e_{11}, e_{22} = nonshared environmental path coefficients; a_{21}, c_{21}, e_{21} = specific additive genetic path coefficient, specific shared environmental path coefficient, specific nonshared environmental path coefficient influence on phenotype1 and phenotype2 simultaneously; r_G = correlation between genetic factors; r_C = correlation between shared environmental factors; r_E = correlation between nonshared environmental factors.

males and females, as well as by specifying the correlation between their genetic effects (r_g ;Orstavik et al., 2007). r_g is freely estimated when fitting the model that accounts for qualitative and quantitative genetic differences, which means that genes that influence a phenotype differ between males and females. Then, r_g was set to 1 to fit the quantitative model, which means that the same genes influence the trait, while their effect varies in magnitude. The no-sex-difference model means that r_g is equal to 1 and the parameters for different genders are also identical. As the effects of C and D cannot be concurrently assessed in twins reared together, the ACE model and ADE model were fitted separately, and the one with a lower Bayesian Information Criterion (BIC) value was chosen as the best model (Mather et al., 2016). Then nested models that dropped the genetic (A or D) and environmental (C) components were fitted and the performances of the ACE/ADE model and its nested models were compared. A nested model was defined as the best-fit model if the LRT p value was >.05, and the Akaike's information criterion (AIC) was smaller than that of the ACE/ADE model.

Similar to univariate SEM, bivariate SEM can be applied to assess the variances of A, C, D and E between two phenotypes using Cholesky decomposition (Figure 1, sex-differences are not shown). A bivariate SEM was fitted based on the results of the univariate SEM. We reported phenotypic correlation (rp) and proportion of genetic and environmental contributions to the phenotypic correlation for BMI and lipid phenotypes. rp means the correlation coefficient of two phenotypes, and proportions of genetic correlation means the proportion of shared genetic factors contributing to phenotypic correlations. In the SEM analysis, raw data of BMI were used and logarithmic transformation of serum lipids was performed, and models were adjusted for age. The SEM was fitted with OpenMx package (version 2.18.1) in R (version 4.0.2).

GWAS

GWAS analysis of BMI, TC, TG, HDL-C, LDL-C were conducted for 457 participants (2 individuals treated with lipid-lowering medicine were excluded) using the genomewide efficient mixed-model association (GEMMA, version 0.98, Linux) program, which uses linear mixed models to explain kinship among samples, population stratification and other confounding factors in genetic association tests (Zhou & Stephens, 2012). Logarithmic transformation of serum lipids data was performed to meet the normal distribution, and the models were adjusted for age, age square, sex and the first 10 principal components (PCs). CPASSOC analysis based on univariate GWAS results can identify SNPs that influence BMI and lipid traits simultaneously (Zhu et al., 2015). This analysis was conducted assuming heterogeneity is present or not present, because the effect size and direction of a SNP may be different between two phenotypes. The results of CPASSOC were further compared with the results of bivariate GWAS performed by GEMMA (Zhou & Stephens, 2014). Genomewide significance was set at $p < 5E\text{-}8$, but $p < 1E\text{-}5$ was adopted as a suggestive significance level owing to the limited sample size in this analysis (Li et al., 2019; Ran et al., 2013). The CPASSOC analysis (version 1.0.1) were performed in R (version 4.0.2). SNPs with suggestive significance were selected for the validation stage, which involved subjects with sequencing data. If a certain SNP was not in the validation data, another SNP in linkage disequilibrium ($r^2 > .6$) was selected. Validation model was adjusted for age, sex and region. $p < .05$ was set as significance level. Both of the two stages used an additive genetic model.

Enrichment Analysis

Significant SNPs in the GWAS analysis were mapped to the loci or the nearest gene on the chromosomes. Enrichment analysis of GO pathways was conducted using Gorilla (http://cbl-gorilla.cs.technion.ac.il/) (Eden et al., 2009). Pathways with a p value < .001 were considered significant biological pathways.

Results

Structural Equation Modeling

The basic epidemiological characteristics of all 2394 individuals, including 727 MZ twin pairs and 470 DZ twin pairs in the SEM analysis, are described in Table 1. The analysis involved 1548 men and 846 women aged 49.6 and 46.6, respectively. More than half of men were current smokers and drinkers, while the corresponding percentages of women were relatively lower. TC and LDL-C levels were comparable between genders. Men had higher BMI and TG level, while lower HDL-C level than women did.

The univariate and bivariate SEM model selection procedures are shown in Supplementary Table S1 and S2 respectively. Univariate SEM results showed that BMI and the four lipid phenotypes were all affected by genetic factors. The ACE model was better than ADE for all traits except for LDL-C, and there were qualitative sex differences in the variation of BMI, and quantitative sex differences in the variation of TG, HDL-C and LDL-C (Supplementary Table S1). For example, the heritability of BMI was 71% (95% CI [.66, .75]) in males but only 39% (95% CI [.15, .71]) in females. The shared environmental factors had a

https://doi.org/10.1017/thg.2022.39 Published online by Cambridge University Press

Table 1. Epidemiological characteristics of 2394 Chinese twins for SEM analysis

	All	Male	Female	*p* value
n (%)	2394	1548 (64.7)	846 (35.3)	
Age (years, mean ± *SD*)	48.6 ± 12.0	49.6 ± 11.7	46.6 ± 12.2	.852
Zygosity (MZ), *n* (%)	1454 (60.7)	950 (61.4)	504 (59.6)	<.001
Region, *n* (%)				
Sichuan	256 (10.7)	165 (10.7)	91 (10.8)	–
Shandong	606 (25.3)	397 (25.6)	209 (24.7)	.818
Jiangsu	610 (25.5)	389 (25.1)	221 (26.1)	.883
Zhejiang	678 (28.3)	461 (29.8)	217 (25.7)	.427
Heilongjiang	244 (10.2)	136 (8.8)	108 (12.8)	.119
Smoking status, *n* (%)				
Never	1323 (55.3)	495 (32.0)	828 (97.9)	–
Former	269 (11.2)	266 (17.2)	3 (0.4)	<.001
Current	802 (33.5)	787 (50.8)	15 (1.8)	<.001
Drinking status, *n* (%)				
Never	1019 (42.6)	432 (27.9)	587 (69.4)	–
Former	82 (3.4)	69 (4.5)	13 (1.5)	<.001
Current	1293 (54.0)	1047 (67.6)	246 (29.1)	<.001
Physical activity level, *n* (%)				
Low	577 (24.1)	346 (22.4)	231 (27.3)	–
Medium	356 (14.9)	207 (13.4)	149 (17.6)	.599
High	1461 (61.0)	995 (64.3)	466 (55.1)	.002
BMI (kg/m^2, mean ± *SD*)	24.52 (3.27)	24.63 (3.04)	24.33 (3.65)	.024
TC (mmol/L, mean ± *SD*)	4.87 (1.02)	4.87 (1.06)	4.87 (0.95)	.790
TG (mmol/L, mean ± *SD*)	1.84 (2.23)	1.99 (2.59)	1.59 (1.30)	<.001
HDL-C (mmol/L, mean ± *SD*)	1.34 (0.35)	1.31 (0.36)	1.40 (0.34)	<.001
LDL-C (mmol/L, mean ± *SD*)	2.56 (0.76)	2.55 (0.75)	2.57 (0.79)	.580

Note: SEM, structural equation modeling; MZ, monozygotic; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. *p* values were corrected for the correlation between co-twins using multinomial logistic regression for categorical variables and random-effects models for continuous variables.

greater impact on the variation of BMI in females (34%, 95% CI [.4, .56]) than males (0%). Similar results were also obtained for HDL-C level. However, sex differences seemed to be smaller in the variation of TC, TG and LDL-C, of which the heritability was 50−70% (Supplementary Table S3).

The results of the bivariate SEM are shown in Table 2, and there were genetic correlations between BMI and the four serum lipids in both genders. For example, the correlation coefficient between BMI and serum TC was 0.16 (95% CI [.11, .21]) and 0.17 (95% CI [.10, .25]) for males and females respectively. Genetic effects accounted for 76% of male and 65% of female phenotypic correlations between BMI and TC. The genetic contribution that affected the covariation of BMI and the four serum lipids accounted for more than 60%, exceeding the environmental contribution.

GWAS

The study sample with genotype data consisted of 302 men and 155 women whose epidemiological characteristics are similar to those of participants eligible for SEM analysis (Supplementary Table 4). The paired subjects were randomly divided into two groups, considering the similarity of the genetic material between them. The remaining unpaired subjects were all included in group 1. Finally, there were 241 subjects in group 1 and 216 subjects in group 2. The results of the two groups were combined using METAL. We excluded SNPs with a missingness rate > 5% or minor allele frequency (MAF) < 0.05 after imputation due to the small sample size in each group. Finally, 5,191,111 and 5,144,874 SNPs were included in group 1 and group 2 analyses respectively.

The results of the two analysis strategies (CPASSOC assuming no heterogeneity and CPASSOC assuming heterogeneity) in the three groups (group 1, group 2 and combined) are shown in Supplementary Tables S5−S8. We only showed the SNP with the smallest *p* value nearest to one gene. The results performed by GEMMA bivariate linear mixed models were similar to CPASSOC (assuming heterogeneity), indicating the results were robust, so we did not show these results. The Manhattan plot of results of CPASSOC (assuming heterogeneity) are shown in

https://doi.org/10.1017/thg.2022.39 Published online by Cambridge University Press

Table 2. Results of best bivariate structural equation model for BMI-lipid levels

				Components of variance				Proportion of contribution	
	Phenotype	Best model		A	C	E	r_p	Genetic	Environmental
Male	BMI + TC	ACE	BMI	0.71 (0.66, 0.75)	–	0.29 (0.25, 0.34)	.16 (.11, .21)	76%	24%
			TC	0.48 (0.33, 0.64)	0.16 (0.01, 0.30)	0.36 (0.32, 0.41)			
	BMI + TG	AE	BMI	0.71 (0.66, 0.75)	–	0.29 (0.25, 0.34)	.37 (.32, .41)	73%	27%
			TG	0.60 (0.55, 0.65)	–	0.40 (0.35, 0.45)			
	BMI + HDL-C	ACE	BMI	0.71 (0.66, 0.75)	–	0.29 (0.25, 0.34)	−.29 (−.34, −.24)	77%	23%
			HDL-C	0.70 (0.66, 0.74)	–	0.30 (0.26, 0.34)			
	BMI + LDL-C	AE	BMI	0.71 (0.66, 0.75)	–	0.29 (0.25, 0.34)	.15 (.09, .20)	64%	36%
			LDL-C	0.57 (0.51, 0.62)	–	0.43 (0.38, 0.49)			
Female	BMI + TC	ACE	BMI	0.38 (0.14, 0.67)	0.35 (0.06, 0.57)	0.27 (0.22, 0.33)	.17 (.10, .25)	65%	35%
			TC	0.48 (0.33, 0.64)	0.16 (0.01, 0.30)	0.36 (0.32, 0.41)			
	BMI + TG	ACE	BMI	0.37 (0.15, 0.65)	0.36 (0.10, 0.56)	0.27 (0.22, 0.33)	.26 (.19, .33)	63%	37%
			TG	0.66 (0.59, 0.71)	–	0.34 (0.29, 0.41)			
	BMI + HDL-C	ACE	BMI	0.35 (0.13, 0.64)	0.38 (0.11, 0.59)	0.27 (0.22, 0.33)	−.20 (−.27, −.12)	84%	16%
			HDL-C	0.43 (0.20, 0.72)	0.31 (0.02, 0.52)	0.27 (0.22, 0.32)			
	BMI + LDL-C	ACE	BMI	0.37 (0.13, 0.66)	0.36 (0.08, 0.58)	0.27 (0.22, 0.33)	.17 (.10, .24)	70%	30%
			LDL-C	0.71 (0.64, 0.76)	–	0.29 (0.24, 0.36)			

Note: BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; A, additive genetic effect; C, shared environmental effect; E, nonshared environmental effect; r_p = phenotypic correlation coefficient.

Figure 2 and Supplementary Figures S1–S4. In total, we obtained 913 SNPs that might be associated with BMI and lipid traits simultaneously.

Initially, there were 325 participants who had whole-genome sequencing data. We excluded individuals who had recently taken lipid- or weight-lowering medicine, and we randomly selected one individual from the paired twins to form a validation set, which comprised 289 individuals. 845 SNPs that were found or were in the linkage disequilibrium ($r^2 > .6$) with SNPs in the first stage were selected for validation. Validation results are shown in Supplementary Table S9, and only the SNP with the smallest *P* value nearest to one gene were reported. Finally, we identified 12, 18 16, 16, 18 genes that could be associated with BMI, TC, TG, HDL-C and LDL-C, and 7 genes (*LOC105378740*, *LINC02506*, *CSMD1*, *MELK*, *FAM81A*, *ERAL1*, *MIR144*) that could be associated with both BMI and lipid traits simultaneously, as shown in Table 3.

By providing the seven genes above, the Go enrichment analysis performed by Gorilla obtained four biological pathways that are mainly involved with the *MIR144* gene. These pathways regulate reverse cholesterol transport and high-density lipoprotein particle clearance, which is mainly involved in lipid homeostasis.

Since we found sex differences in the genetic effects in the variation of BMI and lipid traits in the SEM analysis, we conducted a bivariate GWAS stratified by gender. The *MIR144* gene was also significant and enriched in biological pathways in males, but was not significant in females. Furthermore, no significant metabolic pathways were obtained in female participants.

Discussion

The results showed that BMI and the four serum lipid traits (TC, TG, HDL-C, and LDL-C) were influenced by genetic factors, and there seemed to be sex differences in some phenotypes. Phenotypic correlations were observed between BMI and lipid levels, in which genetic factors accounted for a moderate proportion. Based on a bivariate GWAS analysis, seven genes were found to be associated with the combination of BMI and lipids. The enrichment analysis revealed some biological pathways related to BMI and lipid metabolism.

Previous studies reported that estimated heritability of BMI ranged from .47–.90 (median .75) in twin studies and .24–.81 (median .46) in family studies (Elks et al., 2012) respectively. In our univariate SEM analysis, the heritability of BMI was found to be .71 (95% CI [.66, .75]) for males and .39 (95% CI [.15, .71]) for females. However, conclusions regarding whether heritability in BMI differed by sex were not consistent. The meta-regression conducted by Elks et al. (2012) confirmed a null effect of sex, but some studies have even indicated that females had a higher heritability than males (Harris et al., 1995; Herskind et al., 1996; Schousboe et al., 2003). The results of this study were similar to those of another study conducted in Chinese twins, which found that the heritability of BMI differs by area and sex. The highest was 67.8% in males of Tianjin and the lowest was 11.2% in females in Heilongjiang province (Zhou et al., 2015). There were qualitative differences between genders, and this means that different genes affect BMI. Only phenotypes such as waist circumference (Randall et al., 2013), waist-to-hip ratio (Yang et al., 2015), and BMI-adjusted waist-to-hip ratio (Winkler et al., 2015) have been found to have gender heterogeneity in genetic factors, which may be explained by the differences in the proportion and distribution of body fat between men and women. Further efforts should be made to investigate whether the mechanisms that affect obesity differ by sex and area.

All four serum lipid traits were related to genetic factors, and their heritability was similar to that reported by previous studies

https://doi.org/10.1017/thg.2022.39 Published online by Cambridge University Press

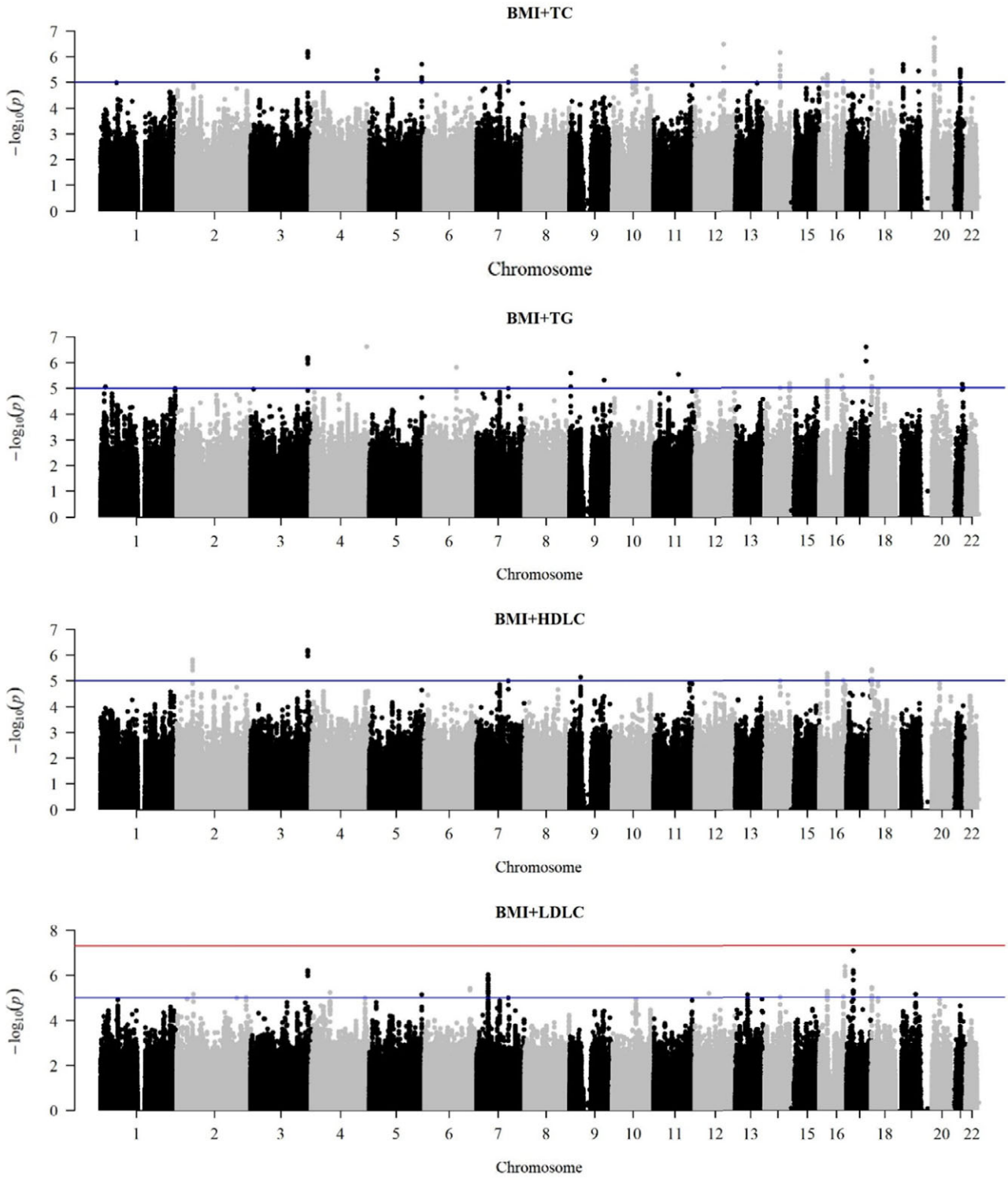

Fig. 2. Manhattan plots for bivariate GWAS results of BMI and lipid traits in the first stage (combined group, cross-phenotype association [CPASSOC], assuming heterogeneity).

(Chien et al., 2007; Heller et al., 1993; Lin et al., 2014; Souren et al., 2007; Weiss et al., 2006). In the bivariate SEM analysis, BMI was positively correlated with TC, TG and LDL-C, and negatively related to HDL-C, which was the same as the conclusions drawn from analyses performed on subjects from different races, genders and regions (Abbasi et al., 2013; Cadby et al., 2018; Mahaney et al., 1995; Pang et al., 2010; Tang et al., 2006; Tao et al., 1992; Zhu et al., 2005). BMI was more strongly correlated with blood pressure, insulin and lipids in men than in women in an observational study (Fall et al., 2015). Lipid metabolomics studies also found that some lipids were associated with BMI in opposite directions across genders, and the strength of the correlation between some lipids and BMI differed significantly between males and females (Beyene et al., 2020). Mendelian randomization studies also indicated that

https://doi.org/10.1017/thg.2022.39 Published online by Cambridge University Press

Table 3. Genes that could be associated with BMI and lipid traits in the bivariate GWAS results

Gene	Chr	SNP	Position (hg19)	Associated trait	*p*1	*p*2
LOC105378740	1	rs12565060	56311809	BMI		5.18E-1
				TC	7.26E-5[a]	4.11E-2
				LDL-C	4.99E-6[a]	5.77E-2
	1	rs12563373	56312695	BMI		2.22E-2
				TC	9.80E-5[a]	7.06E-1
				LDL-C	7.91E-6[a]	8.20E-1
LINC02506	4	rs79198200	31878384	BMI		3.53E-2
				HDL-C	3.60E-3[b]	1.36E-2
				LDL-C	8.25E-6[b]	8.94E-1
CSMD1	8	rs56181019	4,271,843	BMI		3.27E-2
				TC	8.10E-6[c]	9.98E-2
				HDL-C	1.29E-3[c]	1.91E-3
MELK	9	rs10738975	36732969	BMI		1.60E-2
				TC	1.69E-1[b]	5.57E-2
				HDL-C	1.82E-6[b]	6.18E-1
				LDL-C	4.71E-2[b]	1.02E-3
FAM81A	15	rs8029197	59749417	BMI		1.39E-2
				TC	7.14E-6[a]	1.46E-1
				TG	7.96E-4[a]	1.52E-3
				HDL-C	1.70E-2[a]	8.50E-2
				LDL-C	6.93E-5[a]	9.30E-1
ERAL1	17	rs89916866	27183155	BMI		9.86E-3
				TG	7.49E-6[d]	1.42E-1
	17	rs59068724	27184386	BMI		1.02E-1
				TG	7.49E-6[d]	4.82E-2
MIR144	17	rs1109024	27189971	BMI		2.75E-2
				TC	3.88E-3[d]	1.59E-2
				TG	7.49E-6[d]	1.34E-1
				LDL-C	5.11E-3[d]	1.03E-2

Note: BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. *p*1 are the *p* values of bivariate GWAS analysis for the BMI and the corresponding lipid trait. *p*2 are the *p* values for the validation stage. [a]*p* value was extracted from combined group assuming no heterogeneity. [b]*p* value was extracted from group1 assuming heterogeneity. [c]*p* value was extracted from group 2 assuming no heterogeneity. [d]*p* value was extracted from group1 assuming no heterogeneity.

the correlations between metabolites and BMI were higher in males than in females (Wurtz et al., 2014). This phenomenon-may partly be explained by gender differences in the distribution characteristics of BMI and blood lipids, as is indicated in our study. Fat is more likely to deposit in the abdomen in males, which is associated with a deleterious cardiometabolic pattern; for example, hypertension, dyslipidemia and insulin resistance (Fall et al., 2015; Schorr et al., 2018). The sex differences in the correlation between obesity and lipids needs to be further explored.

BMI and the four lipid traits were also genetically correlated in our study, and the proportions of genetic correlation of BMI and lipids were over 50%, indicating genetic effects played a more important role. This conclusion was consistent with some previous twin and family studies, which also indicated a genetic correlation between BMI and lipids. A Chinese twin study fitted models with some combinations of obesity phenotypes and lipids and found no statistically significant genetic correlation between BMI and HDL-C (Liao et al., 2015), which might be explained by the small sample size (only 903 individuals) in that research. However, another Chinese twin study found that the correlation between BMI and lipid traits was mainly due to environmental factors, and it even showed that BMI is positively correlated with HDL-C (Pang et al., 2010), which was contrary to the conclusions of other studies. This study fitted a complex multivariate SEM with six phenotypes, which may lead to unstable parameter estimates. Compared with that study, the bivariate model in our study can explain the correlation between phenotypes more clearly. Some lipids were found to be genetically correlated with obesity in metabolomics study (Cadby et al., 2020). SNPs associated with lipids were identified to be correlated with BMI and waist-hip-ratio (Willer et al., 2013). The genetic risk scores of BMI were associated with lipid traits and vice versa (He et al., 2010; Kim et al., 2016). These studies suggested that BMI and lipids were genetically correlated, and it is of vital importance to find out the genetic background.

After conducting a bivariate GWAS analysis and validation stage, we identified seven genes (*LOC105378740*, *LINC02506*,

https://doi.org/10.1017/thg.2022.39 Published online by Cambridge University Press

CSMD1, *MELK*, *FAM81A*, *ERAL1*, *MIR144*) that could be associated with both BMI and lipid traits simultaneously. Enrichment analysis also revealed the essential role of the *MIR144* gene in lipid metabolism. The transcription product of the *MIR144* gene is microRNA-144 (miR-144). Studies have shown that miR-144 inhibits the expression of the *ABCA1* (adenosine triphosphate binding cassette transporter A1) gene, reduces the level of HDL-C and regulates cholesterol metabolism, thereby accelerating the progression of atherosclerosis (de Aguiar Vallim et al., 2013; Ramirez et al., 2013). miR-144 accelerates plaque formation by promoting the production of pro-inflammatory cytokines (Hu et al., 2014), so it may be closely related to coronary heart disease. Elevation of miR-144 can be observed in coronary heart disease patients, and ST-segment elevation myocardial infarction was significantly associated with elevated *MIR144* expression (Chen et al., 2018). In the F1-zebrafish model, overexpression of *MIR144* led to lipid accumulation and further induced lipid metabolism disorder (Wang et al., 2018). In mice models, miR-144 is one of the most up-regulated miRNAs in response to a high-fat diet (Guedes et al., 2016). Meanwhile, miR-144 is a significant predictor of insulin resistance, and its expression is up-regulated in patients with type 2 diabetes (Yan et al., 2021; Zhu & Leung, 2015). The *MIR144* gene might be closely related to obesity-related metabolic disorders such as dyslipidemia, type 2 diabetes and other metabolic diseases.

The *CSMD1* gene is a putative suppressor of head and neck squamous cell carcinoma in cancer research (Sun et al., 2001). This gene was previously identified to be associated with obesity-related phenotypes (BMI; Zhu et al., 2020), BMI-adjusted waist (Christakoudi et al., 2021), BMI-adjusted waist-hip-ratio (Liu et al., 2013), blood pressure (Hong et al., 2010), glucose (Hebbar et al., 2021), lipids (Hebbar et al., 2021) and metabolic syndrome (Nock et al., 2009), however, the mechanism is not yet clear. The *FAM81A* gene was previously found to be related to BMI-adjusted visceral adipose (Fox et al., 2012) and phosphatidylethanolamine measurement (Rhee et al., 2013) in GWAS analysis, which may be associated with obesity and lipid metabolism. The other four genes were not formally related to these phenotypes. Deletion of the *ERAL1* gene resulted in mitochondrial dysfunction, growth retardation and apoptosis (Dennerlein et al., 2010). The *MELK* gene is mainly involved in cell cycle regulation (Davezac et al., 2002), apoptosis, proliferation (Lin et al., 2007) and intracellular signal transduction (Gaudet et al., 2011). Both the *LOC105378740* and *LINC02506* genes are transcribed to long non-coding RNAs and their biological functions are currently unclear.

We also conducted a bivariate GWAS stratified by gender. The *MIR144* gene was also significant in male subjects, but was not in female subjects. And in female participants, we did not obtain genes that were previously found to be associated with obesity or lipids, or significant biological pathways. On the one hand, it may be due to gender differences in the genetic factors affecting BMI and blood lipids; on the other hand, the sample size of the female subjects was too small ($n = 155$) to find true positive associations. So the gender differences in genes are still worthy of further exploration.

Compared to other research, this study offers deeper insight into the genetic association between obesity and blood lipids from both macro and micro perspectives using data from the Chinese Twin Registry. This study had several strengths. Sex differences could be explored by including opposite-sex twins in this analysis. A GWAS was conducted using a mixed linear model, which can effectively control the population stratification and relatedness. However, this study had some limitations. First, the study participants were not randomly selected from the twin population cohort. Therefore, the results of the SEM cannot be generalized. The heritability obtained for BMI and HDL-C in females was smaller than in a previous study. Whether sex differences play a role in the variation and covariation of obesity and lipid phenotypes requires further investigation. Second, the sample size may be too small and may result in high false-negative rates in GWAS analyses. We did not acquire any positive results at the significance level of 5E-8 even when we combined the two stage subjects. Furthermore, combining GWAS results of the two groups of twins who were genetically similar using the METAL program and performing enrichment analysis with these suggestive genes would introduce bias. Two bivariate analysis methods (CPASSOC and GEMMA) were used to ensure that the findings were robust, and the results of the first stage were externally validated. Cautions should be still exercised when extrapolating these findings to the general population. Third, the influence of X and Y chromosomes were not taken into consideration in gene analysis. Therefore, further replication stages and more extensive analyses are needed to confirm these findings, and we can pay attention to other obesity phenotypes and lipid metabolism components.

In this study, BMI was confirmed to be genetically correlated with serum lipid levels in a Chinese population. The genetic mechanism underlying this association is complex and involves multiple genes, and appears to differ slightly by sex. Obese patients may also have dyslipidemia; therefore, it is important to consider changes in metabolic status as well as sex differences during the process of clinical diagnosis and treatment. Given that there may be a shared genetic background between obesity and blood lipids, a larger cohort research is required to reveal the biological processes involved in obesity and lipid metabolism disorders to confirm molecular therapeutic targets and make plans for prevention and treatment.

Supplementary Material. To view supplementary material for this article, please visit https://doi.org/10.1017/thg.2022.39.

Acknowledgements. We appreciate the support from the participants and the Center for Disease Control and Prevention in Qingdao, Zhejiang, Jiangsu, Sichuan and Heilongjiang.

Author Contributions. Weihua Cao, Wenjing Gao and Liming Li designed this study. Jun Lv, Canqing Yu, Tao Huang, Dianjianyi Sun, Chunxiao Liao and Yuanjie Pang contributed to interpretation of the findings and drafting the article. Zengchang Pang, Liming Cong, Hua Wang, Xianping Wu, Yu Liu and Biqi Wang helped collect data and conducted data quality control. Ji Ke contributed to data analysis and wrote the manuscript. All the authors contributed toward revising the manuscript.

Financial Support. This study was based at the Chinese National Twin Registry (CNTR), and was supported by the National Nature Science Foundation of China (82073633, 81973126, 81573223), Special Fund for Health Scientific Research in the Public Welfare (201502006, 201002007) and Peking University Outstanding Discipline Construction Project of Epidemiology and Biostatistics. We appreciate the support from the participants and the Center for Disease Control and Prevention in Qingdao, Zhejiang, Jiangsu, Sichuan and Heilongjiang.

Conflict of Interest. The authors declare no conflict of interest.

Ethical Standards. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Biomedical Ethics Committee at Peking University (IRB00001052-13022/14021).

References

Abbasi, F., Blasey, C., & Reaven, G. M. (2013). Cardiometabolic risk factors and obesity: Does it matter whether BMI or waist circumference is the index

of obesity? *American Journal of Clinical Nutrition*, *98*, 637–640. http://doi.org/10.3945/ajcn.112.047506

Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., Swartz, A. M., Strath, S. J., O'Brien, W. L., Bassett, D. R. Jr., Schmitz, K. H., Emplaincourt, P. O., Jacobs, D. R. Jr., & Leon, A. S. (2000). Compendium of physical activities: An update of activity codes and MET intensities. *Medicine & Science in Sports & Exercise*, *32*, S498–S504. http://doi.org/10.1097/00005768-200009001-00009

Al-Attar, S. A., Pollex, R. L., Ban, M. R., Young, T. K., Bjerregaard, P., Anand, S. S., Yusuf, S., Zinman, B., Harris, S. B., Hanley, A. J., Connelly, P. W., Huff, M. W., & Hegele, R. A. (2008). Association between the FTO rs9939609 polymorphism and the metabolic syndrome in a non-Caucasian multi-ethnic sample. *Cardiovascular Diabetology*, *7*, 5. http://doi.org/10.1186/1475-2840-7-5

Alshehry, Z. H., Mundra, P. A., Barlow, C. K., Mellett, N. A., Wong, G., McConville, M. J., Simes J., Tonkin A. M., Sullivan D. R., Barnes E. H., Nestel P. J., Kingwell, B. A., Marre, M., Neal, B., Poulter, N. R., Rodgers, A., Williams, B., Zoungas, S., Hillis G. S., ... **Meikle, P. J.** (2016). Plasma lipidomic profiles improve on traditional risk factors for the prediction of cardiovascular events in type 2 diabetes mellitus. *Circulation*, *134*, 1637–1650. http://doi.org/10.1161/CIRCULATIONAHA.116.023233

Beyene, H. B., Olshansky, G., T. Smith, A. A., Giles, C., Huynh, K., Cinel, M., Mellett, N. A., Cadby, G., Hung, J., Hui, J., Beilby, J., Watts, G. F., Shaw, J. E., Moses, E. K., Magliano, D. J., & Meikle, P. J. (2020). High-coverage plasma lipidomics reveals novel sex-specific lipidomic fingerprints of age and BMI: Evidence from two large population cohort studies. *PLOS Biology*, *18*, e3000870. http://doi.org/10.1371/journal.pbio.3000870

Bragg, F., Tang, K., Guo, Y., Iona, A., Du, H., Holmes, M. V., Bian, Z, Kartsonaki, C., Chen, Y., Yang, L., Sun, Q., Dong, C., Chen, J., Collins, R., Peto, R., Li, L., & Chen, Z., & **China Kadoorie Biobank (CKB) Collaborative Group.** (2018). Associations of general and central adiposity with incident diabetes in Chinese men and women. *Diabetes Care*, *41*, 494–502. http://doi.org/10.2337/dc17-1852

Cadby, G., Melton, P. E., McCarthy, N. S., Almeida, M., Williams-Blangero, S., Curran, J. E., VandeBerg, J. L., Hui, J., Beilby, J., Musk, A. W., James, A. L., Hung, J., Blangero, J., & Moses, E. K. (2018). Pleiotropy of cardiometabolic syndrome with obesity-related anthropometric traits determined using empirically derived kinships from the Busselton health study. *Human Genetics*, *137*, 45–53. http://doi.org/10.1007/s00439-017-1856-x

Cadby, G., Melton, P. E., McCarthy, N. S., Giles, C., Mellett, N. A., Huynh, K., Hung, J., Beilby, J., Dubé, M. P., Watts, G. F., Blangero, J., Meikle, P. J., & Moses, E. K. (2020). Heritability of 596 lipid species and genetic correlation with cardiovascular traits in the Busselton family heart study. *Journal of Lipid Research*, *61*, 537–545. http://doi.org/10.1194/jlr.RA119000594

Chen, B., Luo, L., Wei, X., Gong, D., & Jin, L. (2018). Altered plasma miR-144 as a novel biomarker for coronary artery disease. *Annals of Clinical & Laboratory Science*, *48*, 440–445. https://www.ncbi.nlm.nih.gov/pubmed/30143484

Chien, K. L., Hsu, H. C., Chen, W. J., Chen, M. F., Su, T. C., & Lee, Y. T. (2007). Familial aggregation of metabolic syndrome among the Chinese: Report from the Chin-Shan community family study. *Diabetes Research and Clinical Practice*, *76*, 418–424. http://doi.org/10.1016/j.diabres.2006.09.026

Christakoudi, S., Evangelou, E., Riboli, E., & Tsilidis, K. K. (2021). GWAS of allometric body-shape indices in UK Biobank identifies loci suggesting associations with morphogenesis, organogenesis, adrenal cell renewal and cancer. *Scientific Reports*, *11*, 10688. http://doi.org/10.1038/s41598-021-89176-6

Davezac, N., Baldin, V., Blot, J., Ducommun, B., & Tassan, J. P. (2002). Human pEg3 kinase associates with and phosphorylates CDC25B phosphatase: A potential role for pEg3 in cell cycle regulation. *Oncogene*, *21*, 7630–7641. http://doi.org/10.1038/sj.onc.1205870

de Aguiar Vallim, T. Q., Tarling, E. J., Kim, T., Civelek, M., Baldan, A., Esau, C., & Edwards, P. A. (2013). MicroRNA-144 regulates hepatic ATP binding cassette transporter A1 and plasma high-density lipoprotein after activation of the nuclear receptor farnesoid X receptor. *Circulation Research*, *112*, 1602–1612. http://doi.org/10.1161/CIRCRESAHA.112.300648

Dennerlein, S., Rozanska, A., Wydro, M., Chrzanowska-Lightowlers, Z. M., & Lightowlers, R. N. (2010). Human ERAL1 is a mitochondrial RNA chaperone involved in the assembly of the 28S small mitochondrial ribosomal subunit. *Biochemical Journal*, *430*, 551–558. http://doi.org/10.1042/BJ20100757

Eden, E., Navon, R., Steinfeld, I., Lipson, D., & Yakhini, Z. (2009). GOrilla: A tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics*, *10*, 48. http://doi.org/10.1186/1471-2105-10-48

Elks, C. E., den Hoed, M., Zhao, J. H., Sharp, S. J., Wareham, N. J., Loos, R. J., & Ong, K. K. (2012). Variability in the heritability of body mass index: A systematic review and meta-regression. *Frontiers in Endocrinology*, *3*, 29. http://doi.org/10.3389/fendo.2012.00029

Emdin, C. A., Khera, A. V., Natarajan, P., Klarin, D., Zekavat, S. M., Hsiao, A. J., & Kathiresan, S. (2017). Genetic association of waist-to-hip ratio with cardiometabolic traits, type 2 diabetes, and coronary heart disease. *JAMA*, *317*, 626–634. http://doi.org/10.1001/jama.2016.21042

Fall, T., Hagg, S., Ploner, A., Magi, R., Fischer, K., Draisma, H. H., Sarin A. P., Benyamin B., Ladenvall C., Åkerlund M., Kals M., Esko T., Nelson C. P., Kaakinen M., Huikari V., Mangino M., Meirhaeghe A., Kristiansson K., Nuotio M. L., ... **ENGAGE Consortium.** (2015). Age- and sex-specific causal effects of adiposity on cardiovascular risk factors. *Diabetes*, *64*, 1841–1852. http://doi.org/10.2337/db14-0988

Fan, M., Lv, J., & He, P. (2014). Chinese guidelines for data processing and analysis concerning the International Physical Activity Questionnaire. *Zhonghua Liu Xing Bing Xue Za Zhi*, *35*, 961–964. https://www.ncbi.nlm.nih.gov/pubmed/25376692

Flegal, K. M., Graubard, B. I., Williamson, D. F., & Gail, M. H. (2007). Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA*, *298*, 2028–2037. http://doi.org/10.1001/jama.298.17.2028

Fox, C. S., Liu, Y., White, C. C., Feitosa, M., Smith, A. V., Heard-Costa, N., Lohman K., GIANT Consortium, MAGIC Consortium, GLGC Consortium, Johnson, A. D., Foster, M. C., Greenawalt, D. M., Griffin, P., Ding, J., Newman, A. B., Tylavsky, F., Miljkovic, I., Kritchevsky, S. B., ... **Borecki, I. B.** (2012). Genome-wide association for abdominal subcutaneous and visceral adipose reveals a novel locus for visceral fat in women. *PLOS Genetics*, *8*, e1002695. http://doi.org/10.1371/journal.pgen.1002695

Gao, L., Wu, L., Zhang, M., Zhao, X., Cheng, H., & Mi, J. (2018). Gender-specific association of the rs6499640 polymorphism in the FTO gene with plasma lipid levels in Chinese children. *Genetics and Molecular Biology*, *41*, 397–402. http://doi.org/10.1590/1678-4685-GMB-2017-0107

Gao, W., Cao, W., Lv, J., Yu, C., Wu, T., Wang, S., Meng, L, Wang, D., Wang, Z., Pang, Z., Yu, M., Wang, H., Wu, X., Dong, Z., Wu, F., Jiang, G., Wang, X., Liu, Y., Deng, J., ... **Li, L.** (2019). The Chinese National Twin Registry: A 'gold mine' for scientific research. *Journal of Internal Medicine*, *286*, 299–308. http://doi.org/10.1111/joim.12926

Gaudet, P., Livstone, M. S., Lewis, S. E., & Thomas, P. D. (2011). Phylogenetic-based propagation of functional annotations within the Gene Ontology Consortium. *Briefings in Bioinformatics*, *12*, 449–462. http://doi.org/10.1093/bib/bbr042

Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration (BMI Mediated Effects), Lu, Y., Hajifathalian, K., Ezzati, M., Woodward, M., Rimm, E. B., & Danaei, G. (2014). Metabolic mediators of the effects of body-mass index, overweight, and obesity on coronary heart disease and stroke: A pooled analysis of 97 prospective cohorts with 1.8 million participants. *Lancet*, *383*, 970–983. http://doi.org/10.1016/S0140-6736(13)61836-X

Guedes, E. C., Franca, G. S., Lino, C. A., Koyama, F. C., Moreira Ldo, N., Alexandre, J. G., Barreto-Chaves, M. L., Galante, P. A., & Diniz, G. P. (2016). MicroRNA expression signature is altered in the cardiac remodeling induced by high fat diets. *Journal of Cellular Physiology*, *231*, 1771–1783. http://doi.org/10.1002/jcp.25280

Harris, J. R., Tambs, K., & Magnus, P. (1995). Sex-specific effects for body mass index in the new Norwegian twin panel. *Genetic Epidemiology*, *12*, 251–265. http://doi.org/10.1002/gepi.1370120303

He, M., Cornelis, M. C., Franks, P. W., Zhang, C., Hu, F. B., & Qi, L. (2010). Obesity genotype score and cardiovascular risk in women with type 2

diabetes mellitus. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *30*, 327–332. http://doi.org/10.1161/ATVBAHA.109.196196

Hebbar, P., Abubaker, J. A., Abu-Farha, M., Alsmadi, O., Elkum, N., Alkayal, F., John, S. E., Channanath, A., Iqbal, R., Pitkaniemi, J., Tuomilehto, J., Sladek, R., Al-Mulla, F., & Thanaraj, T. A. (2021). Genome-wide landscape establishes novel association signals for metabolic traits in the Arab population. *Human Genetics*, *140*, 505–528. http://doi.org/10.1007/s00439-020-02222-7

Heller, D. A., de Faire, U., Pedersen, N. L., Dahlen, G., & McClearn, G. E. (1993). Genetic and environmental influences on serum lipid levels in twins. *New England Journal of Medicine*, *328*, 1150–1156. http://doi.org/10.1056/NEJM199304223281603

Herskind, A. M., McGue, M., Sorensen, T. I., & Harvald, B. (1996). Sex and age specific assessment of genetic and environmental influences on body mass index in twins. *International Journal of Obesity and Related Metabolic Disorders*, *20*, 106–113. https://www.ncbi.nlm.nih.gov/pubmed/8646246

Hong, K. W., Go, M. J., Jin, H. S., Lim, J. E., Lee, J. Y., Han, B. G., Hwang, S. Y., Lee, S. H., Park, H. K., Cho, Y. S., & Oh, B. (2010). Genetic variations in ATP2B1, CSK, ARSG and CSMD1 loci are related to blood pressure and/or hypertension in two Korean cohorts. *Journal of Human Hypertension*, *24*, 367–372. http://doi.org/10.1038/jhh.2009.86

Howie, B. N., Donnelly, P., & Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLOS Genetics*, *5*, e1000529. http://doi.org/10.1371/journal.pgen.1000529

Hu, Y. W., Hu, Y. R., Zhao, J. Y., Li, S. F., Ma, X., Wu, S. G., Lu, J. B., Qiu, Y. R., Sha, Y. H., Wang, Y. C., Gao, J. J., Zheng, L., & Wang, Q. (2014). An agomir of miR-144-3p accelerates plaque formation through impairing reverse cholesterol transport and promoting pro-inflammatory cytokine production. *PLoS One*, *9*, e94997. http://doi.org/10.1371/journal.pone.0094997

Kim, Y. K., Hwang, M. Y., Kim, Y. J., Moon, S., Han, S., & Kim, B. J. (2016). Evaluation of pleiotropic effects among common genetic loci identified for cardio-metabolic traits in a Korean population. *Cardiovascular Diabetology*, *15*, 20. http://doi.org/10.1186/s12933-016-0337-1

Kring, S. I., Holst, C., Zimmermann, E., Jess, T., Berentzen, T., Toubro, S., Hansen, T., Astrup, A., Pedersen, O., & Sorensen, T. I. (2008). FTO gene associated fatness in relation to body fat distribution and metabolic traits throughout a broad range of fatness. *PLoS One*, *3*, e2958. http://doi.org/10.1371/journal.pone.0002958

Li, L., Gao, W., Yu, C., Lv, J., Cao, W., Zhan, S., Wang, S., Wu, C., & Hu, Y. (2013). The Chinese National Twin Registry: An update. *Twin Research and Human Genetics*, *16*, 86–90. http://doi.org/10.1017/thg.2012.148

Li, Y., Cao, K., Zhu, G., Fang, W., Chen, C., Wang, X., Zhao P., Guo J., Ding, T., Guan, L., Zhang, Q., Guo, W., Fei, Z., & Wang, L. (2019). Genomic analyses of an extensive collection of wild and cultivated accessions provide new insights into peach breeding history. *Genome Biology*, *20*, 36. http://doi.org/10.1186/s13059-019-1648-9

Liao, C., Gao, W., Cao, W., Lv, J., Yu, C., Wang, S., Zhou, B., Pang, Z., Cong, L., Wang, H., Wu, X., & Li, L. (2015). Associations of body composition measurements with serum lipid, glucose and insulin profile: A Chinese twin study. *PLoS One*, *10*, e0140595. http://doi.org/10.1371/journal.pone.0140595

Lin, C. C., Peyser, P. A., Kardia, S. L., Li, C. I., Liu, C. S., Chu, J. S., Lin, W. Y., & Li, T. C. (2014). Heritability of cardiovascular risk factors in a Chinese population ¾Taichung Community Health Study and Family Cohort. *Atherosclerosis*, *235*, 488–495. http://doi.org/10.1016/j.atherosclerosis.2014.05.939

Lin, M. L., Park, J. H., Nishidate, T., Nakamura, Y., & Katagiri, T. (2007). Involvement of maternal embryonic leucine zipper kinase (MELK) in mammary carcinogenesis through interaction with Bcl-G, a pro-apoptotic member of the Bcl-2 family. *Breast Cancer Research*, *9*, R17. http://doi.org/10.1186/bcr1650

Liu, C. T., Monda, K. L., Taylor, K. C., Lange, L., Demerath, E. W., Palmas, W., Wojczynski, M. K., Ellis, J. C., Vitolins, M. Z., Liu, S., Papanicolaou, G. J., Irvin, M. R., Xue, L., Griffin, P. J., Nalls, M. A., Adeyemo, A., Liu, J., Li, G., Ruiz-Narvaez, E. A., Chen, W. M., … Fox, C. S. (2013). Genome-wide association of body fat distribution in African ancestry populations suggests new loci. *PLOS Genetics*, *9*, e1003681. http://doi.org/10.1371/journal.pgen.1003681

Lotta, L. A., Wittemans, L. B. L., Zuber, V., Stewart, I. D., Sharp, S. J., Luan, J., Day, F. R., Li C., Bowker, N., Cai, L., De Lucia Rolfe, E., Khaw, K. T., Perry, J. R. B., O'Rahilly, S., Scott, R. A., Savage, D. B., Burgess, S., Wareham, N. J., & Langenberg, C. (2018). Association of genetic variants related to gluteofemoral versus abdominal fat distribution with type 2 diabetes, coronary disease, and cardiovascular risk factors. *JAMA*, *320*, 2553–2563. http://doi.org/10.1001/jama.2018.19329

Mahaney, M. C., Blangero, J., Comuzzie, A. G., VandeBerg, J. L., Stern, M. P., & MacCluer, J. W. (1995). Plasma HDL cholesterol, triglycerides, and adiposity. A quantitative genetic test of the conjoint trait hypothesis in the San Antonio Family Heart Study. *Circulation*, *92*, 3240–3248. http://doi.org/10.1161/01.cir.92.11.3240

Mather, L., Blom, V., Bergstrom, G., & Svedberg, P. (2016). An underlying common factor, influenced by genetics and unique environment, explains the covariation between major depressive disorder, generalized anxiety disorder, and burnout: A Swedish twin study. *Twin Research and Human Genetics*, *19*, 619–627. http://doi.org/10.1017/thg.2016.73

Nock, N. L., Wang, X., Thompson, C. L., Song, Y., Baechle, D., Raska, P., Stein, C. M., & Gray-McGuire, C. (2009). Defining genetic determinants of the metabolic syndrome in the Framingham Heart Study using association and structural equation modeling methods. *BMC Proceedings*, *3*, S50. http://doi.org/10.1186/1753-6561-3-s7-s50

Orstavik, R. E., Kendler, K. S., Czajkowski, N., Tambs, K., & Reichborn-Kjennerud, T. (2007). Genetic and environmental contributions to depressive personality disorder in a population-based sample of Norwegian twins. *Journal of Affective Disorders*, *99*, 181–189. http://doi.org/10.1016/j.jad.2006.09.011

Pang, Y., Kartsonaki, C., Turnbull, I., Guo, Y., Chen, Y., Clarke, R., Bian, Z., Bragg, F., Millwood, I. Y., Yang, L., Huang, Y., Yang, Y., Zhang, X., Chen, J., Li, L., Holmes M. V., & Chen, Z. (2019). Adiposity in relation to risks of fatty liver, cirrhosis and liver cancer: A prospective study of 0.5 million Chinese adults. *Scientific Reports*, *9*, 785. http://doi.org/10.1038/s41598-018-36460-7

Pang, Z., Zhang, D., Li, S., Duan, H., Hjelmborg, J., Kruse, T. A., Kyvik, K. O., Christensen, K., & Tan, Q. (2010). Multivariate modelling of endophenotypes associated with the metabolic syndrome in Chinese twins. *Diabetologia*, *53*, 2554–2561. http://doi.org/10.1007/s00125-010-1907-5

Parr, C. L., Batty, G. D., Lam, T. H., Barzi, F., Fang, X., Ho, S. C., Jee, S. H., Ansary-Moghaddam, A., Jamrozik, K., Ueshima, H., Woodward, M., Huxley, R. R., & **Asia-Pacific Cohort Studies Collaboration.** (2010). Body-mass index and cancer mortality in the Asia-Pacific Cohort Studies Collaboration: Pooled analyses of 424,519 participants. *Lancet Oncology*, *11*, 741–752. http://doi.org/10.1016/S1470-2045(10)70141-8

Ramirez, C. M., Rotllan, N., Vlassov, A. V., Davalos, A., Li, M., Goedeke, L., Aranda, J. F., Cirera-Salinas, D., Araldi, E., Salerno, A., Wanschel, A., Zavadil, J., Castrillo, A., Kim, J., Suárez, Y., & Fernandez-Hernando, C. (2013). Control of cholesterol metabolism and plasma high-density lipoprotein levels by microRNA-144. *Circulation Research*, *112*, 1592–1601. http://doi.org/10.1161/CIRCRESAHA.112.300626

Ran, S., Pei, Y. F., Liu, Y. J., Zhang, L., Han, Y. Y., Hai, R., Tian Q., Lin, Y., Yang, T. L., Guo, Y. F., Shen, H., Thethi, I. S., Zhu, X. Z., & Deng, H. W. (2013). Bivariate genome-wide association analyses identified genes with pleiotropic effects for femoral neck bone geometry and age at menarche. *PLoS One*, *8*, e60362. http://doi.org/10.1371/journal.pone.0060362

Randall, J. C., Winkler, T. W., Kutalik, Z., Berndt, S. I., Jackson, A. U., Monda, K. L., Kilpeläinen, T. O., Esko, T., Mägi, R., Li S., Workalemahu, T., Feitosa, M. F., Croteau-Chonka, D. C., Day, F. R., Fall, T., Ferreira, T., Gustafsson, S., Locke, A. E., Mathieson, I., … Heid, I. M. (2013). Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLOS Genetics*, *9*, e1003500. http://doi.org/10.1371/journal.pgen.1003500

Rankinen, T., Sarzynski, M. A., Ghosh, S., & Bouchard, C. (2015). Are there genetic paths common to obesity, cardiovascular disease outcomes, and

cardiovascular risk factors? *Circulation Research*, *116*, 909–922. http://doi.org/10.1161/CIRCRESAHA.116.302888

Rhee, E. P., Ho, J. E., Chen, M. H., Shen, D., Cheng, S., Larson, M. G., Ghorbani, A., Shi, X., Helenius, I. T., O'Donnell, C. J., Souza, A. L., Deik, A., Pierce, K. A., Bullock, K., Walford, G. A., Vasan, R. S., Florez, J. C., Clish, C., Yeh, J. R., … Gerszten, R. E. (2013). A genome-wide association study of the human metabolome in a community-based cohort. *Cell Metabolism*, *18*, 130–143. http://doi.org/10.1016/j.cmet.2013.06.013

Rohde, K., Keller, M., la Cour Poulsen, L., Bluher, M., Kovacs, P., & Bottcher, Y. (2019). Genetics and epigenetics in obesity. *Metabolism*, *92*, 37–50. http://doi.org/10.1016/j.metabol.2018.10.007

Schorr, M., Dichtel, L. E., Gerweck, A. V., Valera, R. D., Torriani, M., Miller, K. K., & Bredella, M. A. (2018). Sex differences in body composition and association with cardiometabolic risk. *Biology of Sex Differences*, *9*, 28. http://doi.org/10.1186/s13293-018-0189-3

Schousboe, K., Willemsen, G., Kyvik, K. O., Mortensen, J., Boomsma, D. I., Cornes, B. K., Davis, C. J., Fagnani, C., Hjelmborg, J., Kaprio, J., De Lange, M., Luciano, M., Martin, N. G., Pedersen, N., Pietiläinen, K. H., Rissanen, A., Saarni, S., Sørensen, T. I., Van Baal, G. C., & Harris, J. R. (2003). Sex differences in heritability of BMI: A comparative study of results from twin studies in eight countries. *Twin Research*, *6*, 409–421. http://doi.org/10.1375/136905203770326411

Solovieff, N., Cotsapas, C., Lee, P. H., Purcell, S. M., & Smoller, J. W. (2013). Pleiotropy in complex traits: challenges and strategies. *Nature Reviews Genetics*, *14*, 483–495. http://doi.org/10.1038/nrg3461

Souren, N. Y., Paulussen, A. D., Loos, R. J., Gielen, M., Beunen, G., Fagard, R., Derom, C., Vlietinck, R., & Zeegers, M. P. (2007). Anthropometry, carbohydrate and lipid metabolism in the East Flanders Prospective Twin Survey: Heritabilities. *Diabetologia*, *50*, 2107–2116. http://doi.org/10.1007/s00125-007-0784-z

Sun, P. C., Uppaluri, R., Schmidt, A. P., Pashia, M. E., Quant, E. C., Sunwoo, J. B., Gollin, S. M., & Scholnick, S. B. (2001). Transcript map of the 8p23 putative tumor suppressor region. *Genomics*, *75*, 17–25. http://doi.org/10.1006/geno.2001.6587

Tang, W., Hong, Y., Province, M. A., Rich, S. S., Hopkins, P. N., Arnett, D. K., Pankow, J. S., Miller, M. B., & Eckfeldt, J. H. (2006). Familial clustering for features of the metabolic syndrome: the National Heart, Lung, and Blood Institute (NHLBI) Family Heart Study. *Diabetes Care*, *29*, 631–636. http://doi.org/10.2337/diacare.29.03.06.dc05-0679

Tao, S., Li, Y., Xiao, Z., Cen, R., Zhang, H., Zhuo, Y., Zhou, B., Chen, P., Li, Y., Liao, Y., Folsom, A., Stamler, J., Warnick, G. R., & Williams, O. D. (1992). Serum lipids and their correlates in Chinese urban and rural populations of Beijing and Guangzhou. PRC-USA Cardiovascular and Cardiopulmonary Epidemiology Research Group. *International Journal of Epidemiology*, *21*, 893–903. http://doi.org/10.1093/ije/21.5.893

Wang, B., Gao, W., Yu, C., Cao, W., Lv, J., Wang, S., Pang, Z, Cong, L., Wang, H., Wu, X., & Li, L. (2015). Determination of zygosity in adult Chinese twins using the 450K methylation array versus questionnaire data. *PLoS One*, *10* e0123992. http://doi.org/10.1371/journal.pone.0123992

Wang, X., Zheng, Y., Ma, Y., Du, L., Chu, F., Gu, H., Dahlgren, R. A., Li, Y., & Wang, H. (2018). Lipid metabolism disorder induced by up-regulation of miR-125b and miR-144 following beta-diketone antibiotic exposure to F0-zebrafish (Danio rerio). *Ecotoxicology and Environmental Safety*, *164*, 243–252. http://doi.org/10.1016/j.ecoenv.2018.08.027

Weiss, L. A., Pan, L., Abney, M., & Ober, C. (2006). The sex-specific genetic architecture of quantitative traits in humans. *Nature Genetics*, *38*, 218–222. http://doi.org/10.1038/ng1726

Willer, C. J., Schmidt, E. M., Sengupta, S., Peloso, G. M., Gustafsson, S., Kanoni, S., Ganna, A., Chen, J., Buchkovich, M. L., Mora, S., Beckmann, J. S., Bragg-Gresham, J. L., Chang, H. Y., Demirkan A., Den Hertog, H. M., Do, R., Donnelly, L. A., Ehret, G. B., Esko, T., … Global Lipids Genetics Consortium. (2013). Discovery and refinement of loci associated with lipid levels. *Nature Genetics*, *45*, 1274–1283. http://doi.org/10.1038/ng.2797

Winkler, T. W., Justice, A. E., Graff, M., Barata, L., Feitosa, M. F., Chu, S., Czajkowski, J., Esko, T., Fall, T., Kilpeläinen, T. O., Lu, Y., Mägi, R., Mihailov, E., Pers, T. H., Rüeger, S., Teumer, A., Ehret, G. B., Ferreira, T., Heard-Costa, N. L., … Loos, R. J. (2015). The influence of age and sex on genetic associations with adult body size and shape: A large-scale genome-wide interaction study. *PLOS Genetics*, *11*, e1005378. http://doi.org/10.1371/journal.pgen.1005378

Wurtz, P., Wang, Q., Kangas, A. J., Richmond, R. C., Skarp, J., Tiainen, M., Tynkkynen, T, Soininen, P., Havulinna, A. S., Kaakinen, M., Viikari, J. S., Savolainen, M. J., Kähönen, M., Lehtimäki, T., Männistö, S., Blankenberg, S., Zeller, T., Laitinen, J., Pouta, A., … Ala-Korpela, M. (2014). Metabolic signatures of adiposity in young adults: Mendelian randomization analysis and effects of weight change. *PLOS Medicine*, *11*, e1001765. http://doi.org/10.1371/journal.pmed.1001765

Yan, Y. X., Xiao, H. B., Zhang, J., Wang, S., Dong, J., & Wu, L. J. (2021). Pri-miR-144 rs9279 is associated with type 2 diabetes and regulation of stress response. *Journal of Cellular Physiology*, *236*, 561–569. http://doi.org/10.1002/jcp.29883

Yang, J., Bakshi, A., Zhu, Z., Hemani, G., Vinkhuyzen, A. A., Nolte, I. M., van Vliet-Ostaptchouk, J. V., Snieder, H., Lifelines Cohort Study, Esko, T., Milani, L., Mägi, R., Metspalu, A., Hamsten, A., Magnusson, P. K., Pedersen, N. L., Ingelsson, E., & Visscher, P. M. (2015). Genome-wide genetic homogeneity between sexes and populations for human height and body mass index. *Human Molecular Genetics*, *24*, 7445–7449. http://doi.org/10.1093/hmg/ddv443

Zhou, B., Li, L., Lyu, J., Yu, C., Wang, S., Pang, Z., Cong, L., Dong, Z., Wu, F., Wang, H., Wu, X., Jiang, G., Wang, X., Wang, B., Gao, W., & Cao, W. (2015). Heritability of body mass index on Chinese adult twins from nine provinces/cities in China. *Zhonghua Liu Xing Bing Xue Za Zhi*, *36*, 299–303. https://www.ncbi.nlm.nih.gov/pubmed/25975537

Zhou, X., & Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. *Nature Genetics*, *44*, 821–824. http://doi.org/10.1038/ng.2310

Zhou, X., & Stephens, M. (2014). Efficient multivariate linear mixed model algorithms for genome-wide association studies. *Nature Methods*, *11*, 407–409. http://doi.org/10.1038/nmeth.2848

Zhu, H., & Leung, S. W. (2015). Identification of microRNA biomarkers in type 2 diabetes: A meta-analysis of controlled profiling studies. *Diabetologia*, *58*, 900–911. http://doi.org/10.1007/s00125-015-3510-2

Zhu, S., Heymsfield, S. B., Toyoshima, H., Wang, Z., Pietrobelli, A., & Heshka, S. (2005). Race-ethnicity-specific waist circumference cutoffs for identifying cardiovascular disease risk factors. *American Journal of Clinical Nutrition*, *81*, 409–415. http://doi.org/10.1093/ajcn.81.2.409

Zhu, X., Feng, T., Tayo, B. O., Liang, J., Young, J. H., Franceschini, N., Smith, J. A., Yanek, L. R., Sun, Y. V., Edwards, T. L., Chen, W., Nalls, M., Fox, E., Sale, M., Bottinger, E., Rotimi, C., COGENT BP Consortium, Liu, Y., McKnight, B., … Redline, S. (2015). Meta-analysis of correlated traits via summary statistics from GWASs with an application in hypertension. *American Journal of Human Genetics*, *96*, 21–36. http://doi.org/10.1016/j.ajhg.2014.11.011

Zhu, Z., Guo, Y., Shi, H., Liu, C. L., Panganiban, R. A., Chung, W., O'Connor, L. J., Himes, B. E., Gazal, S., Hasegawa, K., Camargo, C. A. Jr., Qi, L., Moffatt, M. F., Hu, F. B., Lu, Q., Cookson, W. O. C., & Liang, L. (2020). Shared genetic and experimental links between obesity-related traits and asthma subtypes in UK Biobank. *Journal of Allergy and Clinical Immunology*, *145*, 537–549. http://doi.org/10.1016/j.jaci.2019.09.035

https://doi.org/10.1017/thg.2022.39 Published online by Cambridge University Press