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## Shared Genetic and Experimental Links between Obesity-Related Traits and Asthma Subtypes in UK Biobank

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ZZ and LL led statistical analysis with HS, YG, LO, BH, CL and SG, most analyses were carried out by ZZ.

CL led mouse RT-PCR study, performed RT-PCR experiment and analysis.

QL led mouse RNA-seq study, RP performed RNA-seq experiment. BEH performed RNA-seq analysis.

HS and LO carried out causal inference analyses. HS led discussion of causal inference with input from ZZ and LL.

YG and ZZ led writing of the first draft of the paper with edits from LL, HS, CL, KH, CAC, RP and LO.

All authors contributed to the interpretation of the results and the writing of the paper.

ZZ and LL had full access to all study data and take responsibility for data integrity and accuracy of data analysis.

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DATA AVAILABILITY

UK Biobank summary GWAS statistics will be available at the UK Biobank website (<http://biobank.ctsu.ox.ac.uk>).

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## Abstract

**Background:** Clinical and epidemiological studies have shown that obesity is associated with asthma and that these associations differ by asthma subtypes. Little is known about the shared genetic components between obesity and asthma.

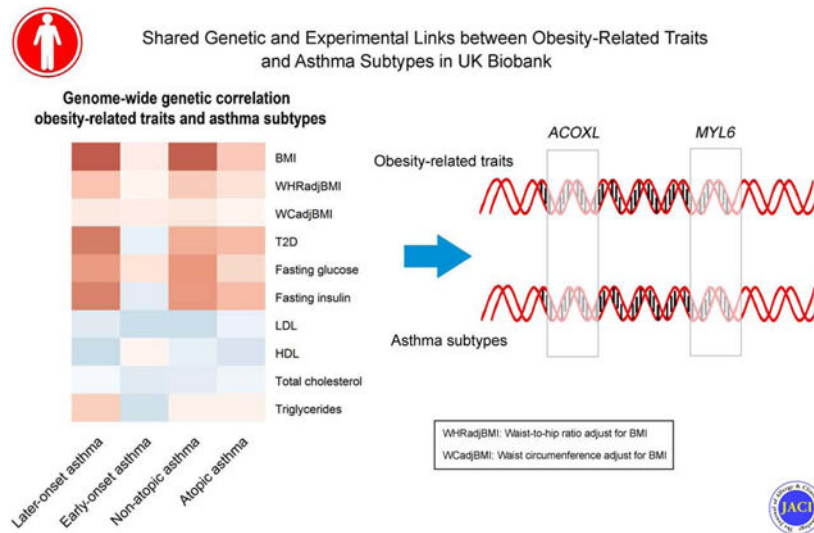
**Objective:** To identify shared genetic associations between obesity-related traits and asthma subtypes in adults.

**Methods:** A cross-trait genome-wide association study (GWAS) was performed using 457,822 individuals of European ancestry from the UK Biobank. Experimental evidence to support the role of genes significantly associated with both obesity-related traits and asthma via GWAS was sought using results from obese vs. lean mouse RNA-seq and RT-PCR experiments.

**Results:** We found a substantial positive genetic correlation between BMI and later-onset asthma defined by asthma age of onset at 16 years of age or older ( $R_g = 0.25$ ,  $P = 9.56 \times 10^{-22}$ ). Mendelian Randomization analysis provided strong evidence in support of BMI causally increasing the risk of asthma. Cross-trait meta-analysis identified 34 shared loci among 3 obesity-related traits and 2 asthma subtypes. GWAS functional analyses identified potential causal relationships between the shared loci and GTEx tissue eQTLs, shared immune- and cell differentiation-related pathways between obesity and asthma. Finally, RNA-seq data from lungs of obese versus control mice found that two genes (*ACOXL* and *MYL6*) from the cross-trait meta-analysis were differentially expressed, and these findings were validated by RT-PCR in an independent set of mice.

**Conclusions:** Our work identified shared genetic components between obesity-related traits and specific asthma subtypes, reinforcing the hypothesis that obesity causally increases the risk of asthma, and identifying molecular pathways that may underlie both obesity and asthma.

## Graphical Abstract



## Capsule Summary:

This large-scale genome-wide cross-trait study discovers shared genetic etiology of obesity- and glycemic-related traits with specific asthma subtypes in adults and reinforces the idea of genetic instruments of obesity causally increases the risk of asthma.

## Keywords

obesity; metabolic disorder; asthma subtypes; shared genetics; genome-wide association study; RNA-seq; RT-PCR

## Introduction

Asthma is a common chronic disease that accounts for a substantial burden of disease worldwide<sup>1</sup>. Metabolic disorders, such as obesity and type 2 diabetes (T2D), have become highly prevalent globally and in the US<sup>2</sup>, and numerous studies have linked metabolic traits with increased asthma risk<sup>3, 4</sup>. Asthma is a highly heterogeneous disease; both age of onset and atopic status play important roles in currently recognized asthma subtypes<sup>5</sup>. Early-onset asthma is highly heritable and associated with atopy, while later-onset asthma is often non-atopic and resistant to treatment<sup>6</sup>. Recent studies indicate that different asthma subtypes are related disproportionately with metabolic traits, such as obesity<sup>7</sup>. For example, obesity appears to have a stronger association with non-atopic asthma than atopic asthma<sup>8</sup>.

Metabolic disorders and asthma are highly heritable traits, and the parallel rise in prevalence of metabolic disorders (especially obesity) and asthma worldwide suggests these conditions share genetic and environmental risk factors<sup>1</sup>. Understanding the shared genetic architecture across various complex traits has been a topic of recent interest. For example, shared genetic risk factors have been identified between sleep disturbance and metabolic traits<sup>9</sup>, and type 2 diabetes and coronary heart diseases<sup>10</sup>. Additionally, we previously performed a cross-trait genome-wide association study (GWAS) to identify shared genetic variants among asthma

and allergic diseases<sup>11</sup>, Alzheimer's disease and metabolic traits<sup>12</sup>, and chronic obstructive pulmonary diseases and cardiovascular traits<sup>13</sup>. Previous studies that have investigated shared genetic associations between obesity-related traits (i.e., obesity and high-density lipoprotein [HDL]) and asthma<sup>14-16</sup> have found that genes such as *GNPDA2*, *PTPRD* and *ROBO1* are associated with both body-mass index (BMI and asthma, these studies were limited by small sample size). Therefore, it remains to be determined to what extent the phenotypic association between obesity-related traits and asthma is due to genetic effects, and whether the shared genetic factors with obesity differ among main asthma subtypes: early-onset and later-onset asthma. In the current study, we conducted a large-scale GWAS cross-trait analysis between asthma (early-onset, later-onset asthma, atopic asthma and non-atopic asthma) and obesity traits (i.e., BMI, BMI adjusted waist-to-hip ratio [WHRadjBMI], and BMI adjusted waist circumferences [WCadjBMI]) using UK Biobank and other publicly available GWAS data for obesity-related metabolic traits, including T2D from the DIAGRAM Consortium<sup>17</sup>, fasting glucose (FG) and fasting insulin (FI) from the MAGIC Consortium<sup>18</sup>, and blood lipids (i.e. low-density lipoproteins [LDL], HDL, total cholesterol [TC], triglycerides [TG]) from the ENGAGE Consortium<sup>19</sup>.

## Methods

### Study Population and Design

The UK Biobank study has been previously described in detail<sup>11, 20</sup>. All participants provided informed consent to the UK Biobank. Our overall study design is shown in Figure 1. To identify genetic variants that contributed to self-reported obesity traits and doctor-diagnosed asthma, we performed GWAS using phenotype data provided for UK Biobank participants (N = 487,409). We restricted subjects to 457,822 individuals of European ancestry (457,822 with BMI measures, 457,690 with WHR and WC measures, 13,435 with early-onset asthma, 33,418 with later-onset asthma, 28,862 with non-atopic asthma, 23,982 with atopic asthma) with high-quality genotyping and complete phenotype/covariate data. We did not remove related samples because we used a linear mixed model (LMM) method for phenotype-genotype association analysis that appropriately accounted for relatedness<sup>21</sup>. Detailed demographic information is provided in Supplementary Table 1.

### Ascertainment of asthma in UK Biobank

Asthma subjects were subset in two ways: by age of onset and atopic status. Early-onset asthma was defined as an individual with doctor-diagnosed asthma that developed before 16 years of age and later-onset asthma as doctor-diagnosed asthma that developed at 16 years of age or older. Non-atopic asthma was defined as doctor-diagnosed asthma without hayfever/allergic rhinitis or eczema. Atopic asthma was defined as doctor-diagnosed asthma with hayfever/allergic rhinitis or eczema. UK Biobank data field 6154 was used to determine doctor-diagnosed asthma or allergic diseases; data field 3786 was used to determine age of asthma onset. There were 21,434 cases who had later-onset and non-atopic asthma; 15,172 cases who had later-onset and atopic asthma; 6,640 cases who had early-onset and non-atopic asthma; and 7,999 cases who had early-onset and atopic asthma.

## GWAS analysis

Genome-wide genotyping was performed in UK Biobank participants using the UK Biobank Axiom array and UK BiLEVE Axiom™ Array from Affymetrix<sup>20</sup>. Genotype imputation was performed using the Haplotype Reference Consortium panel<sup>22</sup>. We selected variants that did not deviate from Hardy-Weinberg Equilibrium ( $P > 1 \times 10^{-6}$ ), have per-variant missing call rates  $< 10\%$ , have per-sample missing rate  $< 10\%$ , and did have minor allele frequency  $> 1\%$  and imputation quality score  $> 0.8$ . Detailed quality control (QC) and imputation information is provided in the Supplementary Appendix. We used BOLT-LMM v2.3<sup>21</sup> to conduct association tests. For continuous outcomes, we carried out LMM association analyses while adjusting for age, age squared, sex, genotyping array and twenty ancestry principal components (PCs) to assess association between the inverse normally transformed phenotype residuals and imputed genotype dosages. For binary phenotypes, we performed LMM association analyses while adjusting for age, sex, genotyping array, twenty ancestry PCs. The effect estimate output of BOLT-LMM linear regression was transformed into log odds ratio.

After association analyses, we used the PLINK clumping function (parameters: `--clump-p1  $5 \times 10^{-8}$  --clump-p2  $1 \times 10^{-5}$  --clump-r2 0.2 --clump-kb 500`) to determine top loci that were independent of each other (i.e., variants with p-value less  $< 1 \times 10^{-5}$ ,  $r^2 > 0.2$  and within 500kb of a given peak's clump).

## Linkage disequilibrium score regression (LDSC) analysis

LDSC estimates genetic correlation between the true causal effects of two traits (ranging from  $-1$  to  $1$ ) from summary statistics using the fact that the GWAS effect size estimate for each SNP incorporates the effects of all SNPs in linkage disequilibrium with that SNP. LDSC applies a self-estimated intercept during the analysis to account for shared subjects between studies<sup>11, 23</sup>.

## Analysis of sex-specific effects

Previous studies have shown that the association between asthma and metabolic traits differs by sex<sup>24</sup> and that girls with early age at menarche have lower lung function and increased risk of asthma in adulthood<sup>25</sup>. Thus, we performed sex-stratified analyses to evaluate the genetic correlation between obesity traits and asthma subtypes according sex and without the effect of age at menarche in female obesity traits and asthma.

## Partitioned genetic correlation analysis

To characterize the genetic correlation at the level of functional categories, we estimated genetic correlations between metabolic traits and asthma (later-onset and non-atopic) using partitioned LDSC in 11 annotation categories: transcribed region, super enhancer, intron, transcription factor binding sites (TFBS), DNaseI digital genomic footprinting (DGF) region, DNase I hypersensitivity sites (DHSs), fetal DHS, and histone marks H3K4me1, H3K4me3, H3K9ac, and H3K27ac<sup>26, 27</sup>. For each annotation category, we re-calculated LD scores for SNPs assigned to that particular category and then used the annotation-specific LD scores for estimating the metabolic trait-asthma genetic correlation.

### Genetic causal inference analysis

We used generalized summary data-based Mendelian Randomization (GSMR)<sup>28</sup> and Latent Causal Variable (LCV)<sup>29</sup> to infer putative direction of causality between BMI and asthma phenotypes. While both GSMR and LCV perform causal inference based on GWAS summary statistics, GSMR estimates effect of exposure on outcome using SNPs significantly associated with exposure<sup>28</sup>, whereas LCV estimates genetic causality proportion (GCP) using all common SNPs and is robust to confounding by pleiotropy<sup>29</sup>. A significant effect of exposure on outcome and a GCP close to 1 are strong evidence of causal relationship. Because samples shared across GWAS induce bias in causal inference<sup>30</sup>, we avoided sample overlap by using summary statistics obtained from our UK Biobank analysis for asthma phenotypes and publicly available summary statistics from the GIANT consortium for BMI<sup>31</sup>. We did not use other obesity-related phenotypes (i.e., WHRadjBMI and WCadjBMI) for causal inference analyses, as these phenotypes are adjusted for heritable covariates and are thus prone to collider bias<sup>32, 33</sup>. We removed strand-ambiguous SNPs and SNPs in the MHC region (chr6:25-34M) only in MR analyses due to its strong pleiotropic effect.

### Cross-trait meta-analysis

Cross Phenotype Association (CPASSOC) combines effect estimates and standard errors of GWAS summary statistics to test the hypothesis of association between a SNP with two traits<sup>34</sup>. We used heterogenous version of CPASSOC (SHet) that is based on a fixed effect model and is more powerful when there is a heterogenous effect present between studies, which is common<sup>35</sup>. SHet uses the sample size for a trait as a weight and accounts for correlation due to overlapping or related subjects within and among different studies.

### Fine mapping credible-set analysis

In order to identify the most credible genes within the clumping results, we identified a credible set of variants that were 99% likely to contain the causal variant at each of the shared loci obtained from the cross-trait meta-analysis using the Bayesian-likelihood fine-mapping algorithm<sup>36</sup>. This algorithm maps the primary signal using a flat prior with steepest descent approximation.

### Overrepresentation enrichment analysis

To identify biological pathways that significant genes after cross-trait meta-analysis ( $P_{\text{meta}} < 5 \times 10^{-8}$ ) were involved in, we used the WebGestalt tool<sup>37</sup> to assess overrepresented enrichment of gene sets in Gene Ontology (GO) biological process categories.

### Colocalization analysis

We used the R package coloc<sup>38</sup> to determine whether cross-trait meta-analysis association signals of shared loci co-localized at the same variant. After extracting summary association data for variants within 500kb of the index SNP at each of the shared loci, we calculated the posterior probability that the two traits were associated with different causal variants (H3) or that the two traits were associated and shared one common causal variant (H4). Loci were considered to be co-localized if the posterior probability H4 was  $> 0.7$ . In addition, since the



GTEX eQTL signals by themselves are pervasive, we conducted the colocalization analysis between cross-trait meta-analysis results and 48 single GTEX tissue cis-eQTL (version 7) to determine if the same genetic variants were related to expression and the diseases.

### Mouse lung gene expression changes with obesity

We obtained two independent measures of differential lung expression, one with RNA-Seq and one with RT-PCR, to identify gene expression changes in lung between (1) mice on a high fat diet (HFD) vs. a low fat diet (LFD) mice and (2) autosomal recessive diabetes mutation (*db/db*) vs. wild-type mice. Such differentially expressed genes were sought as providing evidence linking obesity to asthma vis-à-vis potential changes in lung tissue. A detailed description of gene expression analyses is provided in the Supplementary Appendix.

## Results

### Single Trait GWAS Results

We identified 1,636 genome-wide significant independent loci associated with BMI, 1,074 with WHRadjBMI, 1,260 with WCadjBMI, 77 with later-onset asthma, 219 with early-onset asthma, 101 with non-atopic asthma, and 198 with atopic asthma (Supplementary Table 2-8, Supplementary Figure 1-7). The genomic inflation factor intercepts from LDSC were 1.12 for BMI, 1.19 for WHRadjBMI, 1.17 for WCadjBMI, 1.03 for later-onset asthma, 1.06 for early-onset asthma, 1.04 for non-atopic asthma and 1.05 for atopic asthma (Supplementary Figure 8-14). Given the small genomic inflation factor intercept values, we attributed the lift-off of QQ plots (high lambda GC) to polygenicity and not population stratification.

### Genetic Correlation between Metabolic Traits and Asthma

We estimated the genetic correlation of metabolic traits with four asthma subtypes. As shown in Figure 2 and Supplementary Table 9, obesity traits were positively genetically correlated with later-onset asthma ( $R_g=0.25$ ,  $P=9.56\times 10^{-22}$  for BMI; and  $R_g=0.08$ ,  $P=1.19\times 10^{-3}$  for WHRadjBMI), but not with early-onset asthma. Sex-specific analyses found no significant difference between the later-onset asthma/BMI genetic correlation in males and females, but did find a significant difference between later-onset asthma and WHRadjBMI genetic correlation (Supplementary Table 10-11). When we extended our analysis to atopic and non-atopic asthma, we observed a positive genetic correlation between obesity traits and non-atopic asthma ( $R_g=0.24$ ,  $P=5.75\times 10^{-17}$  for BMI; and  $R_g=0.07$ ,  $P=0.02$  for WHRadjBMI), but only a modest association between BMI and atopic asthma ( $R_g=0.08$ ,  $P=1.70\times 10^{-3}$ ). In addition, T2D, FG, and FI had significant positive genetic correlations with later-onset asthma ( $R_g$ : 0.20, 0.15, and 0.19;  $P$ :  $1.39\times 10^{-6}$ , 0.02, and  $9.21\times 10^{-3}$ , respectively) and non-atopic asthma ( $R_g$ : 0.12, 0.16, and 0.15;  $P$ :  $4.19\times 10^{-3}$ ,  $5.72\times 10^{-3}$ , and 0.04, respectively) but not with early-onset asthma. We did not find significant genetic correlations between lipids (i.e., HDL, LDL, TC, TG) and any asthma subtypes. To evaluate whether certain annotation categories contributed disproportionately to the observed whole-genome genetic correlation between later-onset/non-atopic asthma and obesity-related traits, we performed LDSC for 11 functional annotation categories (Supplementary Table 12-13). Generally, the partitioned LDSC by functional categories were consistent and had similar magnitude as genome-wide genetic correlations. For

example, BMI was significantly correlated with later-onset asthma ( $R_g$  ranges from 0.21 to 0.25,  $P < 0.05/110$ ) and non-atopic asthma ( $R_g$  ranges from 0.18 to 0.26,  $P < 0.05/110$ ) for nine functional categories (i.e., transcribed region, TFBS, DGF, DHSs, fetal DHS, H3K4me1, H3K4me3, H3K9ac, and H3K27ac) after correcting for multiple comparisons. The Intron category had  $R_g = 0.37$  but was not statistically significant after multiple comparisons correction ( $P = 0.0043$ ). No functional category had a disproportionate contribution to the genetic correlation between later-onset/non-atopic asthma and obesity-related traits. Thus, our results provided evidence that obesity-related traits and later-onset/non-atopic asthma are genetically correlated and motivated us to further investigate the genetic components shared between these traits.

### Causal Inference

We performed causal inference between BMI (GIANT consortium) and UK Biobank asthma phenotypes using GSMR and LCV. A strong positive causal effect of BMI on asthma phenotypes (later-onset asthma, non-atopic asthma, and atopic asthma) but not vice versa was observed with GSMR (Table 1). For example, the estimated effect of BMI on later-onset asthma was significant with an  $OR = 1.21$  ( $P = 6.3 \times 10^{-7}$ ), but the estimated effect of later-onset asthma on BMI was not ( $OR = 1.00$ ;  $P = 0.70$ ), where the odds ratio (OR) in the former case represents the increase in the odds of having asthma, per standard deviation increase in BMI. Our estimated causal effect of BMI on asthma was comparable to that obtained by Skaaby *et al.* ( $OR = 1.07$ , 95% CI: [1.03, 1.10] based on a GWAS of sample size  $N = 162,124$  and 26 instruments)<sup>39</sup>, and that obtained by Granell *et al.* ( $OR = 1.55$ , 95% CI: [1.16, 2.07] based on a GWAS of sample size  $N = 4,835$  and 32 instruments)<sup>40</sup>.

Because BMI and asthma are polygenic traits<sup>11, 31</sup>, inferred causal relationships between them are prone to confounding due to pleiotropy. Therefore, we also applied LCV, a method robust to pleiotropy confounding, to infer the genetic causality proportion (GCP) between BMI and asthma phenotypes. GCP is a unitless measure of causality and equal to 1 under full causality. Reassuringly, we found that the LCV estimates of GCP for all inferred causal relationships were close to 1 (Table 1). For example, the estimated GCP for the causal direction BMI  $\rightarrow$  later-onset asthma was 0.90 (S.E. 0.09), not significantly different from 1. Taken together, both GSMR and LCV provided strong evidence in support of the putative model that BMI causally increases the risk of asthma. Detailed causal inference results are provided in Supplementary Table 14.

### Cross-Trait Associations Between Metabolic Traits and Asthma

In total, we identified 8 independent loci shared between BMI and later-onset asthma and 10 shared between BMI and non-atopic asthma ( $P_{single\ trait} < 1 \times 10^{-5}$  and  $P_{meta} < 5 \times 10^{-8}$ ) (Table 2). The credible set of SNPs for each of these shared loci was also identified (Supplementary Tables 15 and 16). Our results found both similarities and differences in the shared genetic components of obesity-related traits with later-onset and non-atopic asthma. Three out of the 8 significant shared loci between BMI and later-onset asthma were also significantly shared between BMI and non-atopic asthma at the same top variant (including *12q13.2* [*ERBB3*, index SNP: *rs4759229*], *1p35.2* [*COL16A1*, index SNP: *rs6681149*], and *17q25.1* [*UNC13D*, index SNP: *rs111365807*]). Interestingly, the strongest shared signal of BMI with



later-onset and non-atopic asthma was at chromosome *12q13.2* (*ERBB3*, index SNP: *rs4759229*) ( $P_{\text{meta}}=1.67\times 10^{-23}$  for later-onset asthma and  $P_{\text{meta}}=1.75\times 10^{-21}$  for non-atopic asthma). Other shared genetic components between BMI and later-onset asthma included *1q24.1* (*POU2F1* or *Oct-1*), *2q13* (*ACOXL*), *3q26.32* (*TBL1XR1*), *4q12* (near *USP46*), and *6q24.2* (*PLAGL1*). *POU2F1* (*Oct-1*) and *TBL1XR1* are both involved in glucocorticoid receptor regulation, while loss of *TBL1XR1* drives glucocorticoid resistance<sup>41</sup>, and thus, may play a functional role in the glucocorticoid resistance observed in obese asthmatics<sup>42</sup>. Shared loci between BMI and non-atopic asthma were *1p36.23* (*REER*), *6p21.32* (near *HLA-DQB1*), *7p21.1* (near *ABCB5*), *11q13.2* (*ACTN3*), *17q21.1* (*PSMD3*), *17q25.3* (near *TIMP2* and *USP36*), and *18q12.3* (near *PIK3C3*), genes involved in cell cycle processes including cellular differentiation, apoptosis, or proliferation<sup>43-45</sup>.

We identified 6 and 7 independent shared loci of WHRadjBMI with later-onset and non-atopic asthma, respectively (Table 2). The credible set of SNPs for these shared loci is provided in Supplementary Tables 17 and 18. Similar to the results of BMI, we observed 3 common loci shared among WHRadjBMI, later-onset asthma, and non-atopic asthma (two shared at the same index SNP, and one at different index SNP): *15q22.33* (*SMAD3*, index SNP: *rs10152595*,  $P_{\text{meta}}=2.56\times 10^{-20}$  for later-onset asthma and  $P_{\text{meta}}=1.40\times 10^{-20}$  for non-atopic asthma), *19q13.32* (*FOXA3*, index SNP: *rs8103278*,  $P_{\text{meta}}=1.30\times 10^{-11}$  for later-onset asthma and  $P_{\text{meta}}=5.12\times 10^{-11}$  for non-atopic asthma), and *11q23.1* (near *LAYN*, index SNP for later-onset asthma: *rs4616071*, index SNP for non-atopic asthma: *rs11213940*,  $P_{\text{meta}}=5.85\times 10^{-12}$  for later-onset asthma and  $P_{\text{meta}}=1.73\times 10^{-13}$  for non-atopic asthma).

We investigated shared loci for three glycemetic metabolic traits (i.e., T2D, FG, and FI) that were genetically correlated with asthma (Table 2) and identified the credible set of SNPs for these shared loci (Supplementary Table 19-20). A locus at chromosome 11q12.2 was common for FG, later-onset asthma, and non-atopic asthma (*FADS2*, index SNP: *rs174583* for FG with both asthma subtypes). Interestingly, *FADS2* (index SNP: *rs174584* for T2D with both asthma subtypes) was also a suggestive shared loci between T2D and both asthma subtypes when we set the single trait GWAS threshold at  $P<1\times 10^{-3}$  and  $P_{\text{meta}}<5\times 10^{-8}$  (Supplementary Table 21-22). No significant shared loci were identified for FI with later-onset or non-atopic asthma.

### Colocalization Analysis

To determine whether obesity traits colocalized at the shared loci with asthma, we performed colocalization<sup>38</sup> analysis (Supplementary Table 23). Seven out of the eight independent loci that were in common between BMI and later-onset asthma colocalized at the same candidate causal variant (PPH4>0.7) and one (*3q26.32*) colocalized with different candidate causal variants (PPH3>0.7). Among the ten loci shared between BMI and non-atopic asthma, 4 colocalized at the same candidate causal variant within each loci (PPH4>0.7), three colocalized with different candidate causal variants (PPH3>0.7), and three loci did not colocalize. Most shared loci between WHRadjBMI and both asthma subtypes colocalized at the same candidate causal variants or colocalized with different candidate causal variants. Further, FG was colocalized at *rs174583* with both later-onset and non-atopic asthma (PPH4=0.99).

We next conducted colocalization analysis to identify shared genetic components from the cross-trait meta-analysis between the two asthma subtypes and BMI (Supplementary Tables 24-25 and Supplementary Figures 15-16), WHRadjBMI (Supplementary Tables 26-27 and Supplementary Figures 17-18), and FG (Supplementary Tables 28-29 and Supplementary Figures 19-20), while including GTEx eQTL results across 48 tissues. We observed a significant amount of colocalized signals between metabolic traits and asthma in specific tissues, including skeletal muscle, esophagus mucosa and transformed fibroblast cells. The shared variant between BMI and asthma at *12q13.2* (*rs4759229*) was also a potential causal eQTL variant for *ERBB3*, *MYL6B*, or *SUOX* across 28 GTEx tissues.

### Pathway Analysis

To investigate the biological pathways represented by shared genes, we assessed enrichment of independent loci for each trait and shared genes between obesity traits and asthma subtypes in GO biological process categories and observed several significant shared pathways (FDR:  $q < 0.05$ ) (Supplementary Table 30). Common pathways for genes shared between asthma and BMI or WHRadjBMI, included cell differentiation, cell proliferation, cell migration, and inflammatory response.

### Lung Tissue Gene Expression in Obese Mice

We evaluated whether shared associated genes (Table 2) were differentially expressed in lung tissue using results from RNA-Seq studies of two obese mouse models: HFD mice and *db/db* genetically obese mice. Compared to their age-matched controls, both HFD and *db/db* mice are obese and exhibit innate airway hyperresponsiveness, a hallmark feature of asthma<sup>46</sup>. We found 3 genes from 34 loci to be differentially expressed in lung tissues for these mouse models compared to their corresponding controls. In the set of *db/db* vs. WT mice, we found that *ACOXL* was significantly differentially expressed ( $P=2.93 \times 10^{-6}$ ) (Figure 3A and Supplementary Table 31). In HFD vs. LFD mice, genes near the top GWAS loci, *MYL6* (sentinel SNP: *rs4759229*) (Figure 3B) and *DPP3* (sentinel SNP: *rs540874*) were differentially expressed ( $P=7.99 \times 10^{-5}$  and  $1.28 \times 10^{-5}$ ) (Supplementary Table 32).

To confirm RNA-Seq findings for these 3 genes, we performed a RT-PCR in independent sets of mice. *ACOXL* gene expression decreased in lungs of *db/db* mice compared to WT mice (Figure 3C). Transcript levels of *MYL6* in lung tissue from HFD mice which were fed a HFD for 4 months were increased significantly over lean control ( $1.38 \pm 0.02$ -fold,  $P < 0.05$ , Figure 3D). We also found that mRNA levels of *MYL6* in lung tissue reached peak values at 4 months after feeding WT mice a HFD diet, and this effect was attenuated at 5 months (Figure 3E). No significant difference of *Dpp3* expression in the lung was found between HFD-fed and lean mice via RT-PCR.

### Discussion

In the present study, we found positive genetic correlation between obesity traits and glycemic traits with later-onset and non-atopic asthma, but not with early-onset and atopic asthma. Sensitivity analysis showed a significantly higher genetic correlation between later-onset asthma and WHRadjBMI in male than female, but not for BMI and WCadjBMI,

suggesting the differences in genetic background of obesity traits in relation to asthma. Cross-trait meta-analysis identified independent shared loci between obesity traits and asthma subtypes, highlighting the role of airway remodeling via changes of cell proliferation, differentiation, migration, apoptosis, and accumulation of extracellular matrices (ECM) proteins.

Our study found more evident genetic correlation of obesity traits with later-onset asthma and non-atopic asthma than with early-onset asthma and atopic asthma. In epidemiological studies, results for early-onset asthma were inconsistent. For example, Guibas et al. found that BMI was not associated with asthma in preschool children<sup>47</sup>, while a recent meta-analysis observed a significant association between high body weight and asthma in children<sup>48</sup>. Unlike early-onset asthma, later-onset asthma was usually non-atopic and occurred in parallel with obesity<sup>6</sup>. The most important difference between patients with atopic and non-atopic asthma is the different airway inflammation patterns<sup>49</sup>. Atopic asthma is characterized by high eosinophils, mast cells, and T lymphocytes, while non-atopic asthma displays high neutrophils and mast cells<sup>49</sup>. Muc et al. reported that the main change in obese asthmatics was the shifts of immune response from T helper (Th) 2 (a typical atopic immunological profile) to Th1<sup>50</sup>. Thus, the type of airway inflammation driven mainly by neutrophil<sup>51</sup> in obese subjects might partially explain the stronger relationship of obesity with later-onset and non-atopic asthma.

Obesity was closely related with metabolic disorders and age at menarche genetically and phenotypically. Although BMI was inversely correlated with age at menarche in the current study (Supplementary Table 26), sensitivity analysis after adjusting for age at menarche showed consistent genetic correlation between BMI and asthma (Supplementary Table 10). Further, even though both BMI and WHRadjBMI were consistently correlated with T2D, FG, FI, HDL, and TG (Supplementary Table 33), we only observed significant genetic correlations of later-onset and non-atopic asthma with glycemic metabolic traits (T2D, FG and FI) other than lipid traits, which indicated different shared genetic background with asthma for glucose metabolism and lipid metabolism.

We obtained strong and unequivocal evidence supporting the model that BMI causally increased the risk of asthma, providing insights into the pathological mechanisms of asthma. However, we emphasize that our inferred causal relationships are based on GWAS summary statistics from cross-sectional studies. Unadjusted confounders (e.g. population stratification) and genetic pleiotropy may bias the causal inference. In addition, longitudinal studies are necessary to confirm the inferred causal relationships.

According to previous studies, the hallmark pathological features of asthma includes airway inflammation and structure changes (or airway remodeling)<sup>52</sup>. While it is well known that obesity is not only mass loading but also a state of chronic low-grade inflammation<sup>53</sup>, it is reasonable to speculate that inflammation plays a role in it. Shared loci identified from cross-trait meta-analysis also supported the involvement of inflammation in the shared etiologies between obesity and asthma. For example, SMAD3 protein, encoded by *SMAD3*, is one of the intracellular mediators and important transducer of TGF- $\beta$  signaling<sup>54</sup> and TGF- $\beta$ /SMAD3 signaling regulates inflammatory response and T cells activation and

differentiation<sup>55, 56</sup> *ERBB3* encodes ERBB3 protein which is the receptor for neuregulin (NRG). Neuregulin-1 (NRG-1)/ERBB signaling plays a role in the control of proinflammatory activation of monocytes<sup>57</sup>. Additionally, we found a shared locus (*6p21.32, HLA-DRB1-DQA1-DQB1*) at human leukocyte antigen (*HLA*) region which was well-established in playing key roles in the immune system<sup>58</sup>. However, what interests us most was that the shared loci were implemented in cell proliferation, metaplasia, and ECM remodeling, all of which were reported to be important pathology of airway remodeling<sup>59</sup>. Firstly, except the involvement in inflammatory response, *ERBB3* and *SMAD3* are also important regulators of cell proliferation and differentiation. According to Polosa et al., *ERBB3* and its family members occurred in bronchial epithelial cells to regulate epithelial repair and remodeling after mechanical damage to human bronchial epithelial cells *in vitro*<sup>60</sup>. DiRenzo *et al.* reported that elevated TGF- $\beta$ /Smad3 stimulated the secretion of canonical Wnts which in turn enhanced SMC proliferation through  $\beta$ -catenin stabilization<sup>61</sup>. The blockade of TGF- $\beta$ /SMAD3 was suggested to be beneficial for many diseases including obesity and asthma<sup>62, 63</sup>. Secondly, collagen and hyaluronan are components of ECM, and ECM deposition has been identified as an important pathogenic factor for severe asthma<sup>64</sup>. *COL16A1* encodes type XVI collagen which is unregulated by TGF- $\beta$  during tissue repair and causes the abnormal accumulation, a character for tissue fibrosis<sup>65</sup>. *LAYN* encodes a hyaluronan receptor. There was a significant correlation between the severity of asthma as measured by airway hyperresponsiveness and the hyaluronan (HA) levels in BAL fluid, serum, and lung tissue<sup>66</sup>. Thirdly, epithelial metaplasia is another feature of airway remodeling. *FOXA3* was directly related with goblet cell metaplasia<sup>67</sup> and body mass regulation<sup>68</sup>. Airway remodeling characterized by increased smooth muscle cell mass, goblet cell metaplasia, and ECM deposition was implicated in persistent airway hyperresponsiveness, excessive airflow narrowing, and resistance to high-intensity treatment and poor prognosis<sup>69, 70</sup>. Thus, airway remodeling might also be an explanation for treatment resistance and potential future therapeutic target for obese asthma patients who are characterized by more severe symptoms and poorer prognosis.

Surprisingly, we found some shared variants have opposite sign of effect estimates between obesity and asthma even though the overall genetic correlation is positive, which suggests the pathways shared by them are heterogeneous.

Finally, we found multiple top genes from cross-trait GWAS also showed differential gene expression in two independent mouse experiments, the RNA-seq and RT-PCR gene expression in two sets of mice. In the set of *db/db* vs WT, we identified *ACOXL* (Acyl-Coenzyme A oxidase-like) gene, which is proposed to participate in fatty acid  $\beta$ -oxidation, fatty acid metabolic process and oxidation reduction<sup>71</sup>. Fatty acid metabolism, particularly mitochondrial fatty acid oxidation (FAO) emerges as an important regulator of innate and adaptive immunity<sup>72</sup>. Obesity causes a decrease in FAO<sup>73</sup>, and increases inflammation<sup>72</sup>. The pro-inflammatory state found in obesity might be a critical player in the role of lipid mediators in asthmatic patients<sup>74</sup>.

In the set of HFD-fed vs lean controls, *MYL6* (myosin light chain 6) is a protein-coding gene that encodes a myosin light chain and has important roles in airway smooth muscle (ASM) contractile function<sup>75</sup>. An increase in contractility of ASM is a contributing factor in

the excessive airway narrowing in asthma<sup>76</sup>. The contractile response of ASM was enhanced in obese donors due to elevated intracellular calcium responses to contractile agonists<sup>77</sup>, resulting an increased risk of developing asthma.

We investigated the genome-wide genetic relationships of obesity-related traits with four asthma subtypes for the first time. The study has several important strengths. Firstly, the current study has large samples size and the ability to classify different types of asthma. Although sample overlapping was a concern for cross trait analysis, we used methods that are robust to such issue (including LDSC and SHet)<sup>11, 27, 34</sup>. Moreover, we used the strategy of restricting  $P < 1 \times 10^{-5}$  in single trait association as a secondary significance criterion in meta-analysis, which ensures the meta-analysis signals are at least suggestive signals with all of single traits. Finally, our computational analysis provides insights to the molecular mechanisms underlying co-morbid obesity and asthma. However, we also acknowledge potential limitations. First, we restricted the analysis to individuals of European ancestry in order to minimize the population stratification, thus, some findings in this study may not be generalizable to other racial/ethnic populations. Second, although cases of asthma and atopic diseases were identified using self-reported doctor diagnosed data, there was still possibility of misclassification of atopic asthma and non-atopic asthma and future studies using biomarkers (such as immunoglobulin E) in diagnosing atopic status are warranted to validate our findings. We also noted there is no clear-cut age threshold that defines early- and later-onset asthma, thus the definition based on age of 16 cut-point may contains heterogamous effect of asthma for young adult age. Third, we observed little evidence of significant genetic correlation between adult obesity and early-onset asthma; we encourage further studies to investigate the genetic correlation between childhood obesity and early-onset asthma.

## Conclusion

In conclusion, the current study showed substantial evidence of positive genetic correlations of obesity traits and glycemic traits with later-onset and non-atopic asthma, which can further our understanding of the connection between obesity and different asthma subtypes. Our work reinforces common biological pathways related to immune and inflammatory systems between obesity and asthma and suggests new treatment avenues for subgroups of individuals with asthma.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations Used:

**ASM**                      Airway smooth muscle

<b>BMI</b>	Body mass index
<b>CPASSOC</b>	Cross phenotype association
<i>db/db</i>	Autosomal recessive diabetes mutation
<b>DGF</b>	DNaseI digital genomic footprinting
<b>DHS</b>	DNase I hypersensitivity site
<b>ECM</b>	Extracellular matrices
<b>eQTL</b>	Expression quantitative trait loci
<b>FAO</b>	Fatty acid oxidation
<b>FG</b>	Fasting glucose
<b>FI</b>	Fasting insulin
<b>GCP</b>	Genetic causality proportion
<b>GO</b>	Gene Ontology
<b>GSMR</b>	Generalized summary data-based Mendelian Randomization
<b>GWAS</b>	Genome-wide association study
<b>HDL</b>	High-density lipoprotein
<b>HFD</b>	High fat diet
<b>HLA</b>	Human leukocyte antigen
<b>LCV</b>	Latent Causal Variable
<b>LDL</b>	Low-density lipoproteins
<b>LDSC</b>	LD score regression
<b>LFD</b>	Low fat diet
<b>QC</b>	Quality control
<b>T2D</b>	Type 2 diabetes
<b>TC</b>	Total cholesterol
<b>TFBS</b>	Transcription factor binding sites
<b>TG</b>	Triglycerides
<b>WC</b>	Waist circumference
<b>WHR</b>	Waist-to-hip ratio
<b>WT</b>	Wild-type



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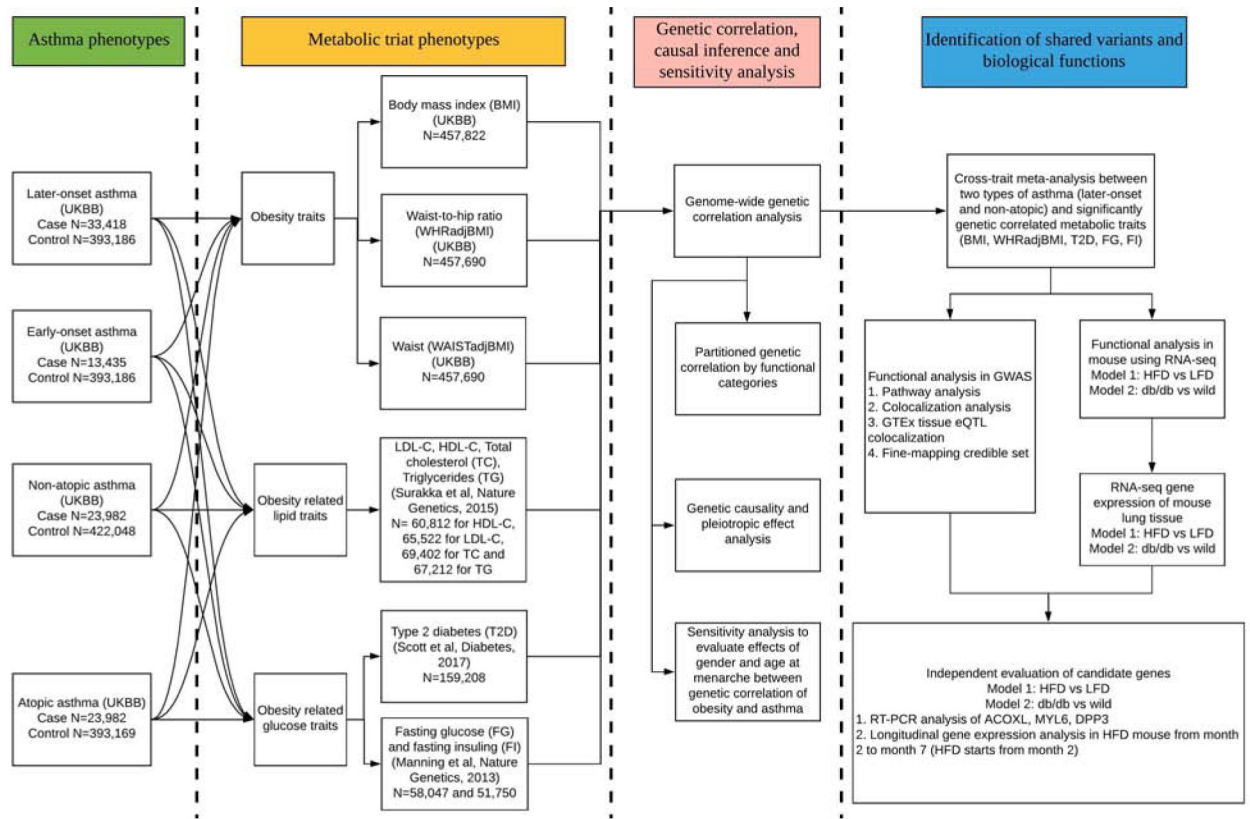
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**Key Messages:**

- This large-scale cross-trait GWAS provides strong evidence for shared genetic components between obesity-related metabolic traits and asthma subtypes
- The strongest positive genetic correlation was observed between obesity and later-onset asthma
- Mendelian Randomization analysis provided strong evidence in support of BMI causally increasing the risk of asthma

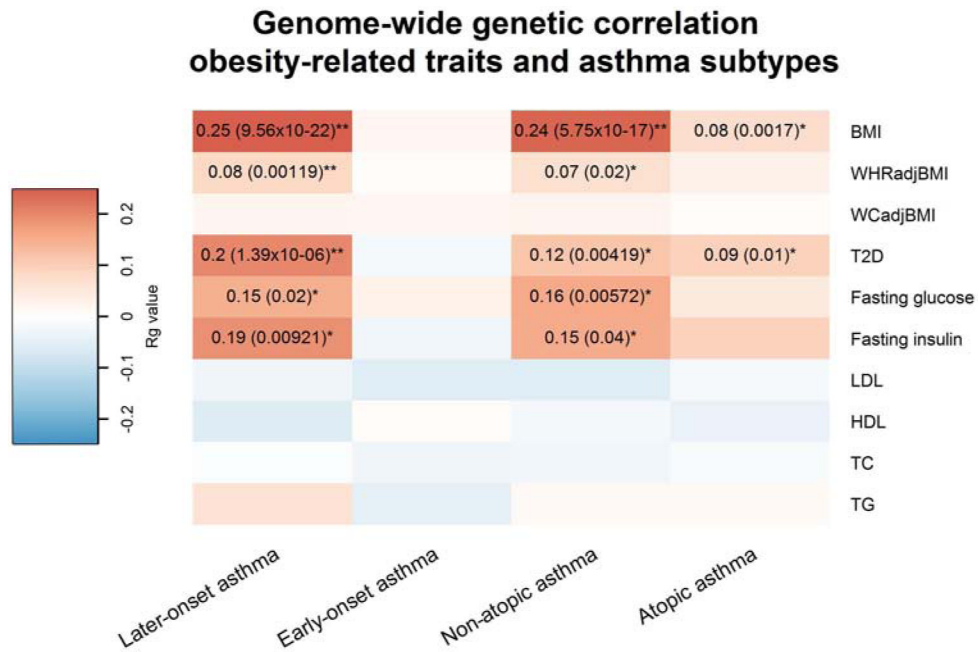


**Figure 1.**

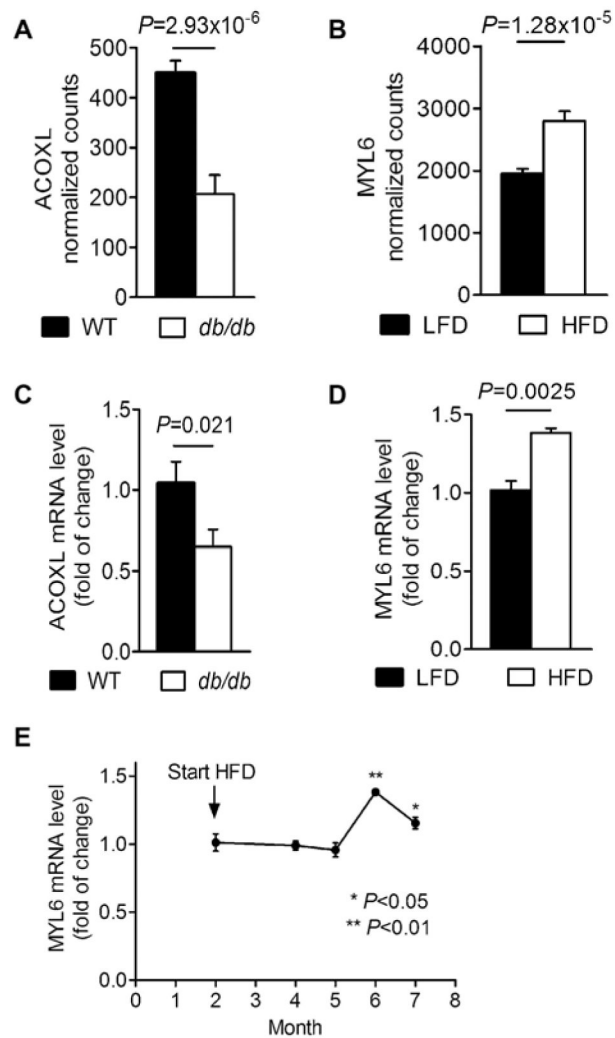
Overall study design.

UKBB: UK Biobank; LDSC: LD score regression. GSMR: generalized summary data-based Mendelian Randomization; LCV: Latent Causal Variable model





**Figure 2.** Genome-wide genetic correlation between 4 types of asthma and metabolic traits. The color of each box scales with the magnitude of the genetic correlation. Pairs of traits with nominal significant genetic correlation ( $p < 0.05$ ) are marked by 1 star, and pairs of traits with significant genetic correlation after correcting for multiple testing ( $p < 0.05/40$ ) are marked by 2 stars.

**Figure 3:**

ACOXL and MYL6 expression in lean and obese mice

(A) RNA-seq detected ACOXL differential expression in lung tissue from wild-type (WT) (*C57BL/6J*) and *db/db* mice ( $n = 4$  mice/group) (B) RNA-seq detected MYL6 differential expression in lung tissue from low fat diet (LFD) and high fat diet (HFD) mice ( $n = 4$  mice/group). (C) RT-PCR detected ACOXL differential expression in lung tissue from WT and *db/db* mice ( $n = 6$  mice/group). (D) RT-PCR detected MYL6 differential expression in lung tissue from LFD and HFD mice ( $n = 4$  mice/group). (E) RT-PCR detected MYL6 expression in lung tissue from HFD mice at different time points ( $n = 4$  mice/time point).

Estimates of causal effect size and genetic causality proportion (GCP) for BMI and asthma phenotypes, obtained by 2-sample bi-directional GSMR and LCV, respectively

**Table 1.**

Trait 1	Trait 2	Causal Odds Ratio (S.E.)		Genetic Causality Proportion (S.E.)
BMI	Later-onset Asthma	forward	1.21 (0.04) $P=6.3\times 10^{-7}$	0.90 (0.09)
		reverse	1.00 (0.01) $P=0.70$	
BMI	Non-atopic Asthma	forward	1.10 (0.04) $P=0.04$	0.86 (0.11)
		reverse	1.00 (0.006) $P=0.75$	
BMI	Atopic Asthma	forward	1.20 (0.03) $P=8.4\times 10^{-6}$	0.68 (0.21)
		reverse	1.00 (0.01) $P=0.76$	

BMI GWAS is from GIANT consortium. The “forward” denotes trait 1 is the exposure and trait 2 the outcome; and the “reverse” denotes trait 2 as the exposure and trait 1 the outcome. Here, we used the default GSMR outlier p-value threshold of 0.01. Causal effect size estimates obtained using a more exclusive threshold ( $p<0.1$ ) can be found in Supplementary Table 34.

**Table 2.**

Cross-trait meta-analysis results between 2 types of asthma and 3 obesity-related metabolic traits ( $P_{\text{meta}} < 5 \times 10^{-8}$ , single trait  $P < 1 \times 10^{-5}$ )

Model	SNP	Genome position	Effect Allele	Ref Allele	P1	P2	PMeta	Genes within clumping region
Later-onset asthma and BMI	rs6427082	chr1:167179419-167393635	T	A	$1.00 \times 10^{-07}$	$1.80 \times 10^{-06}$	$2.66 \times 10^{-13}$	POU2F1
	rs6681149	chr1:32152518-32228004	G	A	$6.70 \times 10^{-06}$	$3.30 \times 10^{-10}$	$2.91 \times 10^{-13}$	BAI2,COL16A1,MIR4254
	rs72836344	chr2:111861838-112023842	G	T	$2.10 \times 10^{-06}$	$3.80 \times 10^{-06}$	$3.62 \times 10^{-09}$	ACOXL,BCL2L1
	rs12493005	chr3:176869498-176928657	C	T	$7.00 \times 10^{-06}$	$3.00 \times 10^{-06}$	$2.44 \times 10^{-11}$	TBL1XR1
	rs7670374	chr4:53272753-53392903	G	A	$4.10 \times 10^{-06}$	$5.70 \times 10^{-07}$	$4.89 \times 10^{-10}$	Intergenic region
	rs6900923	chr6:144277568-144318433	C	G	$3.70 \times 10^{-06}$	$1.30 \times 10^{-06}$	$4.74 \times 10^{-10}$	PLAGL1
	rs4759229	chr12:56379060-56609885	A	G	$1.60 \times 10^{-09}$	$1.10 \times 10^{-13}$	$1.67 \times 10^{-23}$	ERBB3,ESYT1,IKZF4,MYL6,MYL6B,PA2G4,RAB5B,RNF41,RPL41,RPS26,SMARCC2,S.UOX,ZC3H10
	rs11365807	chr17:73757836-73953404	G	C	$3.90 \times 10^{-07}$	$1.40 \times 10^{-07}$	$7.07 \times 10^{-12}$	ACOX1,FBF1,GALK1,H3F3B,MIR4738,MRPL38,TRIM47,TRIM65,UNC13D,UNK,WBP2
	rs72836349	chr2:111861838-112023842	T	C	$2.20 \times 10^{-06}$	$1.50 \times 10^{-13}$	$1.02 \times 10^{-18}$	ACOXL,BCL2L1
	rs1787663	chr11:65211979-65342981	C	A	$5.90 \times 10^{-06}$	$2.30 \times 10^{-06}$	$6.35 \times 10^{-10}$	FAM89B,LTBP3,MALAT1,MIR548A,MIR548BA,MIR612,SCYL1,SSSCA1,SSSCA1,AS1
Later-onset asthma and WHRadjBMI	rs4616071	chr11:111366844-111443192	C	G	$1.40 \times 10^{-06}$	$1.40 \times 10^{-06}$	$5.85 \times 10^{-12}$	BTG4,C11orf88,LAYN,MIR34B,MIR34C
	rs10152595	chr15:67468525-67661784	C	G	$1.10 \times 10^{-11}$	$1.80 \times 10^{-11}$	$2.56 \times 10^{-20}$	AAGAB,IQCH,SMAD3
	rs4842921	chr15:84497207-84637651	G	A	$2.70 \times 10^{-06}$	$3.30 \times 10^{-06}$	$7.60 \times 10^{-11}$	ADAMTSL3
	rs8103278	chr19:46219145-46374916	G	A	$1.70 \times 10^{-06}$	$9.90 \times 10^{-07}$	$1.30 \times 10^{-11}$	DMPK,DMWD,FOXO4,FOXA3,LOC388553,RSPH6A,SIX5,SYMMPK
	rs174583	chr11:61537529-61650747	C	T	$4.60 \times 10^{-16}$	$1.69 \times 10^{-08}$	$4.45 \times 10^{-17}$	FADS1,FADS2,FADS3,FEN1,MIR611,MIR1908,MIR6746,MYRF,TMEM258
Later-onset asthma and FG	rs1381928	chr1:8378599-8895866	G	A	$2.30 \times 10^{-07}$	$2.00 \times 10^{-06}$	$3.90 \times 10^{-10}$	RERE,SLC45A1
	rs6681149	chr1:32152518-32228004	G	A	$2.80 \times 10^{-06}$	$3.30 \times 10^{-10}$	$3.17 \times 10^{-13}$	BAI2,COL16A1,MIR4254
	rs9275570	chr6:32428062-32681631	G	A	$2.90 \times 10^{-18}$	$2.60 \times 10^{-06}$	$1.81 \times 10^{-19}$	HLA-DQA1,HLA-DQB1,HLA-DRB1,HLA-DRB5,HLA-DRB6
	rs28423374	chr7:20501204-20638155	T	C	$9.90 \times 10^{-06}$	$3.70 \times 10^{-07}$	$2.42 \times 10^{-11}$	Intergenic region
	rs540874	chr11:66151649-66483265	A	G	$1.60 \times 10^{-06}$	$5.20 \times 10^{-06}$	$4.09 \times 10^{-09}$	ACTN3,BBS1,CCDC87,CCS,CTSF,DPP3,MRPL11,NPAS4,PELI3,RBM4,RBM4B,RBM14,RBM14-RBM4,SPTBN2,ZDHC24
Non-atopic asthma and BMI	rs4759229	chr12:56379060-56609885	A	G	$6.90 \times 10^{-09}$	$1.10 \times 10^{-13}$	$1.75 \times 10^{-21}$	ERBB3,ESYT1,IKZF4,MYL6,MYL6B,PA2G4,RAB5B,RNF41,RPL41,RPS26,SMARCC2,S.UOX,ZC3H10
	rs11365807	chr17:73757836-73953404	G	C	$1.00 \times 10^{-07}$	$1.40 \times 10^{-07}$	$9.81 \times 10^{-12}$	ACOX1,FBF1,GALK1,H3F3B,MIR4738,MRPL38,TRIM47,TRIM65,UNC13D,UNK,WBP2
	rs3826331	chr17:38128765-38218804	T	C	$1.40 \times 10^{-08}$	$2.90 \times 10^{-06}$	$1.13 \times 10^{-12}$	CSF3,GSDMA,MED24,MIR6884,PSMD3,SNORD124,THRA
	rs9895419	chr17:76667271-76858539	A	G	$7.40 \times 10^{-06}$	$3.60 \times 10^{-06}$	$1.32 \times 10^{-10}$	CYTH1,TIMP2,USP36
	rs8089834	chr18:39468982-39697096	T	C	$7.60 \times 10^{-06}$	$4.20 \times 10^{-09}$	$1.46 \times 10^{-13}$	PIK3C3
Non-atopic asthma and WHRadjBMI	rs10152595	chr15:67468525-67661784	C	G	$2.80 \times 10^{-12}$	$1.80 \times 10^{-11}$	$1.40 \times 10^{-20}$	AAGAB,IQCH,SMAD3

Model	SNP	Genome position	Effect Allele	Ref Allele	P1	P2	PMeta	Genes within clumping region
	rs112104755	chr18:20580534-20580534	A	G	3.50×10 <sup>-06</sup>	9.00×10 <sup>-06</sup>	6.50×10 <sup>-10</sup>	RBBP8
	rs11213940	chr11:111366844-111776891	C	A	4.30×10 <sup>-09</sup>	1.60×10 <sup>-06</sup>	1.73×10 <sup>-13</sup>	ALG9,BTG4,C11orf1,C11orf88,FDXACB1,LAYN,MIR34B,MIR34C,PPP2R1B,SIK2
	rs12491785	chr3:168679771-168905214	C	T	2.50×10 <sup>-07</sup>	7.10×10 <sup>-08</sup>	1.10×10 <sup>-12</sup>	MECOM
	rs73084574	chr7:20344126-20344126	G	A	9.10×10 <sup>-06</sup>	3.20×10 <sup>-08</sup>	4.39×10 <sup>-12</sup>	LOC101927769
	rs73086541	chr7:20371232-20478448	C	A	1.40×10 <sup>-08</sup>	3.10×10 <sup>-11</sup>	6.90×10 <sup>-18</sup>	ITGB8,LOC101927811
	rs73504817	chr19:17152232-17229439	T	C	1.50×10 <sup>-06</sup>	4.90×10 <sup>-11</sup>	1.43×10 <sup>-15</sup>	HAUS8,MYO9B
	rs8103278	chr19:46219145-46374916	G	A	4.40×10 <sup>-06</sup>	9.90×10 <sup>-07</sup>	5.12×10 <sup>-11</sup>	DMPK,DMWD,FBXO46,FOXA3,LOC388553,RSPH6A,SIX5,SYMPK
Non-atopic asthma and FG	rs174583	chr11:61543499-61617676	C	T	1.10×10 <sup>-08</sup>	1.69×10 <sup>-08</sup>	3.54×10 <sup>-10</sup>	FADS1,FADS2,FEN1,MIR611,MIR1908,MYRF,TMEM258

P1 is asthma (later-onset or non-atopic) single trait P-value. P2 is metabolic trait (BMI, WHRadjBMI or FG) single trait P-value. Pmeta is cross-trait meta-analysis P-value. FG is fasting glucose.