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Estimating the causal effect of body mass index on hay fever, asthma, and lung function using Mendelian randomization

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Abstract

**Background:** Observational studies have shown that body mass index (BMI) is positively associated with asthma. However, observational data are prone to confounding and reverse causation. In Mendelian randomization, genetic variants are used as un-confounded markers of exposures to examine causal effects. We examined the causal effect of BMI on asthma, hay fever, allergic sensitization, serum total immunoglobulin E (IgE), forced expiratory volume in one second (FEV1), and forced vital capacity (FVC).

**Methods:** We included 490,497 participants in the observational and 162,124 participants in the genetic analyses. A genetic risk score (GRS) was created using 26 BMI-associated single nucleotide polymorphisms (SNPs). Results were pooled in meta-analyses and expressed as odds ratios (ORs) or β-estimates with 95% confidence interval (CI).

**Results:** The GRS was significantly associated with asthma (OR=1.009; 95% CI: 1.004, 1.013), but not with hay fever (OR= 0.998; 95% CI: 0.994, 1.002), or allergic sensitization (OR=0.999; 95% CI: 0.986, 1.012) per BMI-increasing allele. The GRS was significantly associated with decrease in FEV1: β=-0.0012 (95% CI: -0.0019, -0.0006) and FVC: β=-0.0022 (95% CI: -0.0031, -0.0014) per BMI-increasing allele. Effect sizes estimated by instrumental variable analyses were OR=1.07 (95% CI: 1.03, 1.10) for asthma, a 9 ml decrease in FEV1 (95% CI: 2.0-15 ml decrease), and a 16 ml decrease in FVC (95% CI: 7.0-24 ml decrease) per 1 kg/m² higher BMI.

**Conclusions:** The results support the conclusion that increasing BMI is causally related to higher prevalence of asthma and decreased lung function, but not with hay fever or biomarkers of allergy.

**Keywords:** allergic disease, allergic sensitization, asthma, hay fever, serum specific IgE.
Introduction

Overweight and obesity are in reported observational studies consistently associated with increased prevalence of asthma and (1), to some extent, decreased lung function (2), while the association with hay fever and allergic sensitization is less clear (3-5). Overweight and obesity may affect the lungs in several ways, e.g., through inflammation that may predispose to asthma or through a mechanical effect on lung function. Mechanically, increased body mass index (BMI) may lead to decreased static lung volumes (6), and breathing with smaller tidal volumes which may leave some of the cross-bridged myosin-actin in the airways unbroken (7), thereby further narrowing the airways (8). In addition, the overweight/obesity-related low-grade inflammation may affect lung function and the risk of asthma (9). It is likely that other factors associated with overweight and obesity such as dyslipidaemia and increased mast cell activity are also implicated in the pathogenesis of asthma and decreased lung function. E.g., an increased number of mast cells has been found in adipose tissue, and mast cells participate in asthma by degranulation in response to allergen challenge (10). Upon activation, mast cells secrete peptidases such as tryptase (11,12). Tryptase may increase inflammation and remodeling of airway smooth muscles, and tryptase inhibition has been found to reduce airway inflammation.

However, inferring a causal relationship, e.g., between BMI and asthma, from such observational studies may be hampered by bias such as confounding and reverse causation. Mendelian randomization that is based on observational data is a method for making inferences about causal effects by using genetic instrumental variables (IV). The idea is that if we can replace the actual measured values of an exposure (which may be correlated with confounders etc.) by predicted values of the exposure (via the IV) that are related to the actual exposure but uncorrelated with confounders, we can get an un-confounded estimate.
This has been used previously in a study of approximately 5000 children where a higher BMI was found to increase the risk of asthma (13). However, this has not been examined in adults. Mendelian randomization takes advantage of the random allocation of alleles from parent to child and is a method for examining and estimating possible causal relationships where genetic IVs with well-known effects on an exposure are used as proxies for that exposure (14). To be a valid instrument, the genetic variant must be associated with the exposure, it may only affect the outcome through the exposure, and it cannot be associated with any unmeasured confounders.

We used a genetically determined higher BMI to investigate and quantify the effect of adiposity on asthma, hay fever, biomarkers of allergy (IgE antibodies), and lung function in adults (≥16 years) according to the principles of Mendelian randomization. We included 26 SNPs, including the FTO rs9939609 (15), that are associated with BMI at genome-wide significance levels in a number of genome-wide association studies (GWAS) (16-28) previously validated in one of the included studies (29,30).

Methods

Study populations

We used data on 490,497 participants of European ancestry and aged ≥16 years of whom 162124 had data on all or most of the relevant SNPs from the following seven population-based studies: the Danish Monitoring of trends and determinants in Cardiovascular Diseases (MONICA) study (the Monica10 study) (31), Health2006 (32), Health2008 (33), Inter99 (34), the Study of Health in Pomerania (SHIP) (35), and SHIP-TREND (36), and the UK Biobank (more information in Supplementary Material, incl. Table S1) (37). Each study was approved by local Ethics Committees, and the participants gave their informed consent.
**Genotype**

We preselected 26 BMI-associated SNPs as listed in Table 1 (16-28). None of the cohorts used were in the discovery samples for these SNPs. Descriptions of the genotyping method within each study are provided in Supplementary Material. Minor allele frequencies (MAF) and Hardy–Weinberg equilibrium p-values are shown in Table S2. Some SNPs were in one or a few studies not in Hardy-Weinberg equilibrium at the 0.05 significance level, but except for two SNPs in the UK Biobank, all SNPs were in Hardy-Weinberg equilibrium at the Bonferroni-adjusted significance level (p<0.00027). Each SNP was classified according to the number of BMI-increasing alleles, i.e. 0, 1, or 2. A simple genetic risk score was calculated by adding the number of BMI-increasing alleles (38). In secondary analyses, we used a weighted genetic risk score with weights derived from studies different from our own (16-28). See individual weights in Table 1.

**BMI**

Height and weight were measured, and BMI was calculated as weight divided by height squared, expressed in kg/m².

**Hay fever, asthma, allergic sensitization, and serum total IgE, FEV₁, and FVC**

Information on hay fever and asthma was based on self-report (Supplementary Material incl. Table S3). Our first choice was lifetime/ever diagnoses. Allergic sensitization was defined as serum specific IgE positivity to at least one of a number of inhalant allergens (Supplementary Material incl. Table S3). Serum total IgE levels were measured by the IMMULITE 2000 Allergy Immunoassay System in Inter99 Study, and by the Latex IgE test on the BN II
Nephelometer (Dade Behring Marburg GmbH, Marburg, Germany) in the SHIP Study (39). FEV1 and FVC were measured with spirometry. More details are provided in the Supplementary Material. For additional analyses in UK Biobank data only, we created subgroups of persons who had both asthma and hay fever (as a measure of ‘atopic’ asthma), asthma but not hay fever (as a measure of ‘non-atopic’ asthma), and not asthma or hay fever.

Statistical analyses

Statistical analyses were performed with SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA), STATA, version 13 (StataCorp, College Station, TX, USA), and R-statistical package, version 3.2.3 for Windows (http://www.r-project.org/). The p-values are two-tailed, and statistical significance was defined as p<0.05, but the causality of associations was assessed by the strength of evidence rather than the size of the P-value only. For each of the studies, a dataset was prepared in accordance with the study protocol, and the data were analyzed by a pre-prepared Stata-script that was adapted to each study. To obtain a normal distribution, serum total IgE was transformed by the natural logarithm (log). Except for first stage analyses only (e.g., Figure 3) where BMI was transformed to the logarithm of two, the reported data are according to BMI not log-transformed (incl. first stage estimates to be used in IV-analyses). The results from each study were meta-analyzed with fixed effects analyses and heterogeneity assessed by the I² test. Effect estimates are presented as β-coefficients or odds ratios with 95% confidence intervals (95% CI).

Observational analyses

Observational, i.e. the BMI-outcome associations, analyses were performed using logistic and linear regression analyses that were adjusted for sex and age, and in the FEV1 and FVC
analyses also for height. We also compared results from observational analyses considering only those from UK Biobank that were included in the MR analyses to the whole UK Biobank sample.

**Mendelian randomization analyses**

The SNP data for each study is shown in Table S1. We created a SNP-score which is a single variable that summarizes multiple SNPs in a univariate score. Our primary analyses rely on a simple SNP-score that counts the number of BMI-increasing alleles whereas in a weighted SNP-score, each SNP contributes a weight that reflects the effect of the SNP on BMI. The ‘F-value’ is an analogue of the F-statistic for the joint significance of the SNP/SNP-score in the first stage regression. The F-value is an indicator of statistical power in the MR analysis (40). In the UK Biobank study, the F-value for the SNP-score was 1185 for BMI. The single-SNP F-values ranged from 1 to 397 in the UK Biobank data. The rs1424233 and rs11847697 SNPs had F-values below 10. Analyses excluding the three SNPs with signs of Hardy-Weinberg disequilibrium in the UK Biobank Study were also performed. The requirement that the instrument is not associated with unmeasured confounders can only be falsified, but to substantiate the assumption, we assessed the associations between each single-SNP and age, sex, smoking status, and alcohol status.

Mendelian randomization analyses (first stage: SNP-BMI, second stage: SNP-outcome) were performed using logistic and linear regression analyses that were adjusted for sex and age, and in the FEV1 and FVC analyses also for height. To obtain a normal distribution, serum total IgE was transformed by the natural logarithm (log).

IV analyses were performed by using the R packages “MendelianRandomization” and “ivpack”, and graphs were made by the “rmeta” package in
R. To obtain estimates expressed per 1 kg/m² higher BMI, we used the untransformed BMI in the first stage analysis. As the primary IV analyses, we performed a one-sample analysis, where all relevant studies were included in both the first and second stage analysis. Secondarily, we performed two-sample IV-analyses where the first and second stage sample had no overlap: All studies except for the UK Biobank Study provided data for the first stage and UK Biobank alone provided data for the second stage analysis. To assess potential pleiotropy, we also performed MR Egger regression and median regression analyses (41).

Results

Observational analyses

Descriptive statistics for each study population are found in Table 2.
In sex- and age-adjusted observational analyses (Figure 1), BMI was inversely and significantly associated with hay fever: OR=0.995 (95% CI: 0.994, 0.997, p <0.001) per 1 kg/m² higher BMI and positively and significantly associated with asthma: OR=1.037 (95% CI: 1.035, 1.039, p<0.001) per 1 kg/m² higher BMI. BMI was positively but non-significantly associated with allergic sensitization with OR=1.007 (95% CI: 0.998, 1.016, p=0.131) per 1 kg/m² higher BMI. BMI was positively and significantly associated with log(IgE): β=0.02 (95% CI: 0.01, 0.03, p<0.001) per 1 kg/m² higher BMI (Figure 2). In general, the heterogeneity was low to modest and ranged from 0.0%-53.2%. BMI was inversely associated with FEV1 and FVC with β=-0.012 (95% CI: -0.013, -0.012, p<0.001) and β=-0.024 (95% CI: -0.025, -0.024, p<0.001), respectively per 1 kg/m² higher BMI (these analyses were further adjusted for height) in fixed effect meta-analyses (Figure 2). The heterogeneity was substantial (both I²>90.0%). The study-specific estimates of observational analyses of the association between BMI, FEV1 and FVC are shown in Supplementary Figure S6. Random effect meta-analyses yielded for FEV1: β=-0.008 (95% CI: -0.012,-0.004, p=0.0002) and FVC: β=-0.019 (95% CI: -0.023,-0.014, p<0.0001). Applying a Bonferroni-correction (6 outcomes, p=0.05/6=0.0083) did not change the statistical significance in the observational analyses. The observational results considering only those from UK Biobank that were included in the MR analyses below compared to the whole UK Biobank sample showed similar results. The results of observational analyses excluding UK Biobank were largely similar (Supplementary Figures S2 and S4).

Mendelian randomization analyses

Figure 3 shows the associations of each SNP with the log2-transformed BMI and substantiates their use as valid instruments. Fixed effect meta-analyses of the age- and sex-adjusted association of the BMI-associated SNP-score and BMI showed significantly higher log2(BMI): β=0.007 (95% CI:
0.007, 0.008, p<0.0001) per BMI-increasing allele. However, there was substantial heterogeneity (I²=95.3%) and random effects meta-analysis showed: β=0.007 (95% CI: 0.006, 0.009, p<0.0001) per BMI-increasing allele. To justify the assumption that the instruments are not associated with unmeasured confounders (33,42), we assessed the associations between each single-SNP and the available possible confounders age (all p>0.007 in Kruskal-Wallis’ test), sex (all p>0.021 in the X²-test), smoking status (all p>0.0009 in the X²-test), and alcohol status (all p>0.016 in the X²-test). No associations were statistically significant at the Bonferroni-adjusted significance level (p=0.05/(4*26)=0.00048), however.

In the primary, one-sample sex- and age-adjusted Mendelian randomization analyses (Figure 1), the SNP-score was positively and significantly associated with asthma: OR=1.009 (95% CI: 1.004, 1.013, p<0.001, I²=51.1%) per BMI-increasing allele. The results of MR-analyses excluding UK Biobank were largely similar (Supplementary Figures S1 and S3). Using a weighted SNP-score in the UK Biobank alone, we found for asthma: OR=1.068 (95% CI: 1.034, 1.104, p<0.001) per BMI-increasing allele. There was a high degree of consistency in the predicted effect of each single-SNP on asthma (Figure S5). IV-analysis yielded an OR=1.07 (95% CI: 1.03, 1.10) of asthma per 1 kg/m² higher BMI. There was no clear evidence that the SNP score was associated with hay fever: OR=0.998 (95% CI: 0.994, 1.002, p=0.333, I²=0.0%) per BMI-increasing allele. Likewise, using a weighted SNP-score in the UK Biobank alone, we found for hay fever: OR=0.995 (95% CI: 0.969, 1.022, p=0.711) per BMI-increasing allele. Alternative definitions of hay fever in UK Biobank data (See Supplementary) showed for those taking medication for hay fever at baseline: OR=1.006 (95% CI: 0.994, 1.018, p= 0.316, N=146072), with self-reported hay fever as serious illness at baseline: OR=1.000 (95% CI: 0.992, 1.009, p=0.908, N=146072), or persons with self-reported hay fever or allergic rhinitis at follow-up in 2015: OR=0.998 (95% CI: 0.990, 1.006, p=0.655, N=34556) for hay fever per BMI-increasing allele compared with the rest, respectively.
The SNP-score was non-significantly associated with allergic sensitization with OR=0.999 (95% CI: 0.986, 1.012, p=0.906, I^2=0.0%) per BMI-increasing allele.

The BMI-increasing SNP-score was inversely associated with FEV1 and FVC with β=-0.0012 L (95% CI: -0.0019, -0.0006, p=0.011) and β=-0.0022 L (95% CI: -0.0031, -0.0014, p<0.001), respectively, per BMI-increasing allele in age-, sex- and height-adjusted analyses. Correspondingly, using a weighted SNP-score in the UK Biobank only, we found for FEV1: β=-0.0089 L (95% CI: -0.0155, -0.0023, p=0.008) and for FVC: β=-0.0164 L (95% CI: -0.0249, -0.0078, p<0.001) per BMI-increasing allele. IV-analyses showed a 9 ml decrease in FEV1 (95% CI: 2.0-15 ml decrease) and a 16 ml decrease in FVC (95% CI: 7.0-24 ml decrease) per 1 kg/m^2 higher BMI. The heterogeneity was low (all I^2 <26.9%) in the analyses of the SNP-score vs. FEV1 and FVC, respectively. Fixed effects meta-analysis showed a non-significantly β=0.003 (95% CI: -0.006, 0.012, p=0.547) higher log(IgE) per BMI-increasing allele with low heterogeneity (I^2=0.0%). Except for the association between the SNP-score and FEV1, applying a Bonferroni-correction (6 outcomes, p=0.05/6=0.0083) did not change the statistical significance in the main IV-analyses.

Two-sample Mendelian randomization analyses of hay fever, asthma, FEV1, and FVC supported the one-sample results (Figure 1-2). We found little evidence of pleiotropy for the BMI-associated SNPs on asthma, as indicated by the MR Egger intercept test (p=0.069) and the Egger regression analysis showed an odds ratio of asthma: OR=1.09 (95% CI: 1.03, 1.15, p=0.002) per 1 kg/m^2 higher BMI (43). Likewise, median regression yielded an odds ratio of asthma: OR=1.05 (95% CI: 1.01, 1.09, p=0.007) per 1 kg/m^2 higher BMI.

Analyses excluding the three SNPs with signs of Hardy-Weinberg disequilibrium in the UK Biobank Study showed for hay fever: OR=0.999 (95% CI: 0.995, 1.003, p=0.544), asthma: OR=1.011 (95% CI: 1.006, 1.016, p=0.00003), and FEV1: β=-0.001 (95% CI: -0.002, -0.0004, p=0.004), and FVC: β=-0.003 (95% CI: -0.004, -0.001, p=0.0001) litre per BMI-increasing allele.
In additional analyses of the association between the genetic risk score and hay fever, we performed the analyses with and without the SHIP TREND Study. The estimate and confidence intervals were equal to the third decimal place. Also, we found that persons with both asthma and hay fever (‘atopic’ asthma) vs. persons with neither asthma nor hay fever had largely the same odds ratio per BMI-increasing allele as in the primary analyses where we compared those with asthma to those without asthma regardless of hay fever status. In comparison, persons who had asthma but not hay fever (‘non-atopic’ asthma) vs. persons with neither asthma nor hay fever had a slightly lower odds ratio per BMI-increasing allele that was not statistically significant.

**Discussion**

In a Mendelian randomization meta-analysis, we found that a genetically determined higher BMI was associated with a significantly higher prevalence of asthma and lower lung function (both FEV1 and FVC), but not with hay fever and allergy biomarkers. The observed positive association between BMI and asthma is in line with the results from the majority of previous studies (Table 3) (44-53), including a Mendelian Randomization study of close to 5,000 children where a higher BMI increased the risk of asthma in mid-childhood (13). A review by Baumann et al. concluded that the association between obesity and asthma was particularly seen for non-atopic asthma (54). However, our analysis in the UK Biobank sample using presence/absence of hay fever to define atopic/non-atopic asthma could not fully corroborate that. A number of studies found no statistically significant associations between BMI and asthma (55-57).

The observed negative association between BMI and lung function is in line with a study by Ciprandi et al. comprising 268 individuals who found that lung function was significantly impaired in overweight and obese asthmatic patients, e.g., overweight patients, had double (OR=1.89) and obese patients had triple the risk (OR=3.17) of a pathological FEV1 compared to
normal-weight patients (58). Fenger et al. found in a study of 2308 adults that increasing adiposity was associated with decreasing lung function over a five year period in longitudinal study of 2308 adults (2). A one standard deviation increase in BMI corresponded to a $-78.5 \text{ ml}$ and $-113.6 \text{ ml}$ five-year change in FEV1 and FVC, respectively, in men, and a $-25.5 \text{ ml}$ and $-32.6 \text{ ml}$ five-year change in FEV1 and FVC, in women, respectively.

The results from previous studies on BMI and hay fever are inconsistent (Table 4). The observed lack of association in the current study is in line with the conclusions from two research studies of 3047 adults and 3466 adolescents, respectively (57,59), and a review including 50,086 individuals (54). However, several studies have found both inverse (51,52,58,60,61) and positive associations between BMI/overweight/obesity and hay fever (49,55,56,60,62,63).

Likewise, previous studies examining the association between obesity and allergic sensitization in adults have shown conflicting results (44). There are studies supporting a positive association (4,64,65) and studies reporting no or an inverse association between BMI and allergic sensitization (66-68). The observed non-significantly higher odds ratio of allergic sensitization for a higher BMI is in line with a study by Sybilski et al. who in almost 10000 adults found that obesity and overweight were not associated with the frequency of sensitization to aeroallergens (51). Likewise, Yao et al. found a statistically non-significant higher prevalence of allergic sensitization in 5,351 Taiwanese children (53). Somewhat in contrast, a study by Byberg et al. involving 617 study participants found BMI in early childhood to be positively associated with atopic sensitization in later childhood (48). Also, in a study of 139 individuals, Lokaj-Berisha et al. found high BMI to be associated with atopy (50).

Strengths of the current study include the fact that the SNPs were preselected from GWAS studies of populations different from our own but of the same ethnicity. Since most of the SNPs had small individual effects, we constructed a genetic risk-score where the multidimensional
genetic data were collapsed into one variable. Risk-scores -as opposed to the individual variants- may be stronger instruments (69). Each SNP in the SNP-score had to fulfill the requirements for an IV in order for the score to be a valid instrument (70). All but two of the SNPs had F-values above 10 in the UK Biobank data, and the F-value was 2000 for the SNP-score.

Limitations of the study include the low power in the analyses of allergic sensitization and serum total IgE compared with the power in the analyses of asthma, hay fever, and lung function. The hay fever variable in the UK Biobank included eczema which is a less specific variable for hay fever than most of the other studies. However, we assessed three additional hay fever variables from the UK Biobank with similar results which indicates that the misclassification did not seriously bias our results. The main weaknesses of the three additional hay fever variables were the fact that the first was measured at follow-up in 2015 in a subgroup only; the second only included participants who would classify their hay fever as a serious illness or disability; and the third additional hay fever variable was based on self-reported medication rather than doctor diagnosed hay fever. In addition, the definition of hay fever in the SHIP TREND Study differs. However, the estimate and confidence intervals of the association between the genetic risk score and hay fever were equal to the third decimal place which means that this potential misclassification did not seriously bias our results.

Causal inference may be distorted by a number of violators of the Mendelian randomization assumptions, e.g., pleiotropy where the genetic marker has diverse biological functions. This assumption may be more plausible for specific proteins or serum markers than for a general phenotype such as BMI. However, MR Egger and median regressions showed little sign of pleiotropy in the BMI-associated SNPs and asthma. There was also a high degree of consistency in the predicted effect of each single-SNP on asthma (Figure S5). Also, we found little evidence of associations between the included SNPs and the possible confounders, age, sex, smoking and
alcohol intake in the UK Biobank data. There were no obvious trends or consistencies regarding the
SNPs with signs of Hardy-Weinberg disequilibrium, neither relating to the SNPs nor the studies,
and we consider these chance findings. This was corroborated by analyses excluding the three SNPs
in Hardy-Weinberg disequilibrium in the UK Biobank Study as listed in the results. Weak
instrument bias is a particular concern in small studies when using genetic variants that explain only
little variation in the risk factor (71). Weak instrument bias can be introduced in one-sample MR
studies because the associations of the SNPs with the risk factor and outcome are correlated. The
causal estimate will be biased in the direction of the observational association contrary to a two-
sample Mendelian randomization analysis where any weak instrument bias is in the direction of the
null. Even so, in support of using an overlapping sample in the current study, the SNP-score was
constructed from SNPs not discovered in the included studies, the SNP-score did not use (internal)
weights, and it had a high F-value. The current study included the very large UK Biobank Study
that had acceptable F-values for the large majority of the SNPs. The estimates in UK Biobank data
were similar to the meta-analysis estimates for asthma, hay fever, and lung function. In addition,
results from two-sample Mendelian randomization analyses of hay fever, asthma, FEV1, and FVC
were almost identical to the one-sample analyses which means that weak instrument bias does not
seriously bias our results. The results from using a weighted genetic risk score supported our
findings from using a simple genetic risk score.

In conclusion, we found that genetically determined higher BMI was associated with a
higher prevalence of asthma and lower lung function. Taken together with the traditional
observational results and the existing evidence, the results are supportive of a positive causal
relation between BMI and asthma and an inverse relation with FEV1 and FVC.
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Author contributions

All authors were involved in the conception and design of the study or the collection and processing of samples. TS conducted data analyses and wrote the initial manuscript. All authors were involved in critical appraisal and revision of the manuscript, and all authors approved the final version.

Competing Interests

The authors report no conflicts of interest.
Figure 1. One- and two-sample IV analysis and fixed effects meta-analysis of the observational estimates of age- and sex-adjusted associations between BMI and hay fever, asthma, and allergic sensitization, respectively. The numbers of persons included in the two-sample IV analysis are shown as ‘number in first stage analysis’/‘number in second stage analysis’. Abbreviations: CI, confidence interval; BMI, body mass index; IV, instrumental variable.

Figure 2. One- and two-sample IV analysis and fixed effects meta-analysis of the observational estimates of BMI (kg/m²) with FEV1 (litre), FVC (litre), and log(IgE). The IV estimates are generated from unadjusted first and second stage analyses. Units are noted in parentheses. The numbers of persons included in the two-sample IV analysis are shown as ‘number in first stage analysis’/‘number in second stage analysis’. Abbreviations: CI, confidence interval; BMI, body mass index; IV, instrumental variable.

Figure 3. Age- and sex-adjusted associations of each BMI-associated SNP and the log2-transformed BMI.
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