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Title: Body mass index as a modifiable risk factor for type 2 diabetes: Refining and understanding causal estimates using Mendelian randomisation.

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## ABSTRACT

This study focused on resolving the relationship between body mass index (BMI) and type 2 diabetes. The availability of multiple variants associated with BMI offers a new chance to resolve the true causal effect of BMI on T2D, however the properties of these associations and their validity as genetic instruments need to be considered alongside established and new methods for undertaking Mendelian randomisation. We explore the potential for pleiotropic genetic variants to generate bias, revise existing estimates and illustrate value in new analysis methods. A two-sample Mendelian randomisation (MR) approach with 96 genetic variants was employed using three different analysis methods, two of which (MR-Egger and the weighted median) have been developed specifically to address problems of invalid instrumental variables. We estimate an odds ratio for type 2 diabetes per unit increase in BMI (kg/m$^2$) of between 1.19 and 1.38, with the most stable estimate using all instruments and a weighted median approach (1.26 95%CI (1.17, 1.34)). *TCF7L2*(rs7903146) was identified as a complex effect or pleiotropic instrument and removal of this variant resulted in convergence of causal effect estimates from different causal analysis methods. This indicated the potential for pleiotropy to affect estimates and differences in performance of alternative analytical methods. In a real type 2 diabetes focused example, this study demonstrates the potential impact of invalid instruments on causal effect estimates and the potential for new approaches to mitigate the bias caused.

Observational studies have shown body mass index (BMI) to be associated with risk of type 2 diabetes as well as with a range of diabetes-related metabolic traits (1; 2). However, it is well known that confounding, reverse causation and biases can generate such associations and that even with careful study design, incorrect inference is possible (3). One approach to circumventing these problems is to use genetic association results within a Mendelian randomization (MR) framework (3; 4). In MR analyses, genetic variants act as proxies for an exposure in a manner independent of confounders. If in addition the variants only affect an outcome of interest through the chosen exposure, then they are said to be valid instrumental variables (IVs). This enables evaluation of the causal effect of the exposure on the outcome, escaping some of the limitations of observational epidemiology; (5).

Following the success of genome-wide association studies (GWASs), the number of MR analyses using large numbers of mostly uncharacterized variants associated with complex health outcomes or intermediates is rapidly increasing (6; 7). In the case of BMI, there are now 97 genetic variants reliably associated and there are examples where multiple variants have been used as a composite IV to estimate the causal impact of BMI on health (8). Although using many IVs can increase the power of MR analyses , it brings with it the concern that enlarged sets of genetic variants are more likely to contain invalid IVs due to violations of the assumptions necessary for valid causal inference using traditional methods (9). In particular, horizontal pleiotropy – where a genetic variant affects the outcome via more than one biological pathway (10) – is a concern. Importantly, the properties of these associations and their validity as genetic instruments need to be considered alongside established and new methods for undertaking Mendelian randomisation.

In response to the general issue of using multiple genetic variants in MR, Bowden *et al.* (9) propose both MR-Egger regression, an approach developed from the original Egger regression technique for assessing small study bias in meta-analysis and a weighted weighted median approach (11) as alternatives to the standard MR analysis. The MR-Egger and weighted weighted median approaches both operate using distinct, but critically weaker, versions of the IV assumptions, and therefore have the potential to deliver robust causal effect estimates. The MR-

Egger method also provides a formal statistical test as to whether or not the average pleiotropic effect of the genetic variants is equal to zero (9).

## Research Design and Methods

With increasing evidence for multiple biological pathways underlying type 2 diabetes (12; 13) and increasing numbers of genetic variants available as IVs for BMI, we set out to test the potential for bias in causal estimates from MR using these state-of-the-art approaches. We compared results from MR-Egger regression (9) and weighted weighted median (11) approaches to a traditional inverse-variance weighted (IVW) method (which makes the strong assumption that all variants are valid IVs) (14) in an investigation of the causal relationship between BMI and type 2 diabetes. These methods all undertake two-sample Mendelian randomisation whereby the GWAS results for a disease outcome are unified with those of an exposure of interest and together used to estimate the causal impact of that exposure on disease. We used published data in a two-sample analysis strategy taking SNP-exposure and SNP-outcome associations from different sources (15; 16).

The effect sizes for BMI-associated SNPs with associated standard errors from a mixed-sex cohort of European ancestry were taken from the Genetic Investigation of ANthropometric Traits (GIANT) consortium (17) along with results for type 2 diabetes from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. To avoid sample overlap, GIANT estimates were re-calculated in the absence of DIAGRAM cohorts yielding a maximum sample size at any given locus of 189,079. To aid interpretation of the effects of BMI on type 2 diabetes, effect sizes were transformed to BMI units prior to analysis, assuming one standard deviation (SD) = 4.5kg/m$^{2}$[17]. For the corresponding SNP-outcome association, we took odds ratios (ORs) and confidence intervals from a GWAS meta-analysis conducted by the DIAGRAM Consortium. This genome-wide meta-analysis includes data from 12,171 type 2 diabetes cases and 56,862 controls of mainly European descent imputed at up to 2.5 million autosomal SNPs (DIAGRAMv3) (18). All but one (rs4787491, *INO80E*) of the BMI-associated SNPs ($p$<5x10$^{-8}$) from GIANT had results listed in the DIAGRAMv3 dataset so 96 SNPs with results in both datasets were taken forward for analysis.

SNP-exposure and SNP-outcome associations were combined using the three different approaches outlined above. All analyses were conducted in R 3.2.0 (19). First, an inverse-variance weighted (IVW) method was implemented to provide a weighted average of the causal effect estimates (14). This method assumes that all genetic variants (i.e. 100%) satisfy the IV assumptions (including zero pleiotropy) and uses weights that assume the gene-exposure association estimates are measured without error (the No Measurement Error (NOME) assumption).

Second, we performed MR-Egger regression (9), which assumes NOME but allows each variant to exhibit pleiotropy. MR-Egger estimates remain consistent only if the magnitude of the gene exposure associations across all variants are independent of their pleiotropic effects (the InSIDE assumption) (9). As recommended by Bowden *et al* (9), the extent to which pleiotropy was balanced across the set of instruments as a whole was visually assessed by plotting the causal effect estimates against their precision, using a funnel plot and checking for asymmetry (Figure 1A). The NOME assumption was assessed for MR-Egger via an adaptation of the $I^2$ statistic ($I^2_{GX}$) (20) and adjusted for by combining MR-Egger with the method of Simulation Extrapolation (SIMEX) (21). Using SIMEX, new data sets are created by simulating gene-exposure association estimates under increasing violations of NOME and recording the amount of attenuation in the estimate that occurs. The set of attenuated estimates are then used to extrapolate back to the estimate that would have been obtained if NOME had been satisfied.

Finally, a weighted weighted median estimation method was applied (11). The weighted median provides a consistent estimate of causal effect if at least 50% of the information in the analysis comes from variants that are valid IVs. For a more detailed description of the three methods applied, see Online Appendix (Supplementary methods). A leave-one-out permutation analysis was conducted across all methods to assess the influence of potentially pleiotropic SNPs on the causal estimates (22). In the case of the linear models (IVW and MR-Egger) two additional analyses were conducted (23; 24). Firstly, the extent to which the causal estimate from each SNP

in the set could be considered an outlier was assessed using studentized residuals. Secondly, Cook's distance (25) was used as a measure of the aggregate impact of each SNP on the model.

**Results**

All three approaches provide evidence of a positive causal relationship between BMI and type 2 diabetes. This is demonstrated in Figure 1B where the slope of the lines show the causal effect estimates as predicted by the IVW, MR-Egger and m weighted median approaches. Estimates correspond to an OR for type 2 diabetes per unit increase in BMI (kg/m$^2$) of 1.19, 1.26 and 1.38 for the IVW, weighted median and MR-Egger analyses, respectively and are in line with a previous MR estimate of 1.27 (95%CI 1.18, 1.36) (2) (Table 1). Assessment of the NOME assumption with respect to the MR-Egger estimate gave $I^2_{GX}$=0.83, suggesting an approximate 15% attenuation of the causal estimate towards zero. Bias adjustment via SIMEX gave a corrected MR-Egger causal estimate of 1.46 (95%CI 1.16, 1.84) for type 2 diabetes per unit increase in BMI (kg/m$^2$).

Considering the individual SNP-based contributions to MR analysis, there is one clear outlier in the distribution of effects shown in Figure 1 and that is *TCF7L2*(rs7903146). *TCF7L2*(rs7903146) shows an association with BMI that is in the opposite direction to the overall trend (and weak relative to its effect on type 2 diabetes), resulting in a large negative causal estimate from this SNP alone. The presence of at least some unbalanced pleiotropy was detected within the set of variants, as reflected by the intercept estimate of -0.019 ($p$=0.10) in the MR-Egger analysis.

To illustrate the impact of *TCF7L2*(rs7903146) on causal estimates, we performed a sensitivity analysis in which each SNP in turn was removed from the set in a leave-one-out permutation analysis. We saw a shift in the causal estimates from the IVW (an increase) and MR-Egger (a decrease) as a result of the removal of *TCF7L2*(rs7903146) but no difference in the estimate from the weighted median approach (Table 1; Figure 2). The results of the leave-one-out permutation analysis showed that the impact of removing *TCF7L2*(rs7903146) from the variant set on the IVW and MR-Egger estimates was greater than that of removing almost any other variant, with the exception of *FTO*(rs1558902) (Figure 2A & B). When *FTO*(rs1558902) was removed, causal

estimates from both the IVW and MR-Egger analysis decreased (Table 1; Figure 2). In this instance we also observed movement in the causal effect estimate from the weighted median (Table 1; Figure 2C). The estimate of the intercept from MR-Egger moved closer to zero following both the removal of *TCF7L2*(rs7903146) and *FTO*(rs1558902) (Figure 2D). *TCF7L2*(rs7903146) was also identified as an outlier in both IVW and MR-Egger (studentized residuals, Bonferroni corrected $p<1x10^{-19}$) but *FTO*(rs1558902) was not (Online Appendix (Supplementary Results, Figures S1A/B)). Calculation of Cook's distance showed both variants to have a disproportionate level of influence on the model compared to other variants in the set (Online Appendix (Supplementary Results, Figures S2A/B)) .

These results suggest *TCF7L2*(rs7903146) may be pleiotropic with respect to the outcome, i.e. that it influences type 2 diabetes through an alternative pathway (other than BMI). Evidence from existing literature supports this assertion as the type 2 diabetes risk increasing allele at *TCF7L2*(rs7903146) has been associated with both increased fasting glucose (26) and decreased BMI (17). Under the assumption that *TCF7L2*(rs7903146) demonstrates horizontal pleiotropy with respect to type 2 diabetes, we would expect its inclusion in the variant set to bias the causal estimate predicted by the IVW approach, but not that predicted by MR-Egger or the weighted median. Removing *TCF7L2*(rs7903146) from the variant set causes a slight shift in the causal estimates from the IVW and MR-Egger approaches, bringing them more in line with one another and also with the weighted median estimate which remained stable in this instance. Also of note is the reduction in the 95% confidence interval of the MR-Egger estimate following removal of the *TCF7L2*(rs7903146). This increase in precision following removal of a likely invalid instrument from the set is another potentially favourable quality of this estimator. The relatively small changes observed across all methods as a result of removing *TCF7L2*(rs7903146) are in line with the relatively weak effect of the SNP as shown in Figure 1B.

In contrast, the effect of removing *FTO*(rs1558902) is more noticeable. Regardless of the method used, removing this variant results in a lower causal estimate (Table 1; Figure 2). The substantial influence of *FTO*(rs1558902) was predicable given the strength of its effect relative to the other

variants (Figure 1B), though properties of this effect are not in line with other variants used to instrument BMI as reported elsewhere for physical activity (27), thyroid function (28) and depression (29). The concomitant increase in standard error associated with the estimates here point towards increased uncertainty moving the estimates towards the null in the absence of *FTO*(rs1558902). The weighted median appears robust, even to the removal of *FTO*(rs1558902), as demonstrated by the relatively tight distribution of estimates returned from the leave-one-out permutation analysis (Figure 2C). This is as expected given the tolerance of weighted median approaches to outliers.

**Discussion**

By applying new analytical techniques to an old question – the causal relationship between BMI and type 2 diabetes – we have explored the potential for invalid instruments to bias causal estimates in MR. In this case where BMI is the exposure, the opportunity to use a large instrument list in causal analyses presents both opportunity, through variance explained, but also cost, through complications generated by instrument properties or methods employed. Results here suggest that both *TCF7L2* and *FTO* appear to have genetic variation which predicts BMI reliably, but for which associations with type 2 diabetes do not fully align with that for other variants (given BMI effects and assumed causality).

For *TCF7L2*, only recently suggested to be associated with BMI directly (17), this is not surprising and reinforces the important point that the validity of a specific method's MR estimate depends on whether the genetic variants collectively satisfy its assumptions. In this case, it is possible that the negative association with BMI observed in GIANT is the product of a form of bias where the risk of type 2 diabetes is leading to effective treatment, health benefit and BMI reduction. This is supported by the apparently causal negative relationship between type 2 diabetes and BMI seen in a reciprocal analysis where BMI is the outcome of interest (Online Appendix (Supplementary Results, Figure S3)), though is likely to be more a comment on study design than biological effect.

In this example, the use of recently derived methods (9; 11) designed to overcome problems caused by directional pleiotropy, yields estimates which are more stable in the presence or absence of potentially invalid instruments and confirm the likely magnitude of the average effect of BMI on type 2 diabetes (i.e. from the most likely and stable estimate, an elevation of odds of disease of ~26% for each additional unit of BMI). The comparison of results from different methods for any set of potential instruments is important when assessing the reliability of causal inferences and important for downstream interpretation. In this case, whilst it is impossible to model precisely, one can estimate the hypothetical impact of an average population level change in lifecourse BMI on type 2 diabetes. Given a population size of 64.1 million in the UK in mid 2013(30) and a modelled prevalence of type 2 diabetes (including non-diagnosed cases) of 7.4%(31; 32), the estimated reduction in odds for a 1kg/m2 reduction would potentially yield a reduction in the number of cases from ~4.7-3.6 million (a shift in prevalence to 5.6%).

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There are **no** conflicts to declare.

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## Tables

Table 1 – Estimates from the application of inverse-variance weighted, MR-Egger and weighted median Mendelian randomisation methodologies. Estimates represent the estimated causal effect of body mass index on type 2 diabetes.

| Method | Estimate | 95% CI | p-value |
|---|---|---|---|
| Complete variant set (n=96 SNPs) | | | |
| IVW | 1.20 | 1.09, 1.30 | $8.00 \times 10^{-05}$ |
| MR-Egger | 1.39 | 1.14, 1.68 | $1.53 \times 10^{-03}$ |
| MR-Egger$^{(\alpha)}$ | -0.019 | -0.041, 0.004 | 0.10 |
| Weighted median | 1.26 | 1.17, 1.34 | $5.26 \times 10^{-9}$ |
| *TCF7L2*(rs7903146) removed from the variant set (n=95 SNPs) | | | |
| IVW | 1.22 | 1.16, 1.28 | $1.49 \times 10^{-11}$ |
| MR-Egger | 1.34 | 1.17, 1.51 | $9.71 \times 10^{-06}$ |
| MR-Egger$^{(\alpha)}$ | -0.011 | -0.024, -0.024 | 0.13 |
| Weighted median | 1.26 | 1.19, 1.32 | $3.29 \times 10^{-10}$ |
| *FTO*(rs1558902) removed from the variant set (n=95 SNPs) | | | |
| IVW | 1.16 | 1.06, 1.27 | $1.31 \times 10^{-03}$ |
| MR-Egger | 1.30 | 1.01, 1.65 | 0.04 |
| MR-Egger$^{(\alpha)}$ | -0.012 | -0.038, 0.014 | 0.34 |
| Weighted median | 1.21 | 1.13, 1.28 | $6.81 \times 10^{-08}$ |

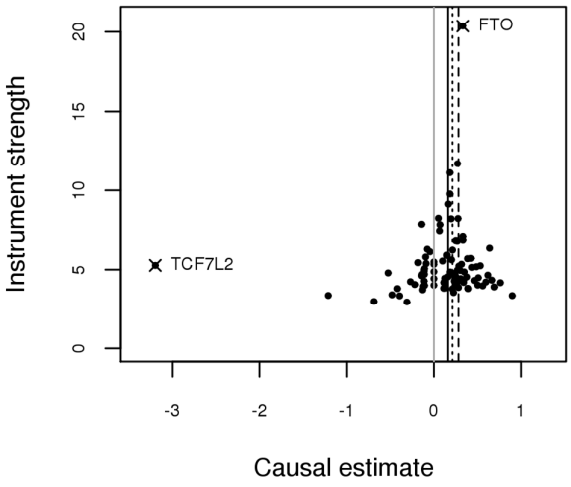
Intercept coefficients MR-Egger$^{(\alpha)}$ represent the average pleiotropic effect of a genetic variant on type 2 diabetes risk. "IVW" refers to inverse variance weighted estimates, SNP refers to single nucleotide polymorphism.

493 **Figures**

494 Figure 1 – Genetic associations with body mass index (BMI) and type 2 diabetes from 96 variants measured in GIANT (17) and DIAGRAM (18),
495 respectively. *TCF7L2*(rs7903146) and *FTO*(rs1558902) are marked with a 'X' and labelled.
496 A - funnel plot of minor allele frequency corrected genetic associations with BMI (interpreted as instrument strength) against causal estimates based
497 on each genetic variant individually, where the causal effect is expressed in logs odds ratio of type 2 diabetes for each unit increase in BMI. The
498 overall causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by inverse-variance weighted (solid black line), MR-Egger (dashed black
499 line) and weighted median (dotted black line) methods are also shown. Grey solid line represent x=0, that is a causal estimate of zero.
500 B - scatter plot of genetic associations with type 2 diabetes against associations with BMI, with causal estimates ($\beta$ coefficients) of BMI on type 2
501 diabetes estimated by inverse-variance weighted (solid line), MR-Egger (dashed line) and weighted median (dotted line) methods.
502
503 Figure 2 – Distributions of regression estimates resulting from leave-one-out permutation analysis. Solid line = estimate from main analysis (n=96
504 variants); dashed line = estimate with *TCF7L2*(rs7903146) removed; dotted line = estimate with *FTO*(rs1558902) removed.
505 A - Causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by an inverse-variance weighted method
506 B - Causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by MR-Egger
507 C - Causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by a weighted median method
508 D – Estimates of the intercept by MR-Egger
509

Figure 1

A


Instrument strength
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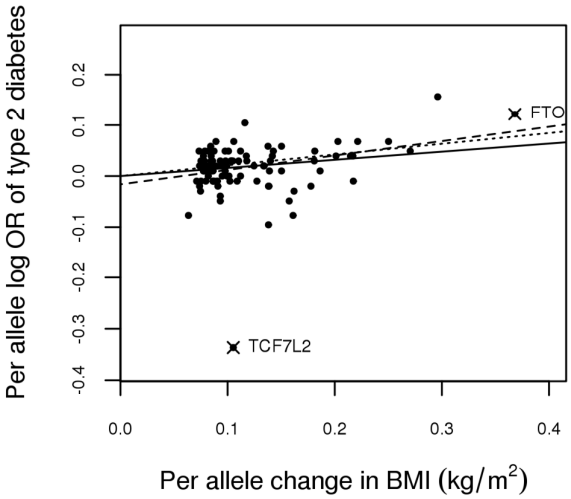
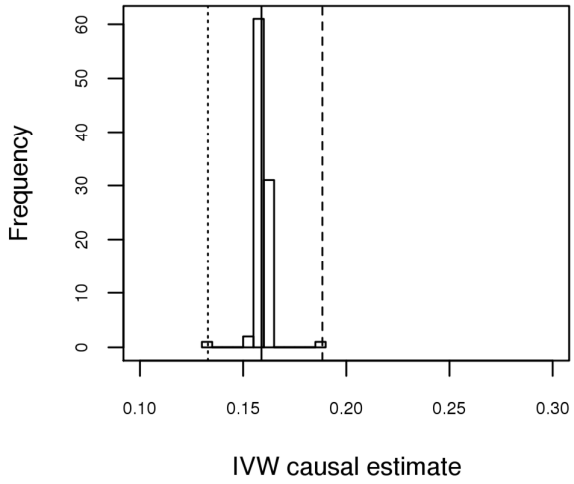
5
10
15
20
FTO
TCF7L2
-3
-2
-1
0
1
Causal estimate


Per allele log OR of type 2 diabetes
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
FTO
TCF7L2
0.0
0.1
0.2
0.3
0.4
Per allele change in BMI (kg/m$^2$)

B

Figure 2


Frequency
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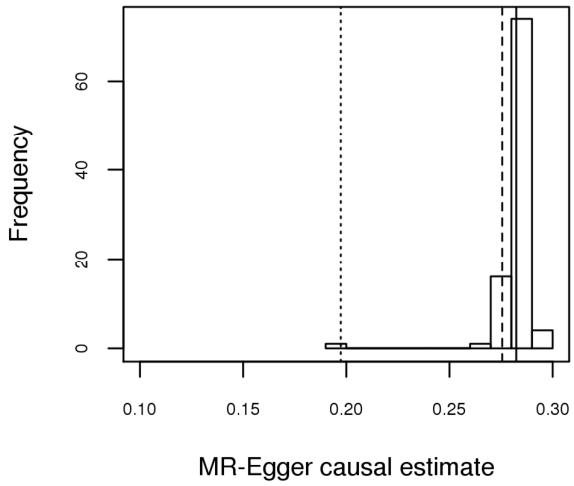
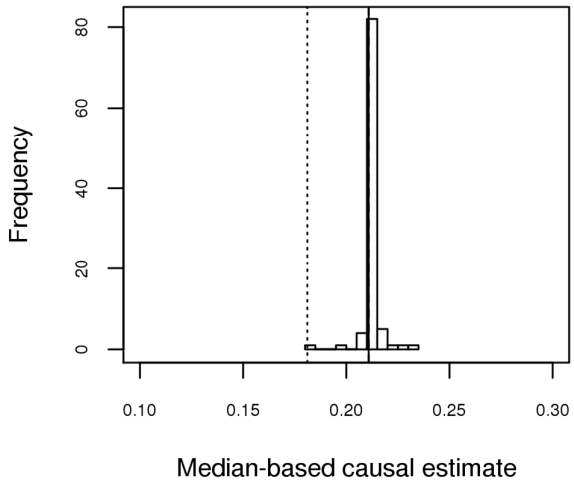
10
20
30
40
50
60
0.10
0.15
0.20
0.25
0.30
IVW causal estimate

A


Frequency
0
20
40
60
0.10
0.15
0.20
0.25
0.30
MR-Egger causal estimate

B


Frequency
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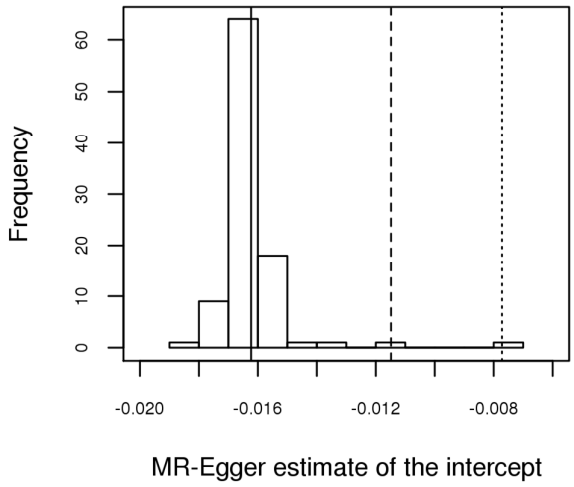
20
40
60
80
0.10
0.15
0.20
0.25
0.30
Median-based causal estimate

C


Frequency
0
10
20
30
40
50
60
-0.020
-0.016
-0.012
-0.008
MR-Egger estimate of the intercept

D

# Online Appendix

## Supplementary methods

### Mendelian randomization framework

Let $\hat{\Gamma}_j$ equal the gene-outcome association estimate for variant j = 1, . . ., J, with associated standard error $\sigma_{Yj}$. Let $\hat{\gamma}_j$ equal the gene-exposure association estimate for variant j, with associated standard error $\sigma_{Xj}$. Let the causal effect of the exposure on the outcome be denoted by $\beta$. An estimate for $\beta$ based on variant j alone can be obtained via the ratio method as

$$\hat{\beta}_j = \frac{\hat{\Gamma}_j}{\hat{\gamma}_j}$$

Two forms for the variance of $\hat{\beta}_j$ are often used:

(i) $Var(\hat{\beta}_j) = \frac{\sigma_{Yj}^2}{\hat{\gamma}_j^2}$

(ii) $Var(\hat{\beta}_j) = \frac{\sigma_{Yj}^2}{\hat{\gamma}_j^2} + \frac{\hat{\Gamma}_j^2 \sigma_{Xj}^2}{\hat{\gamma}_j^4}$,

Using either a first order (i) or second order (ii) Taylor series expansion. We use the variance from (i). This is equivalent to assuming that the gene-exposure association estimates are measured without error and is referred to as the No Measurement Error (NOME) assumption. NOME is equivalent to the assumption $\sigma_{Xj}^2 = 0$ for all j, so that $\hat{\gamma}_j = \gamma_j$ for all j.

### The inverse variance weighted (IVW) method for the overall causal effect estimate

Let $w_j = 1/\text{var}(\hat{\beta}_j)$ where $\text{var}(\hat{\beta}_j)$is defined as in (i) under NOME. The inverse variance weighted (IVW) estimate for the causal effect is given by the standard meta-analytic formula

$$\frac{\sum_j w_j \hat{\beta}_j}{\sum_j w_j}.$$

The $w_j$ terms derived under NOME are also referred to as ‘Toby Johnson’ weights. The IVW estimate assumes that all genetic variants satisfy the instrumental variable assumptions. If this is not true then it could give a biased estimate for $\beta$.The IVW estimate for $\beta$ is consistent even if all genetic variants are invalid, provided that:

- Across all variants, the magnitude of the gene exposure associations are independent of their pleiotropic effects (the InSIDE assumption)
- NOME is satisfied
- The pleiotropic effects have zero mean

### The weighted median method for the overall causal effect estimate

Let $\hat{\beta}_{(1)},\ldots,\hat{\beta}_{(J)}$ equal the J causal effect estimates ordered from smallest ($\hat{\beta}_{(1)}$) to largest ($\hat{\beta}_{(J)}$). Now define

$$w^*_{(j)} = \frac{w_j}{S_J}, \quad \text{where} \quad S_J = \sum_j w_j,$$

and equate $\hat{\beta}_{(j)}$ with a quantile, $p^w_{(j)}$, defined as

$$p^w_{(j)} = \frac{100}{S_J}\left(S_{(j)} - \frac{w_{(j)}}{2}\right).$$

$p^w_{(j)}$ represents the quantile from the weighted empirical distribution function of the ordered estimates $\hat{\beta}_{(1)},\ldots,\hat{\beta}_{(J)}$. The weighted median estimate, $\hat{\beta}_{WM}$ is defined as the 50th percentile of this weighted distribution. Typically the 50th percentile will lie between two estimates ($\hat{\beta}_{(l)}$ and $\hat{\beta}_{(m)}$, say), in which case $\hat{\beta}_{WM}$ is found by linear interpolation.

$\hat{\beta}_{WM}$ is a consistent estimate for $\beta$ provided that at least 50% of the `weight' making up $S_J$ comes from genetic variants that are valid instruments.

### The MR-Egger method for the overall causal effect estimate

The MR-Egger method performs a weighted linear regression of the gene-outcome coefficients on the gene-exposure coefficients:

$$\frac{\hat{\Gamma}_j}{\sigma_{Yj}} = \frac{\beta_{0E}}{\sigma_{Yj}} + \beta_{1E}\frac{\hat{\gamma}_j}{\sigma_{Yj}}$$

The weights used are also derived under the NOME assumption. If all genetic variants are valid instruments, then $\beta_{0E} = 0$. The value of $\hat{\beta}_{0E}$ can be interpreted as an estimate of the average pleiotropic effect across the genetic variants. An intercept term that differs from zero is indicative of overall directional pleiotropy. The MR-Egger estimate for $\beta$, $\hat{\beta}_{1E}$, is consistent even if all genetic variants are invalid, provided that:

- Across all variants, the magnitude of the gene exposure associations are independent of their pleiotropic effects (the InSIDE assumption)

- NOME is satisfied.

If NOME is violated then the MR-Egger estimate of causal effect will be attenuated towards the null. We can assess the strength of NOME violation for MR-Egger through the $I^2_{GX}$ statistic: $I^2_{GX} = \frac{Q-df}{Q}$, where $Q = \sum_{J=1}^{J} \frac{\left(\hat{\gamma}_j / \sigma^2_{\hat{Y}_j} - \bar{\gamma}\right)^2}{\sigma^2_{Xj} / \sigma^2_{\hat{Y}_j}}$ and where $\bar{\gamma}$ equals the arithmetic mean of the $\hat{Y}_j / \sigma^2_{\hat{Y}_j}$ terms . Specifically, the $I^2_{GX}$ statistic quantifies the proportion of the total variation between the $\hat{Y}_j / \sigma^2_{\hat{Y}_j}$ terms that is due to `true' variation between the $\gamma_j / \sigma^2_{\hat{Y}_j}$ terms.

Consequently, when NOME is satisfied $\hat{\gamma}_1, \ldots, \hat{\gamma}_J = \gamma_1, \ldots, \gamma_J$, $I^2_{GX}$ equals 1, and no attenuation occurs. When $I^2_{GX}$ = 0.9 we can expect the MR-Egger estimate to be only 90% of its value had NOME been satisfied. A crude correction for NOME violation would be $\frac{\hat{\beta}_{1E}}{I^2_{GX}}$ , however this can be unstable as $I^2_{GX}$ can sometimes be estimated as zero, even when it is truly large. We used the established method of Simulation Extrapolation (SIMEX) (1) instead, as implemented using the R package simex() (2). Under SIMEX, new data sets are created by simulating gene-exposure association estimates under increasing violations of NOME and recording the amount of attenuation in the estimate that occurs. The set of attenuated estimates are then used to extrapolate back to the estimate that would have been obtained if NOME had been satisfied.

## Supplementary Results

### Outlier analysis – Studentized residuals

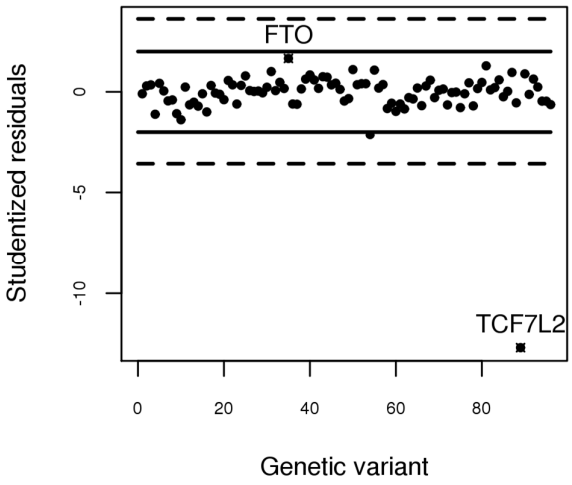



Figure S1A – Studentised residuals applied to the IVW method.

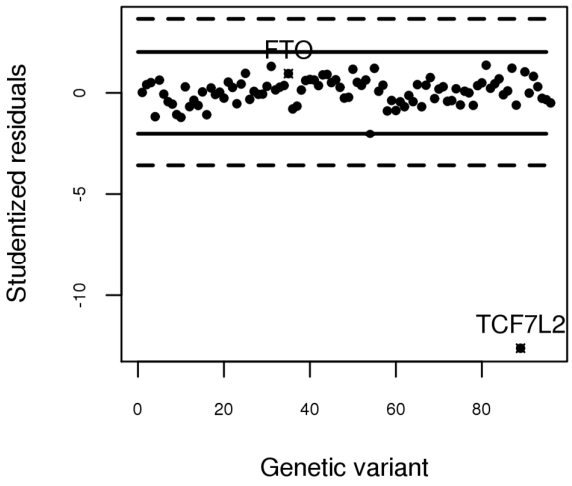



Figure S1B – Studentised residuals applied to the MR-Egger method.s

## Outlier analysis – Cook's distance

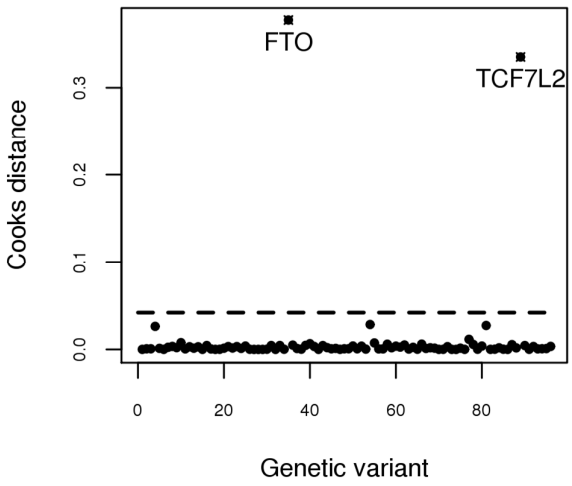



Figure S2A – Cook's distance applied to the IVW method.

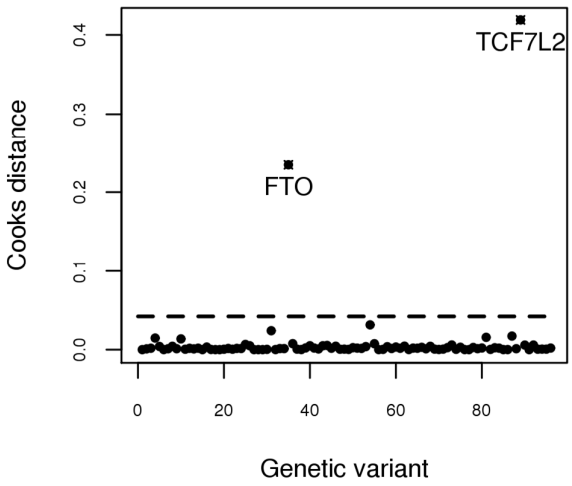



Figure S2B – Cook's distance applied to the MR-Egger method.

## Reciprocal analysis of type 2 diabetes and BMI

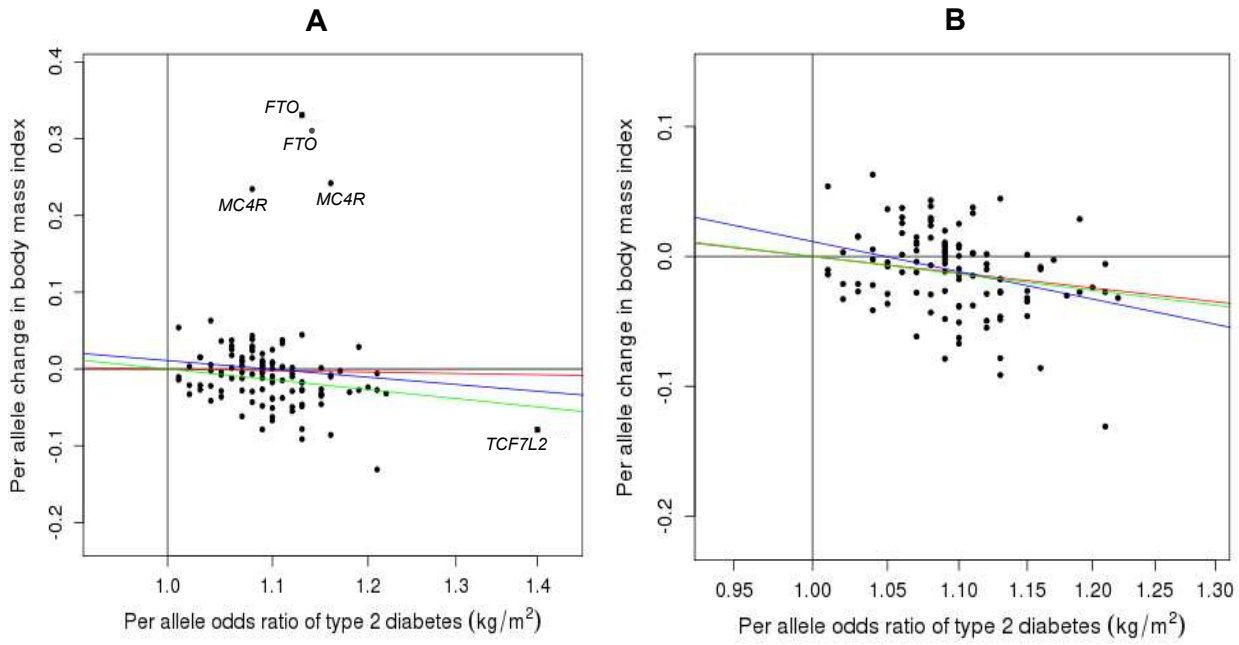



Figure S3 – MR-Egger analysis of the causal impact of type 2 diabetes on BMI.

A - scatter plot of genetic associations with BMI against associations with type 2 diabetes, with causal estimates ($\beta$ coefficients) of type 2 diabetes on BMI estimated by inverse-variance weighted (red line), MR-Egger (blue line) and median-based (green line) methods. For this analysis, all 115 confirmed type 2 diabetes associated loci with OR not equal to 1 from Morris et al (2012)(3) downloaded from DIAGRAM http://diagram-consortium.org/downloads.html) were used.
A - scatter plot of genetic associations with BMI against associations with type 2 diabetes, with causal estimates ($\beta$ coefficients) of type 2 diabetes on BMI estimated by inverse-variance weighted (red line), MR-Egger (blue line) and median-based (green line) methods. For this analysis, 110 confirmed type 2 diabetes associated loci with OR not equal to 1 and no overlapping known BMI loci (excluding *FTO*, *MC4R* and *TCF7L2*) from Morris et al (2012)(3) were again used.

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Title: Body mass index as a modifiable risk factor for type 2 diabetes: Refining and understanding causal estimates using Mendelian randomisation.

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Running title: Body mass index and type 2 diabetes; Mendelian randomisation methods

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**ABSTRACT**

This study focused on resolving the relationship between body mass index (BMI) and type 2 diabetes. The availability of multiple variants associated with BMI offers a new chance to resolve the true causal effect of BMI on T2D, however the properties of these associations and their validity as genetic instruments need to be considered alongside established and new methods for undertaking Mendelian randomisation. We explore the potential for pleiotropic genetic variants to generate bias, revise existing estimates and illustrate value in new analysis methods. A two-sample Mendelian randomisation (MR) approach with 96 genetic variants was employed using three different analysis methods, two of which (MR-Egger and the weighted median) have been developed specifically to address problems of invalid instrumental variables. We estimate an odds ratio for type 2 diabetes per unit increase in BMI (kg/m$^2$) of between 1.19 and 1.38, with the most stable estimate using all instruments and a weighted median approach (1.26 95%CI (1.17, 1.34)). *TCF7L2*(rs7903146) was identified as a complex effect or pleiotropic instrument and removal of this variant resulted in convergence of causal effect estimates from different causal analysis methods. This indicated the potential for pleiotropy to affect estimates and differences in performance of alternative analytical methods. In a real type 2 diabetes focused example, this study demonstrates the potential impact of invalid instruments on causal effect estimates and the potential for new approaches to mitigate the bias caused.


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Observational studies have shown body mass index (BMI) to be associated with risk of type 2 diabetes as well as with a range of diabetes-related metabolic traits (1; 2). However, it is well known that confounding, reverse causation and biases can generate such associations and that even with careful study design, incorrect inference is possible (3). One approach to circumventing these problems is to use genetic association results within a Mendelian randomization (MR) framework (3; 4). In MR analyses, genetic variants act as proxies for an exposure in a manner independent of confounders. If in addition the variants only affect an outcome of interest through the chosen exposure, then they are said to be valid instrumental variables (IVs). This enables evaluation of the causal effect of the exposure on the outcome, escaping some of the limitations of observational epidemiology; (5).

Following the success of genome-wide association studies (GWASs), the number of MR analyses using large numbers of mostly uncharacterized variants associated with complex health outcomes or intermediates is rapidly increasing (6; 7). In the case of BMI, there are now 97 genetic variants reliably associated and there are examples where multiple variants have been used as a composite IV to estimate the causal impact of BMI on health (8). Although using many IVs can increase the power of MR analyses, it brings with it the concern that enlarged sets of genetic variants are more likely to contain invalid IVs due to violations of the assumptions necessary for valid causal inference using traditional methods (9). In particular, horizontal pleiotropy – where a genetic variant affects the outcome via more than one biological pathway (10) – is a concern. Importantly, the properties of these associations and their validity as genetic instruments need to be considered alongside established and new methods for undertaking Mendelian randomisation.

In response to the general issue of using multiple genetic variants in MR, Bowden *et al.* (9) propose both MR-Egger regression, an approach developed from the original Egger regression technique for assessing small study bias in meta-analysis and a weighted weighted median approach (11) as alternatives to the standard MR analysis. The MR-Egger and weighted weighted median approaches both operate using distinct, but critically weaker, versions of the IV assumptions, and therefore have the potential to deliver robust causal effect estimates. The MR-

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Egger method also provides a formal statistical test as to whether or not the average pleiotropic effect of the genetic variants is equal to zero (9).

## Research Design and Methods

With increasing evidence for multiple biological pathways underlying type 2 diabetes (12; 13) and increasing numbers of genetic variants available as IVs for BMI, we set out to test the potential for bias in causal estimates from MR using these state-of-the-art approaches. We compared results from MR-Egger regression (9) and weighted weighted median (11) approaches to a traditional inverse-variance weighted (IVW) method (which makes the strong assumption that all variants are valid IVs) (14) in an investigation of the causal relationship between BMI and type 2 diabetes. These methods all undertake two-sample Mendelian randomisation whereby the GWAS results for a disease outcome are unified with those of an exposure of interest and together used to estimate the causal impact of that exposure on disease. We used published data in a two-sample analysis strategy taking SNP-exposure and SNP-outcome associations from different sources (15; 16).

The effect sizes for BMI-associated SNPs with associated standard errors from a mixed-sex cohort of European ancestry were taken from the Genetic Investigation of ANthropometric Traits (GIANT) consortium (17) along with results for type 2 diabetes from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. To avoid sample overlap, GIANT estimates were re-calculated in the absence of DIAGRAM cohorts yielding a maximum sample size at any given locus of 189,079. To aid interpretation of the effects of BMI on type 2 diabetes, effect sizes were transformed to BMI units prior to analysis, assuming one standard deviation (SD) = 4.5kg/m$^{2}$(17). For the corresponding SNP-outcome association, we took odds ratios (ORs) and confidence intervals from a GWAS meta-analysis conducted by the DIAGRAM Consortium. This genome-wide meta-analysis includes data from 12,171 type 2 diabetes cases and 56,862 controls of mainly European descent imputed at up to 2.5 million autosomal SNPs (DIAGRAMv3) (18). All but one (rs4787491, *INO80E*) of the BMI-associated SNPs ($p$<5x10$^{-8}$) from GIANT had results listed in the DIAGRAMv3 dataset so 96 SNPs with results in both datasets were taken forward for analysis.

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SNP-exposure and SNP-outcome associations were combined using the three different approaches outlined above. All analyses were conducted in R 3.2.0 (19). First, an inverse-variance weighted (IVW) method was implemented to provide a weighted average of the causal effect estimates (14). This method assumes that all genetic variants (i.e. 100%) satisfy the IV assumptions (including zero pleiotropy) and uses weights that assume the gene-exposure association estimates are measured without error (the No Measurement Error (NOME) assumption).

Second, we performed MR-Egger regression (9), which assumes NOME but allows each variant to exhibit pleiotropy. MR-Egger estimates remain consistent only if the magnitude of the gene exposure associations across all variants are independent of their pleiotropic effects (the InSIDE assumption) (9). As recommended by Bowden *et al* (9), the extent to which pleiotropy was balanced across the set of instruments as a whole was visually assessed by plotting the causal effect estimates against their precision, using a funnel plot and checking for asymmetry (Figure 1A). The NOME assumption was assessed for MR-Egger via an adaptation of the $I^2$ statistic ($I^2_{GX}$) (20) and adjusted for by combining MR-Egger with the method of Simulation Extrapolation (SIMEX) (21). Using SIMEX, new data sets are created by simulating gene-exposure association estimates under increasing violations of NOME and recording the amount of attenuation in the estimate that occurs. The set of attenuated estimates are then used to extrapolate back to the estimate that would have been obtained if NOME had been satisfied.

Finally, a weighted weighted median estimation method was applied (11). The weighted median provides a consistent estimate of causal effect if at least 50% of the information in the analysis comes from variants that are valid IVs. For a more detailed description of the three methods applied, see Online Appendix (Supplementary methods). A leave-one-out permutation analysis was conducted across all methods to assess the influence of potentially pleiotropic SNPs on the causal estimates (22). In the case of the linear models (IVW and MR-Egger) two additional analyses were conducted (23; 24). Firstly, the extent to which the causal estimate from each SNP

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in the set could be considered an outlier was assessed using studentized residuals. Secondly, Cook's distance (25) was used as a measure of the aggregate impact of each SNP on the model.

## Results

All three approaches provide evidence of a positive causal relationship between BMI and type 2 diabetes. This is demonstrated in Figure 1B where the slope of the lines show the causal effect estimates as predicted by the IVW, MR-Egger and m weighted median approaches. Estimates correspond to an OR for type 2 diabetes per unit increase in BMI (kg/m$^2$) of 1.19, 1.26 and 1.38 for the IVW, weighted median and MR-Egger analyses, respectively and are in line with a previous MR estimate of 1.27 (95%CI 1.18, 1.36) (2) (Table 1). Assessment of the NOME assumption with respect to the MR-Egger estimate gave $I^2_{GX}$=0.83, suggesting an approximate 15% attenuation of the causal estimate towards zero. Bias adjustment via SIMEX gave a corrected MR-Egger causal estimate of 1.46 (95%CI 1.16, 1.84) for type 2 diabetes per unit increase in BMI (kg/m$^2$).

Considering the individual SNP-based contributions to MR analysis, there is one clear outlier in the distribution of effects shown in Figure 1 and that is *TCF7L2*(rs7903146). *TCF7L2*(rs7903146) shows an association with BMI that is in the opposite direction to the overall trend (and weak relative to its effect on type 2 diabetes), resulting in a large negative causal estimate from this SNP alone. The presence of at least some unbalanced pleiotropy was detected within the set of variants, as reflected by the intercept estimate of -0.019 ($p$=0.10) in the MR-Egger analysis.

To illustrate the impact of *TCF7L2*(rs7903146) on causal estimates, we performed a sensitivity analysis in which each SNP in turn was removed from the set in a leave-one-out permutation analysis. We saw a shift in the causal estimates from the IVW (an increase) and MR-Egger (a decrease) as a result of the removal of *TCF7L2*(rs7903146) but no difference in the estimate from the weighted median approach (Table 1; Figure 2). The results of the leave-one-out permutation analysis showed that the impact of removing *TCF7L2*(rs7903146) from the variant set on the IVW and MR-Egger estimates was greater than that of removing almost any other variant, with the exception of *FTO*(rs1558902) (Figure 2A & B). When *FTO*(rs1558902) was removed, causal

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estimates from both the IVW and MR-Egger analysis decreased (Table 1; Figure 2). In this instance we also observed movement in the causal effect estimate from the weighted median (Table 1; Figure 2C). The estimate of the intercept from MR-Egger moved closer to zero following both the removal of *TCF7L2*(rs7903146) and *FTO*(rs1558902) (Figure 2D). *TCF7L2*(rs7903146) was also identified as an outlier in both IVW and MR-Egger (studentized residuals, Bonferroni corrected $p<1x10^{-19}$) but *FTO*(rs1558902) was not (Online Appendix (Supplementary Results, Figures S1A/B)). Calculation of Cook's distance showed both variants to have a disproportionate level of influence on the model compared to other variants in the set (Online Appendix (Supplementary Results, Figures S2A/B)).

These results suggest *TCF7L2*(rs7903146) may be pleiotropic with respect to the outcome, i.e. that it influences type 2 diabetes through an alternative pathway (other than BMI). Evidence from existing literature supports this assertion as the type 2 diabetes risk increasing allele at *TCF7L2*(rs7903146) has been associated with both increased fasting glucose (26) and decreased BMI (17). Under the assumption that *TCF7L2*(rs7903146) demonstrates horizontal pleiotropy with respect to type 2 diabetes, we would expect its inclusion in the variant set to bias the causal estimate predicted by the IVW approach, but not that predicted by MR-Egger or the weighted median. Removing *TCF7L2*(rs7903146) from the variant set causes a slight shift in the causal estimates from the IVW and MR-Egger approaches, bringing them more in line with one another and also with the weighted median estimate which remained stable in this instance. Also of note is the reduction in the 95% confidence interval of the MR-Egger estimate following removal of the *TCF7L2*(rs7903146). This increase in precision following removal of a likely invalid instrument from the set is another potentially favourable quality of this estimator. The relatively small changes observed across all methods as a result of removing *TCF7L2*(rs7903146) are in line with the relatively weak effect of the SNP as shown in Figure 1B.

In contrast, the effect of removing *FTO*(rs1558902) is more noticeable. Regardless of the method used, removing this variant results in a lower causal estimate (Table 1; Figure 2). The substantial influence of *FTO*(rs1558902) was predicable given the strength of its effect relative to the other

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variants (Figure 1B), though properties of this effect are not in line with other variants used to instrument BMI as reported elsewhere for physical activity (27), thyroid function (28) and depression (29). The concomitant increase in standard error associated with the estimates here point towards increased uncertainty moving the estimates towards the null in the absence of *FTO*(rs1558902). The weighted median appears robust, even to the removal of *FTO*(rs1558902), as demonstrated by the relatively tight distribution of estimates returned from the leave-one-out permutation analysis (Figure 2C). This is as expected given the tolerance of weighted median approaches to outliers.

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## Discussion

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By applying new analytical techniques to an old question – the causal relationship between BMI and type 2 diabetes – we have explored the potential for invalid instruments to bias causal estimates in MR. In this case where BMI is the exposure, the opportunity to use a large instrument list in causal analyses presents both opportunity, through variance explained, but also cost, through complications generated by instrument properties or methods employed. Results here suggest that both *TCF7L2* and *FTO* appear to have genetic variation which predicts BMI reliably, but for which associations with type 2 diabetes do not fully align with that for other variants (given BMI effects and assumed causality).

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For *TCF7L2*, only recently suggested to be associated with BMI directly (17), this is not surprising and reinforces the important point that the validity of a specific method's MR estimate depends on whether the genetic variants collectively satisfy its assumptions. In this case, it is possible that the negative association with BMI observed in GIANT is the product of a form of bias where the risk of type 2 diabetes is leading to effective treatment, health benefit and BMI reduction. This is supported by the apparently causal negative relationship between type 2 diabetes and BMI seen in a reciprocal analysis where BMI is the outcome of interest (Online Appendix (Supplementary Results, Figure S3)), though is likely to be more a comment on study design than biological effect.

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In this example, the use of recently derived methods (9; 11) designed to overcome problems caused by directional pleiotropy, yields estimates which are more stable in the presence or absence of potentially invalid instruments and confirm the likely magnitude of the average effect of BMI on type 2 diabetes (i.e. from the most likely and stable estimate, an elevation of odds of disease of ~26% for each additional unit of BMI). The comparison of results from different methods for any set of potential instruments is important when assessing the reliability of causal inferences and important for downstream interpretation. In this case, whilst it is impossible to model precisely, one can estimate the hypothetical impact of an average population level change in lifecourse BMI on type 2 diabetes. Given a population size of 64.1 million in the UK in mid 2013(30) and a modelled prevalence of type 2 diabetes (including non-diagnosed cases) of 7.4%(31; 32), the estimated reduction in odds for a 1kg/m2 reduction would potentially yield a reduction in the number of cases from ~4.7-3.6 million (a shift in prevalence to 5.6%).

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**Acknowledgements**

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**Author contributions:** NJT conceived and supervised the study, in discussion with GDS, LJC, KHW and RCR. JB and SB contributed to method and script development. LJC conducted the analysis and wrote the manuscript. JB prepared the (Online Appendix (Supplementary methods)). NJT is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**GIANT:** Data on BMI have been contributed by GIANT (Genetic Investigation of ANthropometric Traits). With special thanks to Adam Locke for conducting the additional analyses required for this work.

**DIAGRAM:** Data on type 2 diabetes have been contributed by the DIAGRAM (**DIA**betes **G**enetics **R**eplication **A**nd **M**eta-analysis) consortium and were downloaded from here.

There are **no** conflicts to declare.

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**Tables**

Table 1 – Estimates from the application of inverse-variance weighted, MR-Egger and weighted median Mendelian randomisation methodologies. Estimates represent the estimated causal effect of body mass index on type 2 diabetes.

| Method | Estimate | 95% CI | p-value |
|---|---|---|---|
| Complete variant set (n=96 SNPs) | | | |
| IVW | 1.20 | 1.09, 1.30 | $8.00 \times 10^{-05}$ |
| MR-Egger | 1.39 | 1.14, 1.68 | $1.53 \times 10^{-03}$ |
| MR-Egger(,) | -0.019 | -0.041, 0.004 | 0.10 |
| Weighted median | 1.26 | 1.17, 1.34 | $5.26 \times 10^{-9}$ |
| *TCF7L2*(rs7903146) removed from the variant set (n=95 SNPs) | | | |
| IVW | 1.22 | 1.16, 1.28 | $1.49 \times 10^{-11}$ |
| MR-Egger | 1.34 | 1.17, 1.51 | $9.71 \times 10^{-06}$ |
| MR-Egger(,) | -0.011 | -0.024, -0.024 | 0.13 |
| Weighted median | 1.26 | 1.19, 1.32 | $3.29 \times 10^{-10}$ |
| *FTO*(rs1558902) removed from the variant set (n=95 SNPs) | | | |
| IVW | 1.16 | 1.06, 1.27 | $1.31 \times 10^{-03}$ |
| MR-Egger | 1.30 | 1.01, 1.65 | 0.04 |
| MR-Egger(,) | -0.012 | -0.038, 0.014 | 0.34 |
| Weighted median | 1.21 | 1.13, 1.28 | $6.81 \times 10^{-08}$ |

Intercept coefficients MR-Egger(,) represent the average pleiotropic effect of a genetic variant on type 2 diabetes risk. "IVW" refers to inverse variance weighted estimates, SNP refers to single nucleotide polymorphism.

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**Figures**

Figure 1 – Genetic associations with body mass index (BMI) and type 2 diabetes from 96 variants measured in GIANT (17) and DIAGRAM (18), respectively. *TCF7L2*(rs7903146) and *FTO*(rs1558902) are marked with a 'X' and labelled.

A - funnel plot of minor allele frequency corrected genetic associations with BMI (interpreted as instrument strength) against causal estimates based on each genetic variant individually, where the causal effect is expressed in logs odds ratio of type 2 diabetes for each unit increase in BMI. The overall causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by inverse-variance weighted (solid black line), MR-Egger (dashed black line) and weighted median (dotted black line) methods are also shown. Grey solid line represent x=0, that is a causal estimate of zero.

B - scatter plot of genetic associations with type 2 diabetes against associations with BMI, with causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by inverse-variance weighted (solid line), MR-Egger (dashed line) and weighted median (dotted line) methods.

Figure 2 – Distributions of regression estimates resulting from leave-one-out permutation analysis. Solid line = estimate from main analysis (n=96 variants); dashed line = estimate with *TCF7L2*(rs7903146) removed; dotted line = estimate with *FTO*(rs1558902) removed.

A - Causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by an inverse-variance weighted method

B - Causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by MR-Egger

C - Causal estimates ($\beta$ coefficients) of BMI on type 2 diabetes estimated by a weighted median method

D – Estimates of the intercept by MR-Egger

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A genetic instrument for BMI was constructed using