

Pleiotropic effects of obesity-susceptibility loci on metabolic traits: a meta-analysis of up to 37,874 individuals

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Abstract

Aims/hypothesis Genetic pleiotropy may contribute to the clustering of obesity and metabolic conditions. We assessed whether genetic variants that are robustly associated with BMI and waist-to-hip ratio (WHR) also influence metabolic and cardiovascular traits, independently of obesity-related traits, in meta-analyses of up to 37,874 individuals from six European population-based studies.

Methods We examined associations of 32 BMI and 14 WHR loci, individually and combined in two genetic predisposition scores (GPSs), with glycaemic traits, blood lipids and BP, with and without adjusting for BMI and/or WHR.

Results We observed significant associations of BMI-increasing alleles at five BMI loci with lower levels of 2 h

glucose (*RBJ* [also known as *DNAJC27*], *QPTCL*: effect sizes -0.068 and -0.107 SD, respectively), HDL-cholesterol (*SLC39A8*: -0.065 SD, *MTCH2*: -0.039 SD), and diastolic BP (*SLC39A8*: -0.069 SD), and higher and lower levels of LDL- and total cholesterol (*QPTCL*: 0.041 and 0.042 SDs, respectively, *FLJ35779* [also known as *POC5*]: -0.042 and -0.041 SDs, respectively) (all $p < 2.4 \times 10^{-4}$), independent of BMI. The WHR-increasing alleles at two WHR loci were significantly associated with higher proinsulin (*GRB14*: 0.069 SD) and lower fasting glucose levels (*CPEB4*: -0.049 SD), independent of BMI and WHR. A higher GPS-BMI was associated with lower systolic BP (-0.005 SD), diastolic BP (-0.006 SD) and 2 h glucose (-0.013 SD), while a higher GPS-WHR was associated with lower HDL-cholesterol

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(-0.015 SD) and higher triacylglycerol levels (0.014 SD) (all $p < 2.9 \times 10^{-3}$), independent of BMI and/or WHR.

Conclusions/interpretation These pleiotropic effects of obesity-susceptibility loci provide novel insights into mechanisms that link obesity with metabolic abnormalities.

Keywords BMI · Genetic · Meta-analysis · Metabolic traits · SNP · Obesity susceptibility · Pleiotropy · WHR

Abbreviations

BF%	Body-fat percentage
EPIC	European Prospective Investigation into Cancer and Nutrition
GPS	Genetic predisposition score
GPS-BMI	Genetic predisposition score for BMI
GPS-WHR	Genetic predisposition score for WHR
GWAS	Genome-wide association study
HWE	Hardy–Weinberg equilibrium
LD	Linkage disequilibrium
MRC	Medical Research Council
NSHD	MRC National Survey of Health and Development
QC	Quality control
SNP	Single-nucleotide polymorphism
WHR	Waist-to-hip-ratio

Obesity is a key risk factor for a number of metabolic disorders, including type 2 diabetes, dyslipidaemia and cardiovascular disease, and has been associated with increased mortality [1–6]. Although the exact biological mechanisms linking obesity to these comorbidities are not fully understood, it has been shown that increased adiposity, especially abdominal fat accumulation, over time leads to a cluster of metabolic and cardiovascular abnormalities, including elevated blood glucose, insulin resistance, raised triacylglycerol, lower HDL-cholesterol and high BP [7, 8]. The clustering of obesity with clinical features of metabolic disease might, at least in part, be explained by the presence of shared aetiological factors, such as a set of genetic factors with pleiotropic effects, i.e. genes that affect multiple traits [9, 10]. Examining the pleiotropic effects of obesity-susceptibility loci may, therefore, provide novel insights into underlying biological and molecular mechanisms that link obesity with metabolic abnormalities.

Large-scale genome-wide association studies (GWASs) have identified a number of genetic loci that are robustly associated with obesity phenotypes. To date, there are 32 established loci for BMI, a measure of overall obesity [11–16], and 14 loci associated with BMI-adjusted waist-to-hip ratio (WHR), representing the risk for increased abdominal adiposity independent of overall obesity, that have been identified in populations of European descent [17, 18].

Seven of the 14 WHR-associated loci show stronger associations in women than in men [17].

In the present study, we examined pleiotropic effects of the currently GWAS-established obesity-susceptibility loci on metabolic and cardiovascular traits. We systematically assessed associations of the 32 BMI-increasing and 14 WHR-increasing alleles, individually and combined, with glucose-related traits, blood lipids and BP. We examined whether the variants' associations with related traits are likely to be mediated by their effects on obesity-related traits by testing associations with and without adjusting for BMI and WHR in meta-analyses of up to 37,874 individuals from six population-based studies of European origin.

Methods

Study populations

We combined data from six studies comprising data from up to 37,874 individuals of European origin in meta-analyses: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Cohort study ($n=22,887$) [19]; the LifeLines cohort study ($n=7,846$) [20]; the Fenland study, samples 1 ($n=1,402$) and 2 ($n=3,186$) [21, 22]; the Medical Research Council (MRC) Ely study ($n=1,602$) [23]; and the MRC National Survey of Health and Development (NSHD) study ($n=951$) [24, 25] (electronic supplementary material [ESM] Table 1). This sample represents non-diabetic individuals only, i.e. those with self-reported type 1 or 2 diabetes, individuals using blood-glucose-lowering medication and/or individuals with fasting glucose levels ≥ 7 mmol/l were excluded. All participants underwent an examination for anthropometric and BP measurements, and blood samples were taken for analysis of laboratory markers (see ESM **Methods and Results** for study-specific details). All participants gave their written informed consent and all study protocols were approved by local institutional review boards.

Genotyping

Thirty-two single-nucleotide polymorphisms (SNPs), representing the GWAS-established BMI loci [14], and 14 SNPs for the GWAS-established WHR loci [17] (or their proxies with r^2 for linkage disequilibrium [LD] >0.7) were genotyped in all six studies. Genotype data were derived from various genotyping platforms. Detailed information on selected SNPs, genotyping methods and platforms, quality control (QC) and other filters used for each study are described in ESM Tables 2 and 3 and ESM **Methods and Results**. In short, we excluded low-quality samples and SNPs before imputation in studies with genome-wide data

using standard QC criteria (call rate < 95%, minor allele frequency [MAF] < 1%, Hardy–Weinberg equilibrium [HWE] < 10^{-4} or HWE < 10^{-6}). The quality-controlled data were used to impute genotypes in LifeLines and Fenland study, sample 1. All SNPs or their proxies met QC criteria (call rate > 95%, blind duplicate concordance > 97% and genotype distributions in HWE, $p > 0.001$ [Bonferroni-corrected p value for the 46 SNPs at level $\alpha = 0.05$], imputation score ≥ 0.3) (for details see ESM Table 3 and ESM Methods and Results). In the LifeLines study, rs11847697 (BMI-associated locus near *PRKDI*) and rs6905288 (WHR-associated locus near *VEGFA*) were excluded from analyses because of a low imputation score (i.e. < 0.3).

Statistical analyses

Study-specific analysis In studies with directly genotyped data, SNPs were coded as 0, 1 or 2 according to the number of BMI- or WHR-increasing alleles for each SNP, defined on the basis of the results from the most recent and largest GWAS for BMI and WHR [14, 17]. In the studies with imputed genotype data, genotypes were coded as dosages with fractional values ranging between 0 and 2, corresponding to the estimated number of copies of the effect allele for a given SNP. We used linear regression to examine the associations of each SNP with BMI or WHR, body-fat percentage (BF%), leptin, fasting glucose, 2 h glucose, fasting insulin, proinsulin and HbA_{1c} levels, adjusting for age in men and women separately. Associations with blood-lipid levels were additionally adjusted for the use of lipid-lowering medication and associations with systolic and diastolic BP for the use of BP-lowering medication. As WHR loci were identified after adjusting for BMI [17, 18], we additionally adjusted association analyses of the WHR SNPs for BMI. As distributions for fasting insulin and proinsulin were right-skewed, we log-transformed (natural log) values to approximate a normal distribution prior to the analyses.

To examine whether associations between the obesity-susceptibility loci and metabolic and cardiovascular traits were independent of their effects on adiposity level (referred to as ‘pleiotropic effects’ further in the text), all association analyses were performed with and without additionally adjusting for BMI or WHR (ESM Fig. 1), as appropriate. All analyses were repeated after inverse normal transformation of traits to a mean of 0 and a SD of 1 to allow comparing effect sizes across traits. Phenotypic correlations of BMI/WHR with metabolic traits were calculated using Pearson correlation coefficients.

To assess the combined effect of the obesity-susceptibility loci, we calculated genetic predisposition scores (GPS) for BMI (GPS-BMI) and WHR (GPS-WHR) loci separately by summing the number of effect alleles carried by each individual. Individuals with missing genotypes for more than

10% of the loci (i.e. more than three BMI SNPs or more than one WHR SNP) were excluded from the GPS analyses. As a result, 7,023 individuals were excluded from the GPS-BMI analyses and 3,557 individuals from the GPS-WHR analyses (i.e. 18.5% and 9.4% of the total available sample used for a single-SNP analysis, respectively), resulting in a total sample of 30,790 and 34,179 for the GPS-BMI and GPS-WHR analyses, respectively. In individuals with fewer than 10% of the genotypes missing, the missing genotypes were substituted with the average allele count of the respective SNP in each individual study. In the LifeLines study, in which information on two SNPs was missing, genotypes for rs11847697 (BMI locus near *PRKDI*) and rs6905288 (WHR locus near *VEGFA*) were replaced with the average allele count of the respective SNPs in Fenland study sample 1, because of similarities in study population and genotyping platform used.

In EPIC-Norfolk, the largest study included in our meta-analyses, the GPS-BMI and GPS-WHR were normally distributed with a mean (\pm SD) of 27.6 (\pm 3.4) BMI- and 14.1 (\pm 2.4) WHR-increasing alleles. Distributions were similar in the other studies.

Association analyses for the GPS were performed in men and women combined, in the same way as the single-SNP analyses, and were additionally adjusted for sex. Because of the reported sexual dimorphism of WHR [17], the analysis of the GPS-WHR association with WHR was also performed for men and women separately. To test for sex differences, a SNP \times sex or GPS-WHR \times sex interaction term was added to the model when appropriate. Statistical analyses were performed using SAS, version 9.2, for Windows (SAS Institute, Cary, NC, USA).

Meta-analysis Summary statistics of individual studies (β coefficients and standard errors) were meta-analysed using the inverse-variance method of METAL software (www.sph.umich.edu/csg/abecasis/Metal/) [26].

The statistical significance of the confirmatory associations between single SNPs and BMI and WHR was defined as a nominal significance level of $p < 0.05$. For the exploratory pleiotropic association, we accounted for the number of independent tests using a Bonferroni correction. As such, $p < 2.72 \times 10^{-4}$ ($\alpha = 0.05/[46 \text{ SNPs} \times \text{four trait clusters}]$) and $p < 6.25 \times 10^{-3}$ ($\alpha = 0.05/[two \text{ genetic predisposition scores} \times \text{four trait clusters}]$) were considered statistically significant in the single SNP and the GPS analysis, respectively. The four trait clusters were defined as: (1) adiposity-related traits (BF%, leptin levels); (2) glucose- and insulin-related traits (fasting glucose, 2 h glucose, HbA_{1c}, insulin and proinsulin levels); (3) blood lipids (HDL-cholesterol, LDL-cholesterol, total cholesterol and triacylglycerol levels); and (4) haemodynamic traits (diastolic and systolic BP).

Forest plots of the significant associations were generated using Stata software (version 11; StataCorp, College Station,

TX, USA). Heterogeneity across studies was estimated using the Q statistic for heterogeneity implemented in METAL [26] and quantified by the I^2 index.

Statistical power We used Quanto software (<http://hydra.usc.edu/gxe>) to calculate the statistical power of our study to detect effect sizes of at least the same magnitude as those reported in the discovery studies [14, 17]. We used allele frequencies of BMI- and WHR-related variants and effect sizes reported in the most recent and largest GWASs for BMI and WHR, respectively [14, 17]. For each BMI SNP, our study had a statistical power from 35.5% to 100% to detect effect sizes with a range from 0.06 to 0.39 kg/m² (or larger) at a nominal significance level of 0.05, given a sample size ranging from 27,000 to 35,000 individuals (depending on outcome and on individual SNP) and effect allele frequencies ranging from 10% to 90% (ESM Table 4). More specifically, we had 80% power to detect previously reported associations for 20 of the 32 BMI loci. For each WHR SNP, with effect allele frequencies ranging from 20% to 70%, our study had a statistical power ranging from 67.9% to 100% to detect effect sizes ranging from 0.02 to 0.04 SD/allele at a nominal significance level of $p=0.05$ (ESM Table 4). As such, we had 80% power to detect previously reported associations for 12 of the 14 WHR loci. In addition, our study had sufficient power (i.e. >80%) to detect effect sizes as low as 0.05 SD/allele for associations of individual BMI and WHR SNPs with metabolic traits (ESM Fig. 2).

Results

The association of the obesity-susceptibility loci with adiposity-related traits

Associations with BMI were directionally consistent with results from the discovery studies [11–18] for all BMI-associated loci except the *MTIF3* locus (rs4771122) (ESM Table 5). Associations with BMI reached nominal significance for 23 (72%) of the 32 BMI SNPs ($p<0.05$), which is in close agreement with the number of loci expected based on our power calculation. In addition, the BMI-increasing alleles of 12 BMI SNPs were nominally significantly associated with higher BF% and those of five BMI SNPs with higher circulating leptin levels (ESM Table 5). Furthermore, each additional BMI-increasing allele in the GPS-BMI was associated with a 0.028 SD/allele (Fig. 1a) or 0.12 kg/m² allele⁻¹ higher BMI, which is equivalent to a 347 g heavier body weight in adults of 170 cm in height ($p<1.1\times 10^{-61}$) (ESM Table 5). The GPS-BMI was also significantly associated with a 0.016 SD/allele higher BF% ($p=1.7\times 10^{-19}$)

and 0.022 SD/allele higher leptin levels ($p=3.3\times 10^{-4}$) (Table 1). Of note, the effects sizes of the GPS-BMI were smaller for BF% than for BMI, very likely because BMI is only a proxy for overall adiposity that represents a composite trait of both fat and lean mass.

Associations with WHR adjusted for BMI were directionally consistent with findings of the discovery study for all 14 loci [17], and reached nominal significance for 12 loci (86%) (ESM Table 6). The effects were significantly ($p_{\text{interaction}}<2.4\times 10^{-3}$) larger in women than in men for five of the seven WHR loci for which a sexual dimorphism was observed previously [17]. We did not confirm sex differences for the *HOXC13* (rs1443512) or *RSPO3* (rs9491696) loci (effect size 0.042 in women vs 0.026 SD in men, $p_{\text{interaction}}=0.37$; effect size 0.041 SD vs 0.035 SD, $p_{\text{interaction}}=0.37$, respectively). Yet, we did observe a stronger association of *LY86* (rs1294421) with WHR in women than in men (effect size 0.039 SD vs 0.011 SD, $p_{\text{interaction}}=0.05$). SNPs for three WHR loci were significantly associated with BF% but, interestingly, the WHR-increasing alleles for two of these (*LYPLAL1* and *GRB14*) were associated with lower BF%. No association of the WHR loci with leptin was observed. Each additional WHR-increasing allele in the GPS-WHR was associated with a 0.019 SD or 0.002 WHR-units ($p<1.8\times 10^{-35}$) higher WHR, with the effect being stronger in women than in men (0.03 SD vs 0.007 SD, $p_{\text{interaction}}=8.4\times 10^{-17}$) (ESM Table 6, Fig. 2a). The GPS-WHR was not associated with BF% or leptin levels (Table 1).

Association with glucose- and insulin-related traits

Two of the 32 BMI loci (*QPTCL* and *RBJ* [also known as *DNAJC27*]) showed evidence of association with glucose-related traits, whereas none was associated with insulin-related phenotypes. The BMI-increasing allele at *QPTCL* (rs2287019) was associated with lower 2 h glucose levels, irrespective of adjustment for BMI (without BMI adjustment: -0.108 vs -0.107 SD with BMI adjustment, $p<1.3\times 10^{-5}$). The association of the BMI-increasing allele at *RBJ* (rs713586) with lower 2 h glucose was more pronounced after adjusting for BMI (without BMI adjustment: -0.060 , $p=1.0\times 10^{-3}$; with BMI adjustment: -0.068 SD, $p=1.1\times 10^{-4}$) (Table 2, ESM Table 7). A higher GPS-BMI was associated with higher levels of insulin and proinsulin (0.011 SD/allele for both, $p<2.3\times 10^{-3}$). However, these associations were abolished after adjusting for BMI. Interestingly, a higher GPS-BMI was associated with lower 2 h glucose after, but not before, adjusting for BMI (-0.013 SD/allele, $p=3.6\times 10^{-4}$) (Table 1, Fig. 1b).

Two of the 14 WHR loci (*GRB14* and *CPEB4*) were associated with glycaemic traits. The WHR-increasing allele at *GRB14* (rs10195252) was associated with increased levels of proinsulin, of which the effect size was only slightly

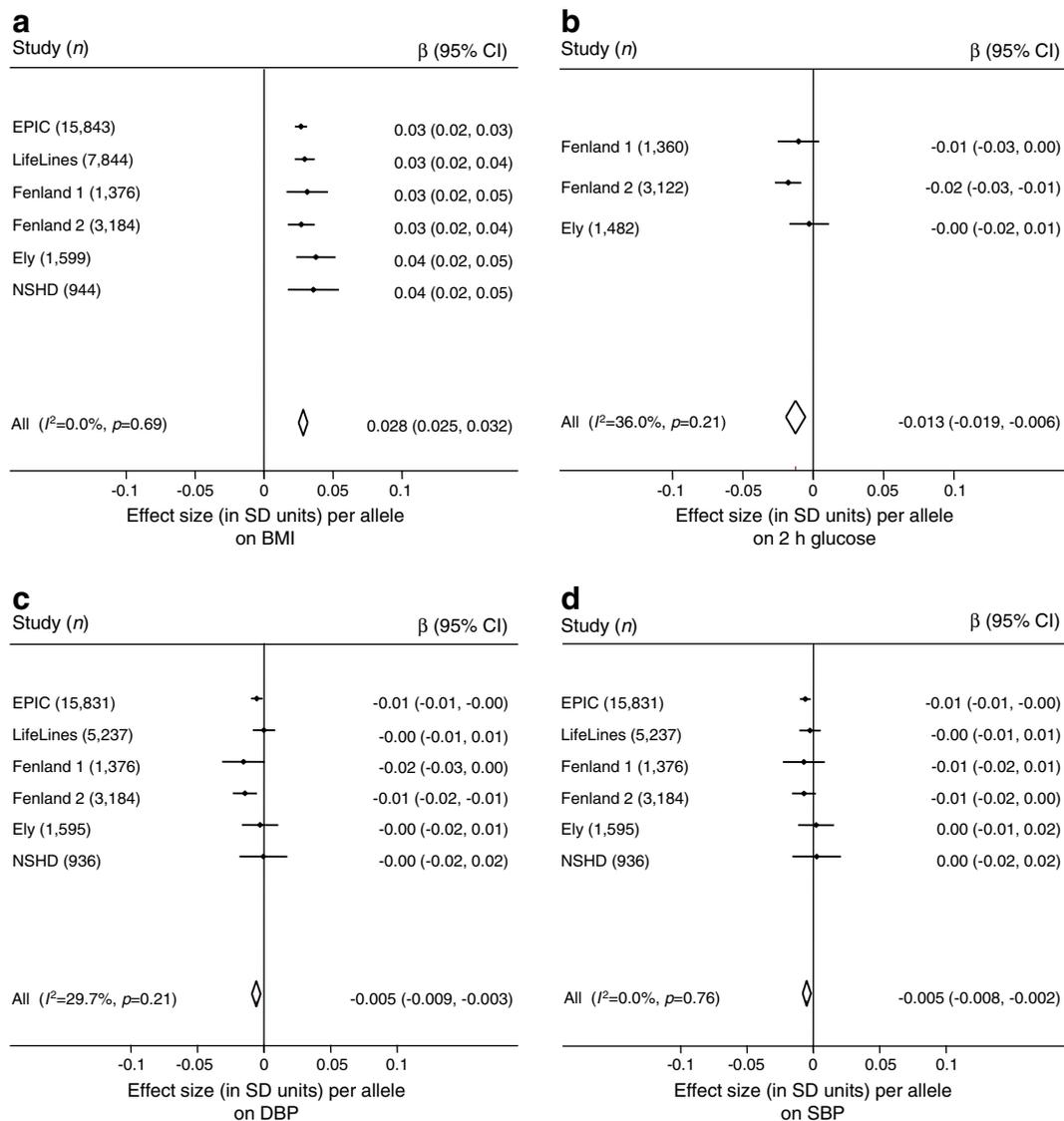


Fig. 1 The combined effects of the BMI-increasing alleles on BMI (**a**), 2 h glucose (**b**) and diastolic BP (DBP) (**c**) and systolic BP (SBP) (**d**). The effect was calculated using linear regression in individual studies and after the meta-analysis of β values and standard errors using the fixed-effects inverse-variance method. The associations were adjusted for age, sex and BMI (except for the analysis of BMI); associations with

diastolic and systolic BP were additionally adjusted for BP-lowering medication. All diabetic patients were excluded from the analysis. As all traits were inverse normally transformed, β values represent the difference in outcome for each additional risk allele under an additive model, expressed in SD units/allele. Risk alleles were defined as the BMI-increasing alleles in the discovery study

attenuated after adjusting for WHR (0.076 SD and 0.069 SD, respectively, $p < 1.2 \times 10^{-5}$). Interestingly, the WHR-increasing allele at *CPEB4* (rs6861681) was associated with lower fasting glucose, but only after adjusting for WHR (-0.049 SD, $p = 2.3 \times 10^{-4}$) (Table 3, ESM Table 8). The GPS-WHR was not associated with glucose-related traits.

Association with lipid levels

Four of the 32 BMI loci showed a significant association with blood lipids. Among these, the BMI-increasing alleles of *SLC39A8* (rs13107325) and *MTCH2* (rs3817334) were

associated with lower HDL-cholesterol levels (-0.079 SD and -0.046 SD), and of *QPCTL* (rs2287019) with higher LDL-cholesterol and total cholesterol levels (0.042 SD and 0.043 SD) (all $p < 1.0 \times 10^{-4}$). Interestingly, the BMI-increasing allele of *FLJ35779* (also known as *POC5*) (rs2112347) was associated with lower levels of both LDL-cholesterol (-0.040 SD) and total cholesterol (-0.039 SD) ($p < 8.6 \times 10^{-7}$) (Table 2, ESM Table 9). All associations remained significant after adjusting for BMI, without attenuation of effect sizes ($p < 1.1 \times 10^{-4}$). A higher GPS-BMI was associated with lower levels of HDL-cholesterol and higher levels of triacylglycerol (-0.015 SD and 0.14 SD, respectively, $p < 1.3 \times 10^{-3}$).

Table 1 The meta-analysis of the association between the traits and genetic predisposition score for the BMI and WHR loci

Trait	GPS-BMI				GPS-WHR					
	n	Unadjusted for BMI		Adjusted for BMI		n	Unadjusted for WHR		Adjusted for WHR	
		β value	p value	β value	p value		β value	p value	β value	p value
Adiposity-related traits										
BMI	30,790	0.028	$1.1 \times 10^{-61\ddagger}$	–	–	–	–	–	–	–
WHR	–	–	–	–	–	34,179	0.019	$7.7 \times 10^{-37\ddagger}$	–	–
BF%	16,089	0.016	$1.7 \times 10^{-19\ddagger}$	–	–	17,235	–0.002	0.20	–	–
Leptin	2,964	0.022	$3.3 \times 10^{-4\ddagger}$	–	–	2,938	0.001	0.79	–	–
Glucose- and insulin-related traits										
Fasting glucose	13,976	0.003	0.19	–0.005	0.03	13,945	–0.001	0.85	–0.003	0.36
2 h glucose	5,964	–0.003	0.38	–0.013	$3.6 \times 10^{-4\ddagger}$	5,938	0.000	1.00	–0.005	0.38
HbA _{1c}	22,152	0.003	0.11	–0.001	0.50	23,583	0.000	0.94	–0.002	0.47
Insulin	5,929	0.011	$2.3 \times 10^{-3\ddagger}$	–0.006	0.05	5,899	0.011	0.02	0.004	0.34
Proinsulin	5,920	0.011	$1.5 \times 10^{-3\ddagger}$	–0.003	0.37	5,891	0.009	0.05	0.003	0.56
Blood-lipid traits										
HDL-cholesterol	26,612	–0.006	$1.6 \times 10^{-4\ddagger}$	0.001	0.44	29,777	–0.015	$7.5 \times 10^{-10\ddagger}$	–0.011	$1.3 \times 10^{-4\ddagger}$
LDL-cholesterol	26,576	0.002	0.39	–0.002	0.27	29,742	0.003	0.17	0.001	0.69
Triacylglycerol	27,229	0.006	$1.3 \times 10^{-3\ddagger}$	–0.003	0.06	30,486	0.014	$6.1 \times 10^{-7\ddagger}$	0.008	$2.4 \times 10^{-4\ddagger}$
Total cholesterol	27,234	0.001	0.64	–0.002	0.17	30,492	0.001	0.57	–0.001	0.61
DBP	28,159	0.002	0.39	–0.006	$4.9 \times 10^{-4\ddagger}$	31,560	0.005	0.03	0.002	0.29
SBP	28,159	0.001	0.47	–0.005	$1.9 \times 10^{-3\ddagger}$	31,560	0.004	0.10	0.002	0.49

β values are shown as change in SD units per BMI- or WHR-increasing allele. Summary statistics of individual studies were meta-analysed. The analyses were adjusted for sex, age and use of medication (BP and lipids traits). The analyses for the WHR SNPs were additionally adjusted for BMI \ddagger Significant association (Bonferroni-corrected significance level, $p < 6.7 \times 10^{-3}$)

DBP, diastolic BP; SBP, systolic BP

However, these associations were abolished after adjusting for BMI (Table 1).

Four WHR loci were significantly associated with lipid traits. The WHR-increasing allele at *GRB14* (rs10195252) was associated with lower HDL-cholesterol levels (–0.033 SD), and those at *RSPO3* (rs9491696), *VEGFA* (rs6905288), and *CPEB4* (rs6861681) were associated with higher triacylglycerol levels (0.029, 0.034, and 0.033 SD, respectively $p < 1.1 \times 10^{-4}$) (Table 3). All these associations were abolished after further adjusting for WHR. Amongst the seven WHR-associated loci that were previously reported to have sex-specific effects (17), the WHR-increasing allele at *VEGFA* was significantly associated with higher LDL-cholesterol levels in women but not in men (0.045 SD in women [$p = 1.1 \times 10^{-4}$] and –0.018 SD in men [$p = 0.16$], $p_{\text{interaction}} = 3.3 \times 10^{-4}$) (ESM Table 10). The association was only slightly attenuated after further adjustment for WHR (0.040 SD in women [$p = 6.3 \times 10^{-4}$] and –0.19 in men [$p = 0.15$]). A higher GPS-WHR was associated with lower HDL-cholesterol levels and higher triacylglycerol levels without and with further adjusting for WHR (–0.015 and 0.014 before and –0.011 and 0.008 SD/allele after the adjustment for

WHR, respectively, $p < 2.4 \times 10^{-4}$) (Table 3, Fig. 2b, c). We did not observe an association of the GPS-WHR with either LDL-cholesterol or total cholesterol levels.

Association with BP

Of the 32 BMI loci, only the association for the *SLC39A8* locus with diastolic BP reached significance, with the BMI-increasing allele being associated with a lower BP (–0.058, $p = 2.5 \times 10^{-4}$). The association was somewhat more pronounced after adjusting for BMI (–0.069 SD/allele, $p = 5.8 \times 10^{-6}$) (Table 2). The GPS-BMI was not associated with BP without adjusting for BMI. After adjustment for BMI, a higher GPS-BMI was associated with lower diastolic and systolic blood BP (–0.006 and –0.005 SD/allele or –0.063 and –0.09 mmHg/allele, respectively, all $p < 1.4 \times 10^{-3}$) (Table 1, Fig. 1c, d, ESM Table 11).

We showed no associations between the individual WHR loci or the GPS-WHR and BP (Tables 1 and 3, ESM Table 12).

There was low ($I^2 < 25\%$) to moderate (I^2 from 25% to 75%) between-study heterogeneity for all above-described

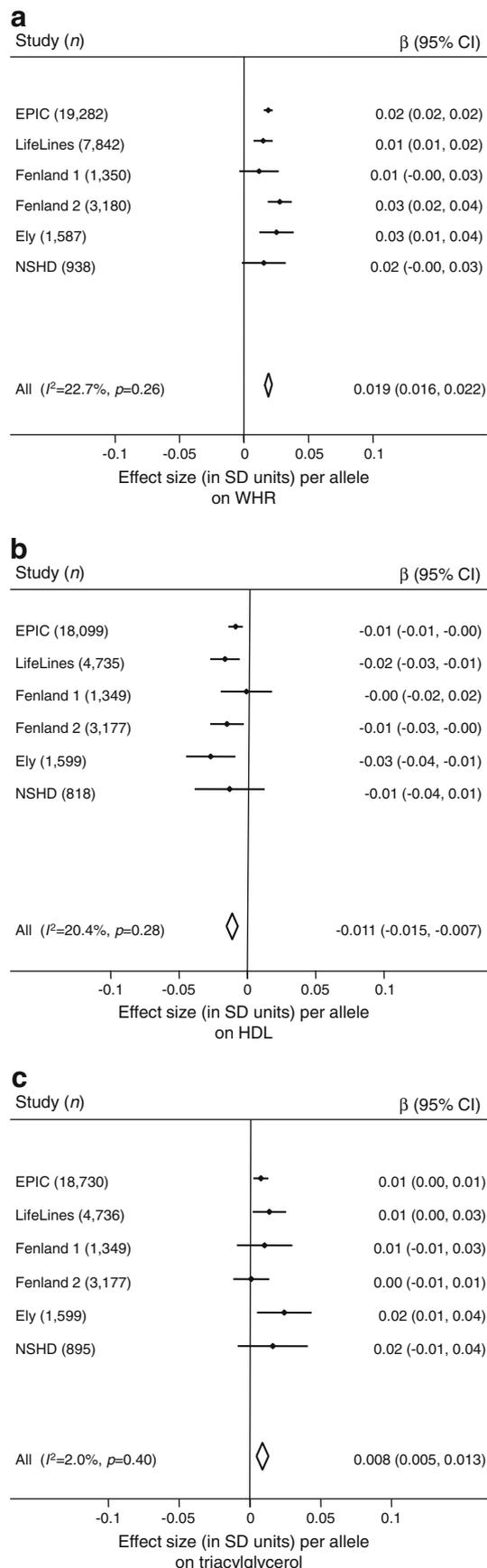


Fig. 2 The combined effects of the WHR-increasing variants on WHR (a), HDL-cholesterol (b) and triacylglycerol (c). The effect was calculated using linear regression in individual studies and after the meta-analysis of β values and standard errors using the fixed-effects inverse-variance method. Associations were adjusted for age, sex, BMI and WHR (except for the analysis of WHR); associations with lipids were additionally adjusted for blood-lipid-lowering medication. All diabetic patients were excluded from the analysis. As all traits were inverse normally transformed, β values represent the difference in outcome for each additional risk allele under an additive model, expressed in SD units/allele. Risk alleles were defined as the WHR-increasing alleles in the discovery study

significant associations (for details see ESM Methods and Results and ESM Tables 13 and 14).

Discussion

In this report, we examined whether the currently GWAS-established obesity-susceptibility loci also influence metabolic and cardiovascular traits, independent of their effect on obesity-related traits. Our meta-analysis in up to 37,874 European individuals revealed pleiotropic effects for five BMI-, and two WHR-associated loci and demonstrated remarkable diversity in their association patterns with both metabolically ‘healthy’ and ‘unhealthy’ obesity phenotypes. Somewhat paradoxically, we further showed that a stronger genetic susceptibility for increased BMI conferred by the 32 loci (GPS-BMI) is associated with lower 2 h glucose levels and lower systolic and diastolic BP after adjusting for BMI. In contrast, a genetic predisposition for increased WHR (GPS-WHR) predisposes to lower HDL-cholesterol and higher triacylglycerol levels, independent of effects on WHR.

We observed directionally consistent associations for a subset of obesity loci with metabolically ‘unhealthy’ conditions [7, 8], such as altered levels of insulin-related traits and blood lipids (ESM Table 15). Adjusting the analyses for adiposity traits abolished some of these associations, but revealed several associations that remained significant or stood out after adjusting for BMI or WHR, suggesting pleiotropic effects. A subset of obesity-susceptibility loci remained associated with metabolically ‘unhealthy’ phenotypes, while for other loci the BMI- and WHR-increasing alleles were associated with metabolically ‘healthy’ profiles (Table 4).

Several of our pleiotropic associations are consistent with previous reports. First, the associations of *QPCTL* with 2 h glucose and blood lipids are in agreement with other studies [27–31]. The *QPCTL* locus harbours the *GIPR* gene, identified in GWASs for 2 h glucose [27–29], which encodes a protein involved in insulin secretion and beta cell function [27–29]. The BMI-associated SNP rs2287019 in *QPCTL* is in strong LD with coding variants in *GIPR* ($r^2 > 0.8$) [14]. Of

Table 2 Association of the individual BMI-related variants with anthropometric, metabolic and cardiovascular traits

SNP nearby gene ^a	BMI	BF%	2 h glucose		HDL-cholesterol		LDL-cholesterol		Total cholesterol		DBP	
			β value	BMI unadj.	β value	BMI adj.	β value	BMI unadj.	β value	BMI adj.	β value	BMI unadj.
	(n=33,269–35,023)	(n=16,454–19,089)	(n=5,964–5,990)	(n=32,038–33,668)	(n=32,001–33,632)	(n=32,717–34,404)	(n=33,248–35,000)					
rs1558902	0.078	0.056	0.027	0.012	0.010	0.003	0.005	0.006	0.018	0.002	0.002	
<i>FTO</i>	$p=1.0 \times 10^{-23†}$	$p=1.9 \times 10^{-7†}$	$p=0.14$	$p=0.48$	$p=0.23$	$p=0.75$	$p=0.55$	$p=0.44$	$p=0.84$	$p=0.84$	$p=0.84$	
rs13107325	0.057	0.072	-0.007	-0.007	-0.079	-0.016	-0.020	-0.035	-0.040	-0.069	-0.069	
<i>SLC39A8</i>	$p=2.3 \times 10^{-4†}$	$p=3.9 \times 10^{-4}$	$p=0.84$	$p=0.83$	$p=1.1 \times 10^{-6†}$	$p=3.0 \times 10^{-5†}$	$p=0.19$	$p=0.02$	$p=0.01$	$p=2.5 \times 10^{-04†}$	$p=5.8 \times 10^{-06†}$	
rs10938397	0.043	0.053	-0.006	-0.019	-0.015	0.012	0.009	0.014	0.010	0.008	-0.002	
<i>GNPDA2</i>	$p=3.9 \times 10^{-8†}$	$p=1.2 \times 10^{-6†}$	$p=0.75$	$p=0.27$	$p=0.08$	$p=0.13$	$p=0.26$	$p=0.09$	$p=0.20$	$p=0.31$	$p=0.85$	
rs2287019	0.016	0.025	-0.108	-0.107	0.035	0.042	0.041	0.043	0.042	0.003	-0.002	
<i>QPCTL</i>	$p=0.13$	$p=0.07$	$p=1.3 \times 10^{-5†}$	$p=6.2 \times 10^{-6†}$	$p=1.6 \times 10^{-3}$	$p=1.0 \times 10^{-4†}$	$p=1.1 \times 10^{-4†}$	$p=4.6 \times 10^{-5†}$	$p=6.7 \times 10^{-5†}$	$p=0.81$	$p=0.84$	
rs713586	0.032	0.026	-0.060	-0.068	0.006	0.025	0.021	0.028	0.024	0.007	-0.001	
<i>RSJ</i>	$p=2.4 \times 10^{-5†}$	$p=0.01$	$p=1.0 \times 10^{-3}$	$p=1.1 \times 10^{-4†}$	$p=0.48$	$p=2.0 \times 10^{-3}$	$p=8.3 \times 10^{-3}$	$p=3.5 \times 10^{-4}$	$p=1.7 \times 10^{-3}$	$p=0.38$	$p=0.94$	
rs2112347	0.016	-0.006	0.004	-0.007	-0.005	-0.040	-0.042	-0.039	-0.041	-0.016	-0.019	
<i>FLJ35779</i>	$p=0.04$	$p=0.54$	$p=0.85$	$p=0.68$	$p=0.59$	$p=6.9 \times 10^{-7†}$	$p=1.9 \times 10^{-7†}$	$p=8.6 \times 10^{-7†}$	$p=2.9 \times 10^{-7†}$	$p=0.05$	$p=0.01$	
rs3817334	0.026	0.016	-0.002	-0.008	-0.046	-0.007	-0.010	-0.021	-0.023	-0.012	-0.018	
<i>MTCH2</i>	$p=1.2 \times 10^{-3}$	$p=0.15$	$p=0.94$	$p=0.66$	$p=8.7 \times 10^{-8†}$	$p=1.9 \times 10^{-6†}$	$p=0.38$	$p=0.01$	$p=5.4 \times 10^{-3}$	$p=0.14$	$p=0.03$	

Results are shown for SNPs/traits (except BMI) if at least one SNP × trait association reached Bonferroni-corrected level of significance

β values are shown as change in SD units per each BMI-increasing allele. Sex-specific summary statistics of individual studies were meta-analysed. The analyses were adjusted for age and medication (BP and lipid traits)

^aGenes within ±500 kb of the lead SNP

[†]Significant associations (Bonferroni-corrected significance level, $p < 2.72 \times 10^{-4}$)

Unadj, unadjusted; adj, adjusted

Table 4 Pleiotropic associations of the individual obesity-susceptibility loci and GPSs with metabolic phenotypes

Phenotypes	Metabolically 'unhealthy' associations	Metabolically 'healthy' associations
Glucose- and insulin-related traits		
Fasting glucose		<i>CPEB4</i>
2 h glucose		<i>QPTCL, RBJ, GPS-BMI</i>
Proinsulin	<i>GRB14</i>	
Blood-lipid traits		
HDL-cholesterol	<i>SLC39A8, MTCH2, GRB14, GPS-WHR</i>	
LDL-cholesterol	<i>QPTCL</i>	<i>FLJ35779</i>
Triacylglycerol	GPS-WHR	
Total cholesterol	<i>QPTCL</i>	<i>FLJ35779</i>
BP traits		
DBP		<i>SLC39A8, GPS-BMI</i>
SBP		GPS-BMI

DBP, diastolic BP; SBP, systolic BP

particular interest is the pattern of association, with the BMI-increasing allele being associated with lower 2 h glucose, as reported previously [27, 30], which is opposite to what would be expected based on known epidemiological correlations. Yet, consistent with epidemiological correlations, the adiposity-increasing allele was associated with higher levels of LDL-cholesterol and total cholesterol. Second, associations between the BMI-increasing allele of rs13107325 in *SLC39A8* with both metabolically 'unhealthy' (lower HDL-cholesterol) and metabolically 'healthy' (lower diastolic BP) phenotypes confirm findings from GWASs for BP and blood lipids [32–34]. The BMI-associated variant rs13107325 is a non-synonymous SNP located in exon 7 [34] of the *SLC39A8* gene, which encodes a zinc transporter involved in zinc transportation into the cell at the onset of inflammation [35, 36]. As indicated recently, zinc deficiency is also associated with lower BP [37]. Third, the associations of the WHR-increasing allele at *GRB14* with metabolically 'unhealthy' phenotypes (higher proinsulin and lower HDL-cholesterol) have been confirmed in the most recent meta-analyses for glycaemic traits (the Meta-Analyses of Glucose and Insulin-related traits Consortium [MAGIC]) [30] and type 2 diabetes (the DIAbetes Genetics Replication And Meta-analysis [DIAGRAM] consortium) [31], and in a GWAS for HDL-cholesterol [38]. Recent analysis of expression quantitative trait locus (eQTL) data implicates *GRB14* as a potential causal gene at the locus, expressed in the liver, skeletal muscle and adipose tissue [39].

Furthermore, some observed associations suggest novel pleiotropic effects. For example, we detected the relationship

of rs2112347 near *FLJ35779* and a more favourable lipid profile. This variant is located approximately 400 kb from *HMGCR3*, involved in lipid metabolism and shown to be associated with total cholesterol [32]. The biological mechanisms by which the remaining obesity-susceptibility loci might affect glucose (*RBJ, CPEB4*) and lipid metabolism (*MTCH2*) are less well understood [40–42] and require further investigation.

We show that a greater susceptibility to increased WHR, as assessed by the GPS, is associated with a metabolically unfavourable lipid profile, even after adjustment for adiposity. In contrast, the associations of a higher GPS-BMI with metabolically unhealthy levels of insulin- or lipid-related traits were abolished after adjusting for BMI. Similar observations of the associations between the obesity risk alleles and insulin resistance, mediated by BMI, were reported previously [43, 44]. Even though trends of associations are consistent across studies, associations of *FTO* with a number of metabolic traits in a BMI-dependent manner do not reach our significance threshold, which accounts for multiple testing, whereas previous studies used nominal levels [44]. In contrast, a higher GPS-BMI was associated with lower levels of 2 h glucose and BP after adjusting for BMI. These results support recent finding of the association of the BMI-increasing alleles collectively with increased beta cell capacity [45]. Particularly intriguing is the novel 'pleiotropic' association of a higher GPS-BMI with decreased BP. While 60–70% of hypertension in adults may be directly attributable to adiposity [4, 46], some discrepancies in the BP–adiposity relationship have also been described. For example, Pima Indians, who have the highest prevalence of reported obesity in the world, have a low prevalence of hypertension [47]. To summarise, the genetic pleiotropic associations may in part explain why some obese individuals remain metabolically healthy despite having excessive accumulation of body fat [48–53]. This phenomenon is described in the current literature as 'healthy obesity' and, to date, little is known about the factors that protect obese individuals from metabolic disturbances [54].

Our results provide evidence for pleiotropic effects of obesity-susceptibility loci on metabolic traits, supporting the hypothesis on the shared nature of genetic susceptibility loci in related complex diseases [10]. A recent analysis of the loci identified in GWASs for common diseases and quantitative traits showed pleiotropic effects for almost 17% of analysed genes and for 4.6% of SNPs [55]. These and our findings indicate that pleiotropy is more common in complex diseases than previously anticipated, and suggest that many proteins play different roles in independent biological pathways. Possible explanations for pleiotropic effects are that positive genetic correlations between phenotypic traits (i.e. affecting the traits in the same direction) occur if they share common biological pathways, while negative genetic

correlations might be ‘evolutionarily’ beneficial when a genetic mutation favourable to one trait can be harmful for another trait [56]. Interestingly, we observed both types of genetic correlation in our study. Among the detected pleiotropic effects of both single-SNP and GPS analyses, 50% of the significant associations were directionally consistent with a BMI- or WHR-increasing allele or a higher GPS associated with metabolically ‘unhealthy’ phenotypes, while the other half showed unexpected relationships with metabolically ‘healthy’ phenotypes (Table 4). In addition, these pleiotropic association signatures extend the genetic associations of the obesity-susceptibility loci to related metabolic traits and diseases and provide a more comprehensive and integrated overview of the potential physiological mechanisms of the loci. These insights may eventually help to prioritise genes for functional follow-up and to guide physiologists in designing their functional studies in experimental settings.

There are some limitations to our study. First, only populations from northern European ancestry and obesity-susceptibility loci identified in European ancestry populations were included in our analyses. Cross-ancestry analyses will be required to investigate whether the observed pleiotropic associations in European ancestry populations generalise to other ancestries. Furthermore, the cross-sectional design and the exclusion of diabetic patients are further potential limitations of our study. Future investigations in large prospective studies can be informative in disentangling whether the identified loci affect other metabolic traits through either first influence obesity risk, or whether the association is truly independent of BMI and WHR.

In conclusion, our results provide evidence that obesity-susceptibility loci have pleiotropic effects on metabolic traits, independent of adiposity. These findings emphasise the importance of detailed physiological characterisation of novel obesity-susceptibility loci to gain new insight into the complex pathogenesis of metabolically related disorders. Importantly, our findings highlight that the effect alleles that predispose to obesity might also predispose to, or protect from, other metabolic disorders.

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