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## Adult weight change and cardiometabolic disease: studies into underlying pathways

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# CHAPTER 2

## **The contribution of tissue-grouped BMI-associated gene sets to cardiometabolic-disease risk: a Mendelian randomization study**

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## ABSTRACT

*Background:* Body mass index (BMI)-associated loci are used to explore the effects of obesity using Mendelian Randomization (MR), but contribution of individual tissues to risks remain unknown. We aimed to identify tissue-grouped pathways of BMI-associated loci and relate these to cardiometabolic disease using MR analyses.

*Methods:* Using Genotype-Tissue Expression (GTEx) data, we performed overrepresentation tests to identify tissue-grouped gene sets based on mRNA expression profiles from 634 previously published BMI-associated loci. We conducted two-sample MR with inverse-variance weighted methods, to examine associations between tissue-grouped BMI-associated genetic instruments and type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD), with use of summary-level data from published genome-wide association studies (T2DM: 74,124 cases, 824,006 controls; CAD: 60,801 cases, 123,504 controls). Additionally, we performed MR analyses on T2DM and CAD using randomly sampled sets of 100 or 200 BMI-associated genetic variants.

*Results:* We identified 17 partly overlapping tissue-grouped gene sets, of which 12 were brain areas, where BMI-associated genes were differentially expressed. In tissue-grouped MR analyses, all gene sets were similarly associated with increased risks of T2DM and CAD. MR analyses with randomly sampled genetic variants on T2DM and CAD resulted in a distribution of effect estimates similar to tissue-grouped gene sets.

*Conclusion:* Overrepresentation tests revealed differential expression of BMI-associated genes in 17 different tissues. However, with our biology-based approach using tissue-grouped MR analyses we did not identify different risks of T2DM or CAD for the BMI-associated gene sets, which was reflected by similar effect estimates obtained by randomly sampled gene sets.

## INTRODUCTION

The prevalence of obesity and obesity-related diseases is increasing worldwide (1). Previous studies identified many genetic variants associated with an increased risk of developing obesity and a high body mass index (BMI) (2-4). A Genome-Wide Association Study (GWAS) by Yengo et al. (2018) identified 656 independent genetic variants associated with BMI, which collectively explained up to 6% of the total variation in BMI (4). High BMI is a well-known risk factor for cardiometabolic disease, such as type 2 diabetes mellitus and cardiovascular disease (5-8), which has been confirmed in Mendelian Randomization (MR) analyses, in which genetic variants associated with an exposure are used as instrumental variables (9).

Characterization of genetic variants that are associated with BMI, and the genes corresponding to these single nucleotide polymorphisms (SNPs), can provide insight in the biological causes of a high BMI. In an earlier MR study on the association between BMI and anxiety (10), SNPs were categorized based on three mechanistic domains through which they were likely to influence BMI: appetite, adipogenesis and cardiopulmonary function, derived from an earlier GWAS on BMI (3). As an alternative and complementary approach to gene categorization based on mechanistic domains, tissue-grouped gene sets of BMI-associated genes can be identified by analyzing the differential expression of BMI-associated genes in tissues: genes which expression is significantly up- or down-regulated in a given tissue compared with other tissues (11).

Enrichment of BMI-associated genes has been observed in tissues of the central nervous system, notably in the hypothalamus, pituitary gland, hippocampus and limbic system (2), which are brain areas involved in appetite regulation, cognition, and emotion, and thereby collectively highlighted the potential biological processes involved in the regulation of body composition. Additionally, a recent study combined genes for BMI, waist-hip ratio (WHR) and WHR adjusted for BMI (WHRadjBMI), which are all proxies of body composition, to identify enrichment of these genes on a tissue level (12). In this study, differential expression of these body composition-related genes was observed in the central nervous system, adipocyte-related tissues, and the digestive and urogenital system (12). Additionally, BMI-associated genes that show differential expression in specific tissues, thereby reflecting potential tissue-grouped causes of a high BMI, may have distinct effects on disease risk as well (13-16). A more detailed investigation of these biological causes will allow a comprehensive overview of potential targets for interventions.

For this reason, we aimed to group BMI-associated genetic instruments based on tissue expression profiles. With these newly identified combinations of BMI-associated genetic instruments, we aimed to conduct two-sample Mendelian Randomization (MR) analyses on cardiometabolic diseases and measures of abdominal adiposity, in order to unravel the underlying pathways of tissue-grouped causes of a high BMI leading to cardiometabolic disease risk.

## METHODS

### *Selection of genetic variants and gene annotation for BMI*

We selected single nucleotide polymorphisms (SNPs) for all genetic loci independently associated with BMI, which were identified in the GWAS meta-analysis by Yengo et al (4).

For the meta-analysis, results of the GWAS on BMI in the UK Biobank were combined with publicly available summary statistics of an earlier GWAS on BMI in the Genetic Investigation of ANthropometric Traits (GIANT) consortium, comprising around 700,000 individuals of mainly European ancestry in total. After meta-analysis, 656 independent SNPs associated with BMI were identified in their main genome-wide significance association ( $p$ -value  $<5 \times 10^{-8}$ ) (see **Supplementary Table 1**).

The 656 SNPs were annotated and mapped to genes based on several lines of evidence using the SNP2GENE function within the Functional Mapping and Annotation (FUMA) v1.3.5e web application (date of accession: 12-11-2019) (17). First, we uploaded a file with the GWAS summary statistics, including the chromosome and position, reference SNP (rs) ID,  $p$ -value, effect allele, non-effect allele, beta, and standard deviation for each SNP, and the sample size of the GWAS.

We did not predefine lead SNPs of a genomic region, but instead used the following default parameters: minimum  $p$ -value of lead SNPs  $<5e^{-8}$ , maximum  $p$ -value cut-off  $<0.05$ ,  $r^2$  threshold to define independent significant SNPs  $\geq 0.6$ , second  $r^2$  threshold to define lead SNPs  $\geq 0.1$ , reference panel population 1000G Phase3 EUR, minimum Minor Allele Frequency (MAF)  $\geq 0$ , and maximum distance between LD blocks to merge into a locus  $<250$  kb. We included variants in the reference 1000G reference panel (non-GWAS tagged SNPs in LD with an independent significant SNP) (18). Gene annotation was based on positional mapping obtained from Annotate Variation (ANNOVAR) with a maximum distance to genes of 10 kilobases (19). For the gene types, we used Ensembl v92 and selected protein coding gene types. A set of prioritized genes is derived from the combined mapping strategies, based on the provided GWAS summary statistics and filter settings. The output from FUMA comprised genome-wide plots, a summary of the results, and result tables, from which we selected the table with independently significant lead SNPs and the table with mapped genes, and merged these to obtain a list with lead SNPs and their mapped genes. Due to the use of a filter to identify independent SNPs, 22 lead SNPs were omitted, resulting in 634 mapped genes of interest which were used to identify the tissue-grouped gene sets. All exact parameters used for gene annotation and mapping can be found in the **Supplementary Methods**.

### *Tissue-grouped gene sets*

Normalized gene expressions (reads per kilo base per million, RPKM) of 54 non-diseased tissue types were available in GTEx version 8 (11), of which we excluded five tissue types because no eQTL analysis was available (bladder, cervix-ectocervix and -endocervix, fallopian tube and kidney medulla). Tissue types included different brain areas, adipose tissue depots, skin, blood, spleen, kidney cortex, and tissues involved in digestion (e.g. esophagus, stomach, small intestine, pancreas and colon) or reproduction (e.g. ovaries, uterus, vagina

and testis). In the GENE2FUNC in FUMA, the average of the normalized expression (zero mean of  $\log_2(\text{RPKM} + 1)$ ) per tissue per gene was shown as expression value, to allow for comparison of expression across tissue types. By this means, we identified differentially expressed gene sets (genes of which the expression is significantly up- or downregulated in a given tissue compared with other tissues) for each of the 54 tissue types available in GTEx v8. Two-sided Student's t-tests with Bonferroni correction were performed per gene per tissue against all other tissues to identify differentially expressed genes in a given tissue. In GENE2FUNC in FUMA, genes are tested against those differentially expressed gene sets by hypergeometric tests to evaluate whether the prioritized genes (genes of interest) are over-represented in differentially expressed gene sets in the 54 specific tissue types. We entered the 634 BMI-associated genes (our mapped genes of interest based on the independent lead SNPs) in the GENE2FUNC function and selected 'All' background genes, Ensembl version v92 and the GTEx v8 54 tissue type (date of accession: 12-11-2019). We selected the tissues that showed significant differential gene expression in GTEx v8 after adjusting for multiple testing using a false discovery rate (FDR) threshold of 5%. Using the summary data retrieved from FUMA, we identified the specific genes that were differentially expressed in each tissue to establish the gene sets used in the tissue-grouped Mendelian Randomization analyses. All tissue-grouped gene sets were considered strong instruments for BMI based on their F-statistics. All exact parameters used to generate the tissue-grouped gene sets can be found in the **Supplementary Methods**.

#### ***Outcome data on type 2 diabetes and coronary heart disease***

As study outcomes for the MR analyses, we used publicly available meta-analysis summary statistics of GWAS on type 2 diabetes mellitus and coronary heart disease by the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) and the Coronary Artery Disease Genome wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics (CARDIoGRAMplusC4D) consortia, respectively. These datasets, based on the largest number of participants to date, contain the per-allele beta-estimates of all investigated SNPs on the outcomes, accompanying standard errors, and the effect alleles.

The report of the DIAGRAM consortium was a meta-analysis of 32 different cohort studies of European ancestry (including the UK Biobank), consisting of 74,124 cases of T2DM and 824,006 controls (20). T2DM was defined as a fasting glucose concentration higher than 6.9 mmol/L or 2-hour plasma glucose of  $\geq 11.1$  mmol/L, treatment with glucose-lowering agents, diagnosis by a general practitioner or medical specialist, or self-report.

Within the CARDIoGRAMC4D consortium, GWAS data of 48 studies were meta-analysed, with 60,801 cases and 123,504 controls (21). Coronary artery disease was defined by a history of myocardial infarction, acute coronary syndrome, chronic stable angina or >50% coronary stenosis.

#### ***Outcome data on anthropometric traits***

The underlying mechanism of the different effects of the tissue-grouped gene sets on disease risk could be via the effect of the tissue-grouped gene sets on body fat distribution measured by waist circumference, or total body fat. By this means, we aimed to give insight

in the underlying mechanisms of tissue-grouped causes of a high BMI and their effect on disease risk. We used publicly available meta-analysis summary statistics of anthropometric traits conducted by the Genetic Investigation of Anthropometric Traits (GIANT) consortium and by the Neale Lab. The data of the GIANT consortium were reported as meta-analysis of 57 multi-ancestry cohorts, with oversampling of cohorts of European-ancestry, genotyped by genome-wide SNP arrays and of 44 cohorts genotyped with the MetaboChip. In the present analysis we used waist circumference (in centimetres, unadjusted for BMI) in up to 224,459 individuals (22).

The GWAS on total body fat (as a percentage) was based on data from the UK Biobank, in up to 331,117 individuals of white British genetic ancestry (23). Closely related individuals, individuals with sex chromosome aneuploidies, and individuals who had withdrawn consent from the UK Biobank study had been removed from the analysis.

### ***Mendelian Randomization analysis***

Methods for MR analyses of summary-level data based on two study samples have been described in detail previously (24, 25). We estimated the associations between all genetic instruments for BMI, the tissue-grouped gene sets and the outcome measures (coronary artery disease, type 2 diabetes mellitus and anthropometric traits). We allowed for use of LD proxies ( $R^2 > 0.8$ ) and we allowed palindromic SNPs with a MAF threshold of 0.3. Using inverse-variance weighted (IVW) analyses, we combined the effects of the individual genetic instruments to obtain a genetically determined association between exposure and outcome under the assumption of absence of horizontal pleiotropy. The IVW analyses resulted in a weighted mean estimate of a genetically determined increase of 1 standard deviation (SD) of BMI ( $\text{kg}/\text{m}^2$ ) on the odds ratio of type 2 diabetes mellitus and coronary artery disease, or on waist circumference (SD, in centimetres), and on total body fat (SD, percentage). In the present study, an increase of 1 SD of BMI ( $\text{kg}/\text{m}^2$ ) corresponded to an increase in BMI of 4.8  $\text{kg}/\text{m}^2$ .

MR analyses could suffer from bias due to pleiotropic effects, when a genetic variant also affects other phenotypes, and thereby influences the outcome via alternative pathways other than through the exposure. Therefore, as sensitivity analyses, we performed MR Egger regression (26) and weighted median estimator (WME) analyses (27). MR Egger accounts for potential pleiotropy and tests for the presence of directional pleiotropy, whereas WME estimates a weighted median effect instead of a weighted mean effect. Similarity of the IVW and MR Egger and WME effect estimates indicates that the results of the MR analysis are robust. In addition to MR Egger and WME, we used the recently described Weighted Mode to detect causal effects, a method that is consistent when the largest number of similar individual causal effect estimates comes from valid instruments, even if the majority of instruments is invalid (28).

For MR analyses, we removed the genetic instrument mapped to TCF7L2 (rs7903146) from the gene sets (and thereby all MR analyses) given its previously described complex and pleiotropic effect (29). Discrepancies between the total numbers of genetic variants in the tissue-grouped gene sets and the numbers of genetic variants in the tissue-grouped gene



sets which were used in the MR analyses are due to the removal of palindromic genetic instruments with intermediate allele frequencies.

The combined effects of the genetic variants in the overall and sensitivity analyses were estimated using the R-based package “TwoSampleMR” (30). The R script for the Mendelian Randomization analyses can be found in the **Supplementary Methods**.

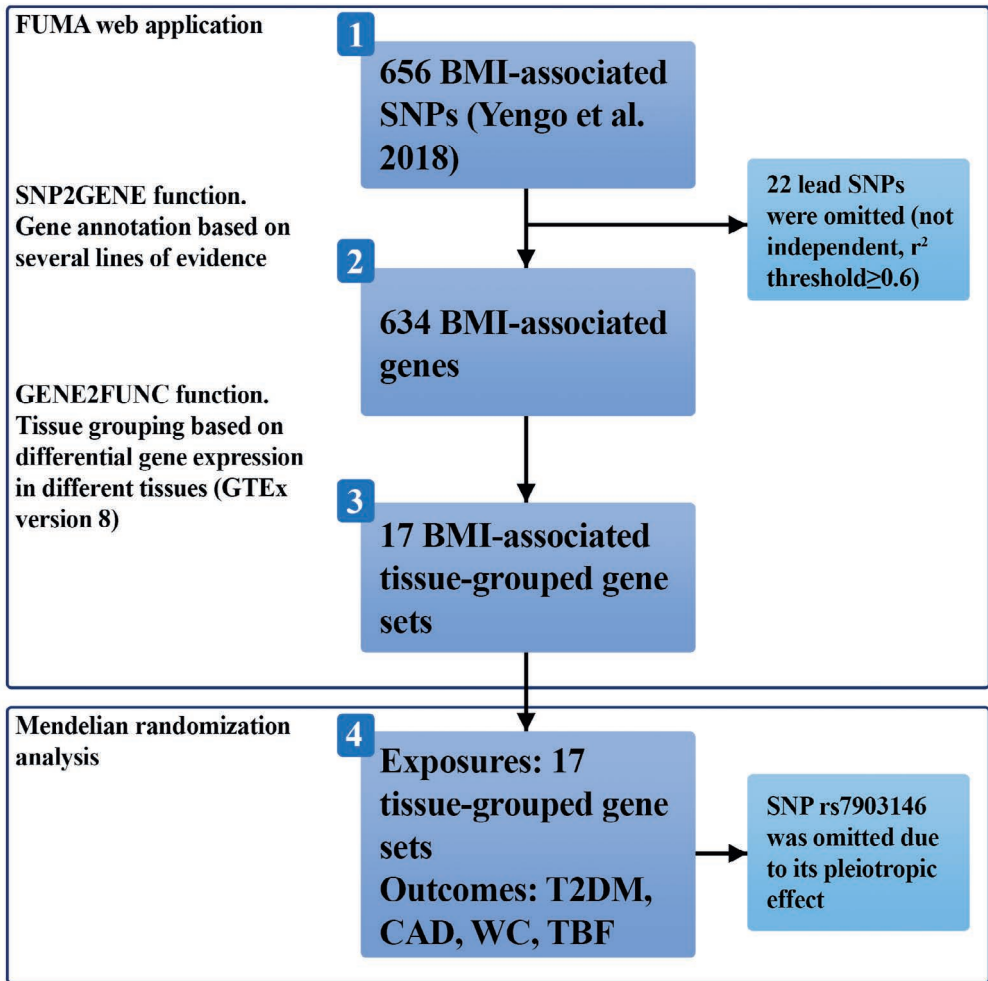
### ***Mendelian randomization analyses using random samples of genetic variants***

To gain insight in whether GTEx-based subsets of genetic instruments are the result of chance, we additionally determined the effects estimates for CAD and T2DM by randomly selecting genetic variants. We randomly sampled 1000 gene sets, consisting of 100 or 200 genetic variants from the 633 BMI-associated genetic variants and performed MR analyses (IVW) on CAD and T2DM with these random gene sets. The R script for the Mendelian Randomization analyses using random samples of genetic variants can be found in the **Supplementary Methods**.

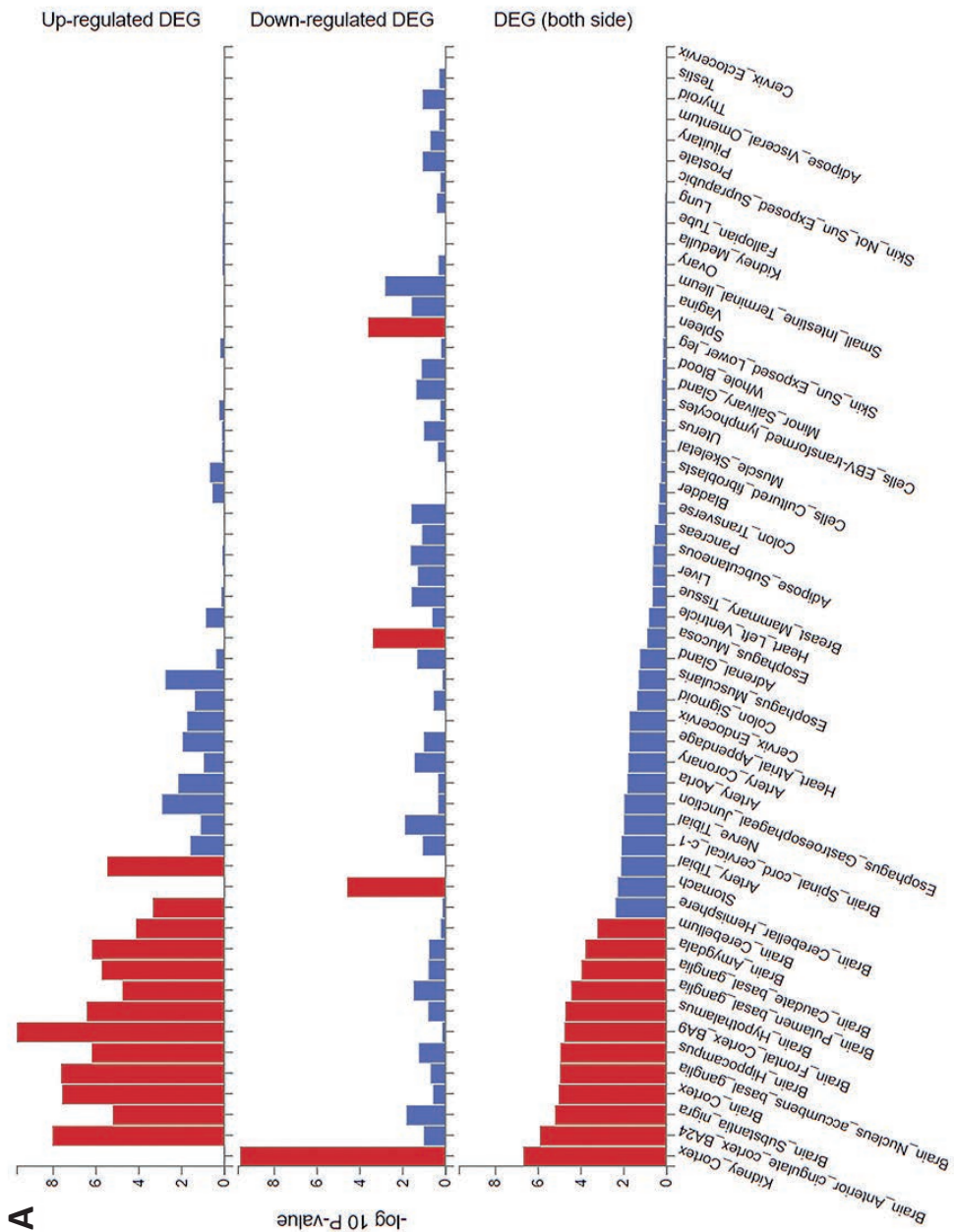
## **RESULTS**

### ***Identification of tissue-grouped expression gene sets***

**Figure 1** shows the analysis procedure we followed to obtain the tissue-grouped BMI-associated gene sets from 656 BMI-associated SNPs. Based on the analysis with GTEx data, we identified 17 tissues in which genes associated with BMI were differentially expressed (**Figure 2A**), of which 12 derived from brain areas. Other tissues were derived from the arteries, digestive system, spleen, or kidney cortex. Differentially expressed genes in each tissue were identified to establish the gene sets (see **Figure 2A**). There was substantial overlap in the genes that were differentially expressed in the brain tissues, but none had a completely overlapping expression profile. Gene symbols and their expression in the different tissue-grouped gene sets are provided in **Supplementary Table 1**. Clustering of the BMI-associated genes in the tissue-grouped gene sets is shown in **Figure 2B**. It should be noted that a substantial number of genetic instruments was not differentially expressed in any of the identified tissues.



**Figure 1.** The analysis procedure. 1) We selected 656 single nucleotide polymorphisms (SNPs) independently associated with BMI (4), 2) Using the SNP2GENE function in the FUMA web application, gene annotation was performed for the selected SNPs based on positional mapping obtained from Annotate Variation (ANNOVAR) (19). In this step, 22 SNPs were omitted based on a  $r^2$  threshold of  $\geq 0.6$ . 3) The 634 BMI associated genes were grouped into 17 tissue-grouped gene sets using the GENE2FUNC function in Functional Mapping and Annotation (FUMA). These gene sets were based on differential gene expression in different tissues with the use of GTEx version 8. 4) The 17 tissue-grouped gene sets were used as exposures in Mendelian Randomization (MR) analyses, with the outcomes type 2 diabetes mellitus (T2DM), coronary artery disease (CAD), waist circumference (WC) and total body fat (TBF). The genetic instrument rs7903146 was removed from the gene sets (and thereby all MR analyses) given its pleiotropic effect.



**Figure 2.** A) Based on the analyses using GTEx v8 54 tissue types data, we identified 17 tissues in which body mass index (BMI)-associated genes were differentially expressed, indicated by red bars. Figure 2A was directly taken from the online tool Functional Mapping and Annotation (FUMA). B) Clustering of the BMI-associated genes in the tissue-grouped gene sets. Differentially expressed genes in a given tissue are shown in red, blue indicates no differential expression in a given tissue.

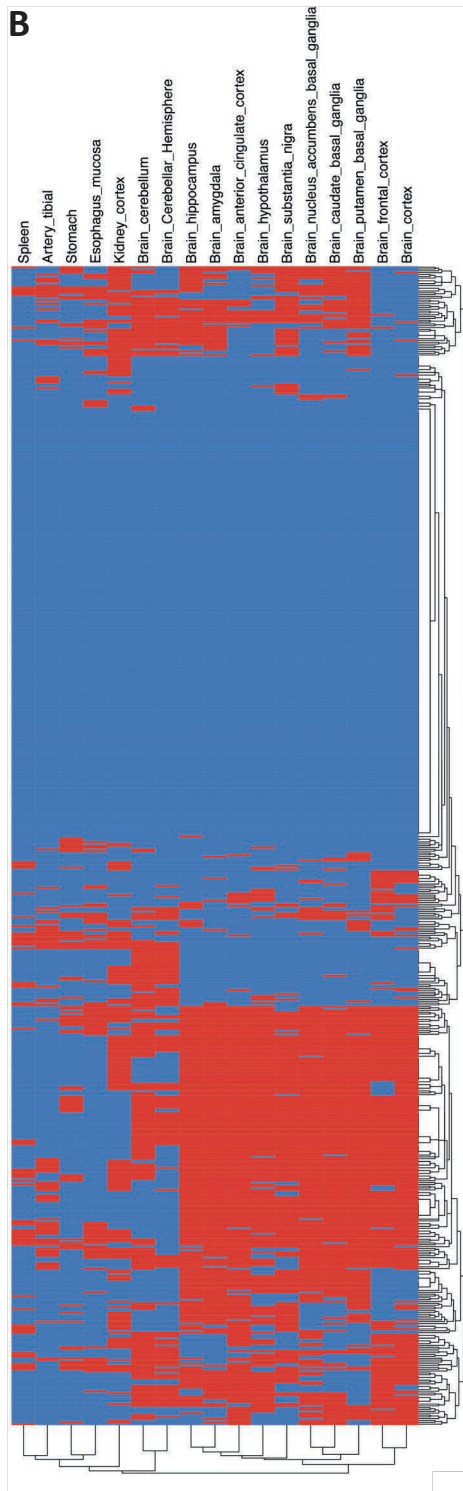


Figure 2. Continued.

## ***Tissue-grouped Mendelian Randomization analyses***

### *Type 2 diabetes mellitus and coronary artery disease*

We performed Mendelian Randomization with the tissue-grouped gene sets on T2DM and CAD (**Table 1**). Considering all BMI genetic instruments, an increase in BMI was associated with an increased risk of both T2DM (per SD BMI OR 2.71, 95% CI 2.49; 2.94) and CAD (per SD BMI, OR 1.48, 1.37; 1.59).

In the tissue-grouped MR analyses, all BMI-associated tissue-grouped gene sets were associated with an increased risk of T2DM (**Table 1**), with ORs ranging from 2.42 (2.04; 2.89) for the brain caudate basal ganglia to 2.94 (2.49; 3.47) for the brain cerebellar hemisphere. For CAD, the ORs were ranging from 1.32 (1.02; 1.70) for the spleen, to 1.58 (1.40; 1.78) for the brain putamen basal ganglia. The sensitivity analyses (MR Egger, WME and Weighted Mode) provided similar effects of the tissue-grouped gene sets on T2DM and CAD, although effect estimates were slightly larger compared with the IVW results (see **Supplementary Table 2**).

**Table 1.** Association between the BMI-overall and BMI-associated tissue-grouped gene sets and risk of type 2 diabetes and coronary heart disease using (tissue-grouped) inverse-variance weighted analyses.

	Type 2 diabetes			Coronary artery disease		
	$n_{\text{genes}}$	Beta (SE)	Odds ratio (95% CI)	$n_{\text{genes}}$	Beta (SE)	Odds ratio (95% CI)
BMI – all genetic variants	616	1.00 (0.04)	2.71 (2.49; 2.94)	613	0.39 (0.04)	1.48 (1.37; 1.59)
Artery tibial	86	0.93 (0.13)	2.54 (1.99; 3.50)	86	0.43 (0.10)	1.53 (1.26; 1.87)
Brain amygdala	231	0.90 (0.08)	2.45 (2.08; 2.89)	230	0.41 (0.07)	1.51 (1.32; 1.72)
Brain anterior cingulate cortex	232	0.93 (0.08)	2.54 (2.19; 2.94)	231	0.43 (0.06)	1.54 (1.37; 1.73)
Brain caudate basal ganglia	222	0.89 (0.09)	2.42 (2.04; 2.89)	221	0.40 (0.07)	1.49 (1.30; 1.69)
Brain cerebellar hemisphere	160	1.08 (0.09)	2.94 (2.49; 3.47)	159	0.34 (0.08)	1.41 (1.22; 1.63)
Brain cerebellum	214	1.00 (0.08)	2.72 (2.33; 3.19)	213	0.37 (0.07)	1.45 (1.28; 1.65)
Brain cortex	213	0.95 (0.08)	2.59 (2.22; 3.03)	212	0.43 (0.06)	1.53 (1.36; 1.73)
Brain frontal cortex	207	0.97 (0.08)	2.63 (2.25; 3.07)	206	0.43 (0.06)	1.54 (1.37; 1.73)
Brain hippocampus	237	0.96 (0.08)	2.60 (2.22; 3.05)	237	0.44 (0.06)	1.55 (1.37; 1.75)
Brain hypothalamus	214	0.97 (0.08)	2.65 (2.27; 3.09)	213	0.40 (0.06)	1.49 (1.33; 1.68)
Brain nucleus accumbens basal ganglia	224	0.99 (0.08)	2.70 (2.31; 3.16)	223	0.40 (0.06)	1.48 (1.31; 1.68)
Brain putamen basal ganglia	237	0.92 (0.08)	2.52 (2.16; 2.95)	237	0.46 (0.06)	1.58 (1.40; 1.78)
Brain substantia nigra	226	0.95 (0.08)	2.58 (2.20; 3.03)	225	0.44 (0.06)	1.55 (1.37; 1.75)
Esophagus mucosa	99	1.00 (0.13)	2.72 (2.12; 3.50)	99	0.33 (0.09)	1.39 (1.17; 1.65)
Kidney cortex	191	1.00 (0.09)	2.72 (2.29; 3.22)	190	0.36 (0.07)	1.44 (1.25; 1.65)
Spleen	63	1.00 (0.12)	2.72 (2.17; 3.42)	63	0.28 (0.13)	1.32 (1.02; 1.70)
Stomach	81	0.94 (0.12)	2.55 (2.01; 3.24)	81	0.41 (0.09)	1.51 (1.25; 1.82)

Betas and odds ratios are obtained using Mendelian Randomization analyses. The odds ratio can be interpreted per increase of 1 standard deviation of BMI ( $\text{kg}/\text{m}^2$ ) which is equivalent to an increase of  $4.8 \text{ kg}/\text{m}^2$ .  $n_{\text{genes}}$  indicates the number of genes in each gene set. Abbreviations: BMI, body mass index; SE, standard error; CI, confidence interval.



### Waist circumference and total body fat

We examined the associations of the tissue-grouped gene sets with the anthropometric traits waist circumference and total body fat as outcomes to explore potential underlying mechanisms of cardiometabolic disease (see **Table 2**). Considering all BMI genetic instruments, an increase of 1 SD of BMI was associated with an increase in waist circumference of 0.83 cm (95% CI 0.80; 0.85) and total body fat of 0.61% (95% CI 0.60; 0.63). All BMI-associated tissue-grouped gene sets had a positive effect on waist circumference and total body fat. Overall, the alternative MR methods MR-Egger, WME and Weighted Mode analyses indicated a positive effect of the tissue-grouped gene sets on waist circumference and total body fat (see **Supplementary Table 3**).

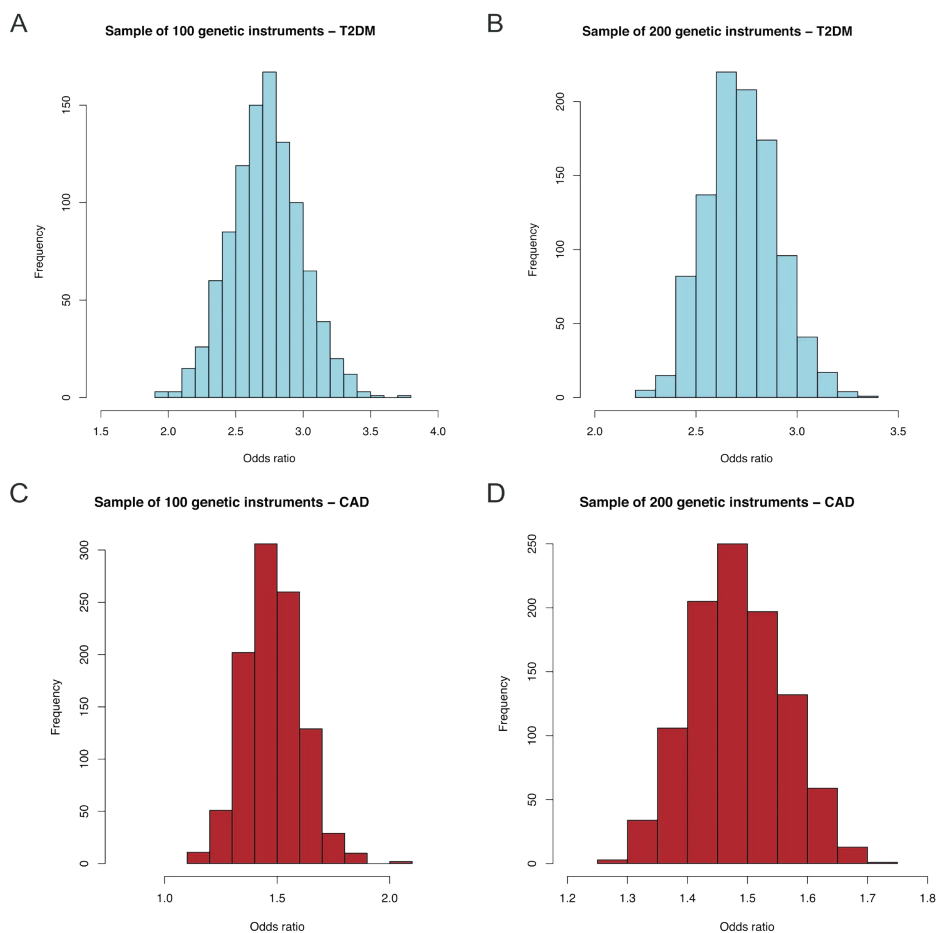
**Table 2.** Association between the BMI-overall and BMI-associated tissue-grouped gene sets and waist circumference and total body fat using (tissue-grouped) inverse-variance weighted analyses.

	Waist circumference (cm)		Total body fat (%)	
	$n_{\text{genes}}$	beta (95% CI)	$n_{\text{genes}}$	beta (95% CI)
BMI – all genetic variants	610	0.83 (0.80; 0.85)	615	0.61 (0.60; 0.63)
Artery tibial	84	0.84 (0.78; 0.90)	86	0.61 (0.57; 0.65)
Brain amygdala	229	0.84 (0.80; 0.88)	231	0.64 (0.61; 0.67)
Brain anterior cingulate cortex	230	0.85 (0.82; 0.89)	232	0.65 (0.63; 0.68)
Brain caudate basal ganglia	219	0.86 (0.82; 0.90)	221	0.64 (0.61; 0.67)
Brain cerebellar hemisphere	156	0.85 (0.81; 0.90)	160	0.61 (0.58; 0.64)
Brain cerebellum	210	0.85 (0.81; 0.89)	214	0.64 (0.61; 0.67)
Brain cortex	212	0.86 (0.82; 0.90)	213	0.65 (0.62; 0.68)
Brain frontal cortex	205	0.85 (0.82; 0.89)	206	0.66 (0.63; 0.69)
Brain hippocampus	236	0.84 (0.80; 0.88)	237	0.64 (0.61; 0.67)
Brain hypothalamus	212	0.84 (0.81; 0.88)	214	0.64 (0.61; 0.66)
Brain nucleus accumbens basal ganglia	222	0.86 (0.82; 0.90)	214	0.65 (0.62; 0.68)
Brain putamen basal ganglia	236	0.83 (0.79; 0.86)	237	0.65 (0.62; 0.67)
Brain substantia nigra	224	0.84 (0.80; 0.87)	226	0.63 (0.60; 0.66)
Esophagus mucosa	97	0.84 (0.78; 0.89)	99	0.61 (0.56; 0.65)
Kidney cortex	188	0.82 (0.78; 0.86)	191	0.63 (0.60; 0.66)
Spleen	62	0.85 (0.78; 0.91)	63	0.59 (0.54; 0.64)
Stomach	80	0.82 (0.75; 0.88)	81	0.61 (0.56; 0.66)

Betas are obtained using Mendelian Randomization analyses. The betas can be interpreted per increase of 1 standard deviation of BMI ( $\text{kg}/\text{m}^2$ ).  $n_{\text{genes}}$  indicates the number of genes in each gene set. Abbreviations: BMI, body mass index; CI, confidence interval.

### Mendelian randomization analyses using random samples of genetic variants

The distribution of ORs obtained by random sampling of 100 or 200 genetic variants (from the 633 BMI-associated genetic variants) was similar to the results of the tissue-grouped MR (IVW method), as presented in the histograms in **Figure 3**. For T2DM, the ORs ranged from 1.9 to 3.8 for the sampled gene sets of 100 variants, and from 1.7 to 3.4 for the sampled gene sets of 200 variants, with the most frequent OR around 2.7, which was similar to the estimates that were observed in the tissue-grouped MR analyses. Similar to the tissue-grouped results, the ORs of the randomly sampled gene sets for the outcome CAD were smaller than for T2DM, ranging from 1.2 to 2.1, with the most frequent OR around 1.4.



**Figure 3.** Histograms displaying the distribution of odds ratios (from inverse-variance weighted [IVW] analyses) for the association between randomly sampled sets of 100 or 200 genetic variants and type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD). A) random sample of 100 genetic variants – T2DM, B) random sample of 200 genetic variants – T2DM, C) random sample of 100 genetic variants – CAD, D) random sample of 200 genetic variants – CAD.

## DISCUSSION

The aims of the present study were to identify tissue-grouped pathways of BMI-associated loci and their relation to cardiometabolic disease and anthropometric measures using MR analyses. The results of our study imply that genetic variants associated with BMI show enrichment in different tissues. In earlier studies, enrichment of BMI-associated genes was observed in the central nervous system (2), in accordance with the results of the present study. Expression of genetic variants which were associated with a decreased WHR was enriched in the digestive and urogenital system (12). We observed enrichment of BMI-grouped genes in the esophagus mucosa, stomach, spleen, and kidney cortex.

Instead of performing MR using all genetic instruments for a given exposure, we examined the contribution of associated tissues to risk of cardiometabolic disease, in order to unravel the role of specific tissues in the genetic risk of cardiometabolic disease due to overweight or obesity. Using MR, the associations between BMI and T2DM and CHD have been well established (5-8). Although the use of tissue-grouped gene sets of genetic instruments would mean a reduction in statistical power to identify effects of BMI, we were still able to identify effect estimates similar to the overall MR analysis comprising all genetic instruments, which was also reflected in the F-statistics of the tissue-grouped gene sets (31). Therefore, the increased availability of large GWAS datasets allows to examine the individual contributions of grouped genetic variants in more detail.

The results of the present study imply that genetic variants associated with BMI have similar effects on type 2 diabetes and coronary artery disease risk, regardless of the tissue in which the genes show differential expression, although there was some variation in the observed effect estimates. All BMI-associated gene sets were shown to have an effect on waist circumference and total body fat, which are both known to be associated with increased risk of T2DM and CAD. These results are supported by findings of additional analyses, in which we randomly selected 100 or 200 genetic instruments from the 633 BMI-associated genetic variants. After we repeatedly performed Mendelian randomization analyses on T2DM and CAD with randomly sampled BMI-associated gene sets, the distribution of the effect estimates was similar to the results of the tissue-grouped MR analyses. This suggests that the effect estimates of the BMI-associated gene sets based on tissue expression profiles were similar to BMI-associated gene sets obtained by random sampling of genetic instruments.

The identification of groups of genetic variants that have differential effects on an outcome measure, representing different mechanisms of disease, can be approached from various perspectives. In the present study, we explored the added value of clustering genetic instruments based on a biological perspective. Importantly, in previous research a statistical approach has been proposed (32). The authors were able to group high-density lipoprotein (HDL) cholesterol variants according to their effect on the risk of coronary heart disease and thus to identify groups of genetic variants showing different effects on disease. We therefore hypothesize that grouping the BMI-associated genetic variants by a statistical approach might identify groups of BMI-associated genetic variants that have different effects on disease risk.

A potential limitation of our study is the methodology of the clustering of the genetic instruments in the tissue-grouped gene sets. Currently, the GTEx analyses are performed using a standard overrepresentation test which does not take into account the effect size of the genetic instrument on the exposure. Genetic variants with large effect sizes therefore have the same weight in the clustering as genetic variants with much smaller effect sizes. As the GWAS by Yengo et al. identified many genetic variants for BMI with small effect sizes, this likely will have affected the clustering. Additionally, tissue-grouped gene sets identified by the enrichment analyses by GTEx are influenced by the number of tissue samples available for the different tissues. Subsequently, this has implications for the results of the MR analyses.

Another limitation of our study is the overlap between the genes within the tissue-grouped gene sets, which made it more difficult to mutually compare the tissue-grouped gene sets. Due to the overlap, the gene sets might be less specific to distinguish between the different tissues, however, they are specific for investigating the relation with cardiometabolic disease.

To conclude, this study explored whether we can add insight in causal disease biology when analysing tissue-grouped gene sets and by examining the effects of these gene sets on different outcome measures using MR analyses. In the context of BMI-associated genetic variants, our novel approach does not provide additional insight in the role of specific tissues in the genetic risk for specific cardiometabolic disease due to overweight or obesity.

## CONFLICT OF INTEREST STATEMENT

All authors declare to have no conflict of interest.

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## SUPPLEMENTARY MATERIAL

The supplementary material can be found at <https://academic.oup.com/ije/article/49/4/1246/5851556#supplementary-data>.



