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Do genetic risk scores for body mass index predict risk of phobic anxiety? Evidence for a shared genetic risk factor

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Abstract

Background—Obesity and anxiety are often linked but the direction of effects is not clear.

Methods—Using genetic instrumental variable (IV) analyses in a sample of 5911 female participants from the Nurses' Health Study (NHS, initiated in 1976) and 3697 male participants from the Health Professional Follow-up Study (HPFS, initiated in 1986), we aim to determine whether obesity increases symptoms of phobic anxiety. FTO, MC4R, and a genetic risk score (GRS) based on 32 single nucleotide polymorphisms that significantly predict body mass index (BMI), were used as instrumental variables. "Functional" GRS corresponding with specific biological pathways that shape BMI (adipogenesis, appetite, and cardio-pulmonary), were considered. Phobic anxiety as measured by the Crown Crisp Experimental Index (CCI) in 2004 in NHS and 2000 in HPFS was the main outcome.

Results—In observational analysis, a one unit higher BMI was associated with higher phobic anxiety symptoms (women NHS: beta=0.05; 95% Confidence Interval (CI): 0.030 – 0.068 and men, HPFS, beta = 0.04; 95% CI: 0.016 – 0.071). IV analyses showed that BMI instrumented by FTO was associated with higher phobic anxiety symptoms (p = 0.005) but BMI instrumented by GRS was not (p=0.256). Functional GRS scores showed heterogeneous, non-significant effects of BMI on phobic anxiety symptoms.

Conclusions—Our findings do not provide conclusive evidence in favor of the hypothesis that higher BMI leads to higher levels of phobic anxiety, but rather suggest that genes that influence

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Conflict of Interest:

There is no conflict of interest by any of the authors of this paper.

obesity, in particular FTO, may have direct effects on phobic anxiety, i.e., that obesity and phobic anxiety may share common genetic determinants.

Keywords

Genetics; Mendelian Randomization Analysis; Anxiety; Phobic Anxiety; Body Mass Index; FTO

Introduction

Although anxiety disorders pose a tremendous public health burden (Kessler *et al.*, 2009), their etiology is not well understood. Obesity is hypothesized to have adverse mental health consequences. (Bjerkeset *et al.*, 2008, Bodenlos *et al.*, 2011) Possible pathways include stigmatization and discrimination processes (Cairney *et al.*, 2008), as well as the negative health consequences associated to obesity that impact quality of life and cause distress. (Sareen *et al.*, 2005) Direct negative health consequences of obesity on brain function might act through inflammation processes and deregulation of the hypothalamic–pituitary–adrenal (HPA) axis. (Nousen *et al.*, 2013) Most, but not all, research reporting that obesity or body mass index (BMI) predict worse anxiety symptoms and higher risk of anxiety disorder. (Garipey *et al.*, 2010) These non-experimental studies are inconclusive, however, because of potential confounding from unobserved common causes of adiposity and anxiety or reverse causation from anxiety to weight gain.

To address these limitations, the “Mendelian Randomization” study design uses genetic polymorphisms that increase adiposity to mimic a “natural experiment”. Because genetic information is determined at conception, this approach, also sometimes called “genetic instrumental variables (IV)”, avoids confounding and reverse causation. The genetic IV approach can be used to identify the effect of BMI on anxiety symptoms, provided the genes influencing BMI do not have direct effects on anxiety; to date no genetic determinants for anxiety have been conclusively established. (Smoller, 2011) Any genetic polymorphisms that directly influence anxiety are not valid candidates for the genetic IV analysis, but due to the paucity of evidence on the genetics of anxiety, such a polymorphism would be of compelling interest.

Previous investigations have used a genetic IV approach to evaluate the relationship between adiposity and common mental disorders. (Jokela *et al.*, 2012, Kivimaki *et al.*, 2011, Lawlor *et al.*, 2011)” No prior study has used a genetic IV to estimate the effects of BMI on anxiety symptoms specifically. We focus here on phobic anxiety; prior work from family and twin studies suggests that there is significant shared genetic risk across all forms of anxiety disorders. (Hettema *et al.*, 2001, Hettema *et al.*, 2005, Smoller *et al.*, 2008)

We constructed a polygenic score for BMI based on findings from previously published meta-analyses of genetic determinants of BMI in over 250,000 participants. (Speliotes *et al.*, 2010) We used this score to conduct genetic IV analyses and estimate the effects of BMI on symptoms of phobic anxiety, based on women in the Nurses’ Health Study (NHS) and men in the Health Professionals Follow-Up Study (HPFS) cohorts. We also estimated the conventional observational prospective association between BMI and symptoms of phobic anxiety, but because of the potential for confounding and reverse causation, we anticipated

that this effect estimate would be biased upwards. We further compared IV and conventional effect estimates to evaluate the bias of the conventional effect estimate. We assessed the validity of the genetic IV approach by evaluating whether there was evidence that genotypes associated with BMI directly influenced symptoms of phobic anxiety, independently of the BMI pathway. Finally, we also evaluated the assumptions inherent to genetic IV analysis by examining the possibility that the effects of BMI on symptoms of phobic anxiety differ depending on the physiologic mechanism linking the genetic polymorphisms and BMI.

Methods and Materials

Population

All data are drawn from 7 nested case-control GWAS within the NHS and HPFS cohorts.

Nurses' Health Study (NHS)—The NHS was established in 1976 when 121,700 female registered nurses aged 30–55 years and residing in 11 large U.S. states completed a mailed questionnaire on medical history and lifestyle characteristics. (Colditz and Hankinson, 2005) Blood was collected from 32,826 participants between 1989 and 1990. DNA was extracted from white blood cells using the QIAmp™ (QIAGEN Inc., Chatsworth, CA) blood protocol and all samples were processed in the same laboratory. Genome-wide scans were obtained from 4 independent GWAS of the cohort, initially designed to examine type 2 diabetes (T2D, n=3286), coronary heart disease (CHD, n=1146), breast cancer (BrCa, n=2287) and kidney stone (KS, n=504) disease.

Health Professionals Follow-up Study (HPFS)—The HPFS was initiated in 1986 when 51,529 male health professionals between ages 40 and 75 years and residing in the U.S. completed a questionnaire on lifestyle and medical history. Participants have been followed with repeated questionnaires on lifestyle and health every 2 years.

Between 1993 and 1996, a blood sample was requested from all active participants and collected from 18,225 men. (Chu *et al.*, 2001) DNA was extracted from white blood cells using the QIAmp™ (QIAGEN Inc., Chatsworth, CA) blood protocol; all samples were processed in the same laboratory. Genome-wide scans were obtained from 3 independent GWAS of the cohort, initially designed to examine T2D (n=2487), CHD (n=1313), and KS (n=553) disease.

Exact genotyping, quality control (QC), and imputation protocols varied by sample set (Supplementary Tables S1 and S2). Principal components analyses were conducted to exclude self-reported white individuals that had substantial similarity to non-European reference samples. (Price *et al.*, 2006) Each study imputed up to 2.5 million autosomal SNPs with NCBI build 36 of Phase II HapMap CEU data (release 22) as the reference panel using MACH. Imputation results summarized as allele calls (0,1,2) were used for analysis.

Considering QC and available information on phobic anxiety a total of 5911 genotyped participants were available from NHS (NHS T2D = 2628, NHS CHD = 883, NHS BrCa = 1931, NHS KS = 469) and 3697 participants from HPFS (T2D = 2055, CHD = 1113, KS = 529).

Ethics Statement

The NHS and HPFS were approved by the Human Subjects Committee of Brigham and Women's Hospital, Boston, MA. All participants in this study provided written informed consent. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Genetic Instrument

The genetic IVs were based on 32 SNPs established as genome-wide significant predictors of BMI, based on the largest currently available meta-analysis. (Speliotes et al., 2010) First, we examined the two individual genes previously used in genetic IV analyses for the effect of adiposity on mental health: fat mass and obesity associated gene (FTO), rs1558902, risk allele A and melanocortin 4 receptor gene (MC4R), rs571312, risk allele A." (Kivimaki et al., 2011, Lawlor et al., 2011). For each polymorphism, we constructed an IV as the sum of the number of risk alleles multiplied by the estimated effect of the risk allele on BMI. The estimated effect was based on the beta-estimates from the GWAS meta-analysis. (Speliotes et al., 2010)

Next, we combined information on all 32 SNPs (including polymorphisms in FTO and MC4R) confirmed as genome-wide significant predictors of BMI to construct a Genetic Risk Score (GRS). We calculated the GRS for each individual in our study sample as the sum of risk alleles, with each SNP weighted by the beta estimate from the previous meta-analysis. (Purcell *et al.*, 2009, Speliotes et al., 2010) The beta weights used for each polymorphism are shown in supplementary Table S3.

This GRS provides the most powerful genetic IV available as it combines the strongest known genetic predictors of BMI. We also constructed a GRS without including FTO (GRS_exFTO) because of recent evidence suggesting a direct pathway from FTO to depression which is highly comorbid with anxiety. (Samaan et al., 2013)

We performed a search in PubMed and Google Scholar databases for peer-reviewed publications identifying the functions of the 32 SNPs established as genome-wide significant predictors of BMI; the NCBI Gene database was used as additional reference if published journal articles regarding SNP functions were not available. After SNP functions were identified to the extent possible, SNPs were categorized by key words and overall function into the following 3 mechanistic domains through which they likely influence BMI: appetite, adipogenesis, and cardio-pulmonary function. SNPs that could not be categorized by these known domains were grouped into a category identified as other/unknown. We then created a GRS for each domain, based on the above protocol but restricting only to SNPs associated with the respective mechanisms (detailed in Supplementary Table 3).

Phenotype

BMI was calculated as kg/m^2 . Height was self-reported as part of the baseline questionnaire (NHS: 1976, HPFS: 1988). Weight in pounds was self-reported at each biennial interview, converted to kg and summarized as the mean of all measurements prior to the assessment of

anxiety in 2004 for NHS and 2000 for HPFS (NHS: 14 measurements between 1976 and 2002; HPFS: 6 measurements between 1988 and 1998). In prior work with these cohorts, self-reported weight was validated by study staff in a subsample of 263 men and women and was highly correlated to measured weight in NHS and HPFS ($r=0.97$). (Rimm *et al.*, 1990)

Outcome

Symptoms of phobic anxiety were assessed in men in the year 2000 and in women in 2004 using the validated phobic anxiety subscale of the Crown Crisp Experimental Index (CCI). Previous research demonstrates that the CCI discriminates individuals with diagnosable anxiety disorders from healthy individuals (Crown and Crisp, 1966), correlates reasonably with other measures of anxiety from the Middlesex Hospital Questionnaire (free-floating anxiety $\rho = 0.48$, obsessional $\rho = 0.45$, somatic $\rho = 0.42$, depressive $\rho = 0.27$) (Crown and Crisp, 1966), has high intra-class correlation in monozygotic twins (0.60) and high heritability (0.64) (Young *et al.*, 1971), and is associated with heart disease in both men and women. (Albert *et al.*, 2005, Haines *et al.*, 1987, Kawachi *et al.*, 1994). The measure continues to be used in ongoing research to evaluate the effectiveness of cognitive behavior therapy (Irvine *et al.*, 2011) and in evaluations examining the relation of phobic anxiety with a range of outcomes, including child behavior problems (Leis *et al.*, 2014) and health related quality of life (Oldroyd *et al.*, 2013).

The scale includes 8 questions assessing fear of crowds, heights, enclosed spaces, going out alone, and worrying; items have two or three response options (see Supplementary Table S4 for a detailed description of the items). Following prior work with this measure (Albert *et al.*, 2005) items with 3 response options were scored as no (0), moderate (1), or high (2); items with 2 response options were coded as no symptoms (0), or high (2) symptom level. To create the outcome for our analyses, we summed across items, resulting in an overall continuous score ranging from 0 to 16 with higher scores indicating higher levels of phobic anxiety. For those with missing items, the total score was divided by the fraction of questions answered and then rounded to the nearest whole number, so the possible range of total scores was consistent across all individuals. The score ranged from 0 – 15 in men and 0 – 16 in women. For comparison to other studies we report proportions for a cut-off of 4 or higher, primarily to indicate individuals higher on the spectrum of phobic anxiety symptoms.

Analyses

We use Chi-Square and ANOVA for bivariate analyses. For all linear regression models, we applied weights based on the inverse of the probability that each individual was selected from the original cohort into the genotyped samples, to reconstruct the original cohort from the nested case-control genetic samples and thereby circumvent the bias of selective sampling in this population. (Bowden and Vansteelandt, 2011, Tchetgen Tchetgen *et al.*, 2013). For comparison with the primary IV models, we first report conventional, prospective, observational linear regression estimates (b_{OLS}) of the effects of self-reported average BMI (prior to when phobic anxiety was assessed) on symptoms of phobic anxiety.

IV analyses estimate the effect of BMI on symptoms of phobic anxiety under a set of assumptions illustrated in the causal diagram in Figure 1 (Glymour et al., 2012) (VanderWeele, 2012): 1) the genetic IV is associated with BMI; 2) there are no unmeasured common causes of the genetic IV and symptoms of phobic anxiety; and 3) every pathway originating from the genetic IV influences symptoms of phobic anxiety *only* through its influence on BMI. Under these assumptions, an individual's genotype can be used to estimate the effect of BMI on symptoms of phobic anxiety, at the same time establishing an (indirect) genetic risk factor. If, however, the genotype has a direct effect on symptoms of phobic anxiety, not mediated by BMI, the IV assumptions are not met and the IV based effect estimate is biased away from the causal effect of BMI on symptoms of phobic anxiety.

The IV analysis was executed using split-sample IV analyses, which is preferable to conventional two-stage least squares models because split-sample approaches avoid the potential for weak-instruments bias. (Angrist and Krueger, 1994, Angrist and Krueger, 1995) In our split-sample IV model, we use 1st stage estimates from a previous meta-analysis of the genetic determinants of BMI. This is accomplished by applying the meta-analyzed GWAS beta-weights from Speliotes et al. (Speliotes et al., 2010) when constructing the genetic IVs, as described above. In the 2nd stage of the split-sample IV, we regress the continuous phobic anxiety symptom score on the genetic IV, using a linear model with inverse probability weights (IPW) to correctly reweight the case-control samples to the respective source population from NHS or HPFS. Under the IV assumptions encoded in Figure 1, the coefficients (b_{IV}) in this 2nd stage model are interpretable as the effect of BMI on the continuous phobic anxiety symptom score. More specifically, under the IV assumptions, the resulting effect estimate may be interpreted as the causal effect of a unit increase in BMI on phobic anxiety. This interpretation is premised on the assumption that BMI is a meaningful exposure, and the effect of a unit increase in BMI is the same regardless of the mechanism via which BMI was changed. Because of prior literature calling into question the phenotype of BMI, we also estimate separate IV models for each of the mechanism-related GRS IVs (appetite, adipogenesis, cardio-pulmonary function, and other/unknown) to validate the IV model.

For inference, sandwich estimators were used to account for heteroskedasticity and the IPW weights. All analyses were adjusted for age and age-squared at the time of anxiety assessment, and the first three genetic eigenvectors to control for population substructure unless otherwise indicated. Because this study uses data from different, sex-specific cohorts, we present sex specific results and gender-pooled results using meta-analysis. Results were based on two sided tests and $p < 0.05$ was considered statistically significant.

To test the necessity of IV models we used Wu-Hausman tests. These tests contrast the estimate obtained from observational linear regression to the IV estimates. If there is no confounding of the observed association between BMI and phobic anxiety, both the observational estimate and the IV estimate are consistent, but the IV estimate is less efficient. If the Wu-Hausman test is rejected, it is typically interpreted as evidence that the observational estimate is biased and the IV estimate should be preferred. We used two approaches to evaluate the IV assumptions. First, we used the mechanism-related categories

of genetic risk scores to conduct over-identification tests, by testing the null that the IV estimates from each of the genetic risk scores were identical. Second, we assessed whether the association between the genetic IVs and the continuous phobic anxiety symptom score was attenuated by adjustment for BMI; as would be expected if BMI mediates the effect of the genes on anxiety.

Results

Consistent with prior findings in nationally representative samples (Kessler et al., 2005), the average phobic anxiety symptom score was 43% higher in women than men (Table 1). Although early work with the CCI among psychiatric patients suggested a mean score of 9 to discriminate phobic disorders from other diagnostic disorders (Crisp *et al.*, 1978, Mavissakalian and Michelson, 1981), in healthier (non-psychiatric) populations a cut-off of 4 or higher has been used to indicate individuals higher on the spectrum of phobic anxiety symptoms. When applying this criterion, we obtained a prevalence of phobic anxiety symptoms of 18.2% for men and 30.5% for women slightly lower than the national average reported by Kessler et al. (Kessler *et al.*, 2012). Among men, the first (1986) and last (1998) measures of BMI taken prior to anxiety assessment were correlated at $r = 0.84$. (Cronbach's alpha using the six assessments = 0.91); in women, the first (1976) and last measures of BMI (2002) were correlated with $r = 0.68$ (Cronbach's alpha using the 14 assessments = 0.81).

In observational analysis, average self-reported BMI predicted higher average anxiety symptom score among both men (beta per unit increase in BMI: 0.044 units; 95% Confidence Interval (CI): 0.016, 0.071) and women (beta=0.049; 95% CI: 0.030, 0.068). In the meta-analysis combining results from men and women, the effect estimate was 0.047, 95% CI (0.032, 0.063).

The genetic instrument based on FTO was strongly associated with average self-reported BMI in men ($F=17.11$) and women ($F=10.30$) and explained 0.85% and 0.75% of the variance in BMI respectively (Table 2). MC4R predicted BMI in men ($F=12.2$, $R^2=0.02\%$) but not women ($F=3.43$, $R^2=0.09\%$). The GRS was strongly associated with BMI in both men ($F=18.73$, $R^2=1.07\%$) and women ($F=31.48$, $R^2=2.73\%$). When the GRS was recalculated excluding FTO (the strongest single SNP) the overall score remained strongly associated with BMI.

Table 2 also shows the results from the IV analyses. The IV effects derived using FTO as an instrument were large and statistically significant among men ($b_{IV,men} = 0.32$, 95% CI = 0.00, 0.64), women ($b_{IV,women} = 0.31$, 95% CI = 0.01 – 0.62) and when pooling male and female samples ($b_{IV} = 0.32$, 95% CI = 0.10, 0.54). The FTO-based IV effect estimates were substantially larger than the corresponding observational effect estimates. The IV effect estimate based on the GRS was more similar to the observational effect estimate ($b_{IV} = 0.060$; 95% CI: -0.04, 0.16) and not statistically significant ($p=0.26$). The IV effect estimate based on GRS excluding FTO was close to the null ($b_{IV} = -0.03$, 95% CI = -0.15, 0.10). For each of the IV effect estimates, we performed Wu-Hausman tests comparing the IV estimate to the estimate obtained from observational linear regression models. This test indicated that

IV effect estimates based on FTO alleles in the meta-analyzed sample of men and women differed significantly from the observational effect estimate ($p = 0.02$, Table 2). None of the other IV effect estimates were significantly different from the conventional observational effect estimate ($p < .05$ for all, Table 2).

Testing Assumptions

For each of the IV effect estimates, we performed Wu-Hausman tests comparing the IV estimate to the estimate obtained from observational linear regression models. This test indicated that IV effect estimates based on FTO alleles in the meta-analyzed sample of men and women differed significantly from the observational effect estimate ($p = 0.02$, Table 2). None of the other IV effect estimates were significantly different from the conventional observational effect estimate ($p < .05$ for all, Table 2).

In order to assess the validity of the IV assumptions, we next compared the IV estimates from each of the “functional” GRS IVs. All four scores were significantly associated with BMI among men and women, with the exception of the “other/unknown” mechanism score, which was not significantly related to BMI among men (Table 3). The IV estimates showed considerable heterogeneity across mechanism-related GRS. For example, BMI differences induced by genes regulating appetite were significantly associated with elevated anxiety in women ($b = 0.19$; 95%-CI (0.01, 0.38) but not in men. However, the over-identification test failed to reject (NHS $p = 0.08$, HPFS $p = 0.21$) indicating that differences in the IV effect estimates for the four alternative GRSs may be due to sampling variability. To consider a potential direct effect of FTO on anxiety, as suggested by Samaan et al. (Samaan et al., 2013) we compared the b_{IV} based on FTO to the b_{IV} based on the GRS excluding FTO. This test rejected the null hypothesis in both men (HPFS $p = 0.01$) and women (NHS $p = 0.02$) providing evidence that FTO influences anxiety via a mechanism distinct from any possible effect of the other 31 obesity-associated SNPs

To test the validity of the IV, we next adjusted the regressions of anxiety on each genetic IV for self-reported average BMI. If BMI fully mediated the effects of the genetic IV on phobic anxiety, this adjustment should completely attenuate and possibly reverse the sign of the associations. (Glymour et al., 2012) Indeed, we found that adjustment for BMI attenuated the associations between the genetic risk scores and symptoms of phobic anxiety (Table 2 and Table 3). When pooling men and women, the FTO based gene score significantly predicted phobic anxiety even after adjustment for average self-reported BMI ($p = .02$). Furthermore we found no evidence for statistical interaction between the mediator and the direct pathway (results not shown).

Discussion

In our large sample of adult men and women, we found that the FTO genotype was associated with phobic anxiety. Although FTO is established to influence BMI throughout life, the association between FTO and phobic anxiety did not appear completely mediated by BMI. This independent association of FTO genotype and phobic anxiety may suggest a direct effect –not mediated through BMI – of FTO on phobic anxiety, similar to the one

recently suggested between FTO and depression. (Samaan et al., 2013) This leads to concerns about the validity of FTO as genetic instrument in this study.

Our study is the first to provide genetic IV based estimates of the effect of BMI on phobic anxiety; previous studies have examined composite phenotypes of mental distress or depression. Kivimaki et al. used FTO genotype as an IV for BMI and overweight/obesity and estimated that increases in BMI induced substantial increase in common mental disorder. (Kivimaki et al., 2011) We found similar results using FTO alone, and like Kivimaki et al. also found evidence that this effect was not fully mediated by BMI. However, our measure of BMI is substantially more robust than that used in the Kivimaki study; we used mid-life BMI averaged over multiple successive interview waves. The finding that even this, likely high-quality measure of BMI, did not eliminate the association between FTO and anxiety further supports the suggestion that FTO has a direct effect on phobic anxiety. Lawlor et al. used FTO and MC4R as IVs to estimate the effect of BMI on 3 questions assessing levels of psychological distress and the use of anti-depressants separately; they reported the surprising finding that genetic factors associated with increases in BMI predicted *better mental health*. (Lawlor et al., 2011) We do not find any evidence of a similar pattern when using FTO and MC4R as IVs, but we do find some suggestive evidence that at least some genetically induced differences in BMI are associated with lower symptoms of anxiety. We found this most pronounced in gene scores associated with BMI via other/unknown mechanisms, not used in the analysis from Lawlor et al. Most recently, Jokela et al. used the same genetic loci as in our analyses, and also found a positive association when investigating the effect of BMI on depression using the Modified Beck Depression Inventory. (Jokela et al., 2012)

The suggestion that some genes, e.g., FTO, may have direct effects on phobic anxiety is intriguing, given the paucity of evidence on the genetic determinants of anxiety. In this study, Wu-Hausman specification tests indicated that IV effect estimates based on FTO alleles in the meta-analyzed sample of men and women differed significantly from the observational effect estimate ($p = 0.02$, Table 2) arguing in favor of using IV models in comparison to standard ordinary least squares. But the IV effect estimates based on the GRS_exFTO did not ($p=0.38$). This casts doubt on the validity of FTO as an IV.

Recently, a candidate gene study suggested protective effects of the rs9939609 A variant, a proxy SNP for FTO in high linkage disequilibrium with the SNP used in this study (rs1558902, $R^2=0.90$), on depression in a multi-ethnic sample. (Samaan et al., 2013) Because the genetics of anxiety and depression are thought to be similar (Domschke and Reif, 2012), it is difficult to explain the harmful effect of FTO on phobic anxiety in our study as compared to the protective effect of FTO on depression reported in the Samaan et al. investigation. It is possible that there are differences between ethnic populations as the study of Samaan et al. failed to provide a significant effect when analyzing the white population only. In any case, if there are any effects of FTO on (phobic) anxiety, they are probably small and difficult to detect. At the same time, findings might be the result of type 1 error and wrongfully reject the null hypothesis. Therefore replication of our findings is required in other, preferably larger cohorts and biasing pathways possibly via an effect of FTO on depression and subsequent comorbid anxiety merit scientific investigation.

The current analyses should be interpreted in light of the strong assumptions under which IV estimates correspond with causal effects. We argue that violations of these assumptions are illuminating, especially when the genetic determinants of the outcome are not well understood. The IV assumptions can be falsified with several approaches, and we implemented two of the most powerful assessments: leveraging assumptions about the direction of confounding of the BMI-anxiety association, and comparing effect estimates across alternative genetic IVs. We assumed net positive confounding between BMI and symptoms of phobic anxiety, i.e. that the observational effect estimate would be larger than the true causal effect. Our results suggest that FTO genotype is unlikely to provide a valid IV because the IV effect estimate using FTO is larger than the observational estimate. (Glymour et al., 2012) Over-identification tests comparing effect estimates from different IVs, further support the inference that FTO is not a valid IV for the effects of BMI per se, but rather has some direct effects on phobic anxiety.

Beyond the challenges common to genetic IV studies, the limitations of our single, brief assessment of phobic anxiety should be recognized. Rather than assessing all anxiety phenotypes, the Crown-Crisp Index measures phobic anxiety only. But thus far no conclusive evidence has linked a specific genetic marker with any form of anxiety, nor has evidence suggested that any one form of anxiety disorder has a stronger genetic basis than any other. Furthermore, the Crown-Crisp is well-validated and appears to perform well in this population. But findings will need to be replicated in more diverse populations and for other anxiety disorders. It is furthermore interesting to note that we observed a gender difference in the performance of the GRS when explaining BMI. Although the GRS was calculated using common SNP weights obtained from a gender-heterogeneous sample, the GRS explained 1.07% of the variation in BMI among men and 2.73% among women. While this does not invalidate our study results, it highlights the potential for gender-specific SNP effects and the potential to improve these types of analyses if gender-specific SNPs effects were available.

In conclusion, our findings provide little evidence that adiposity influences phobic anxiety, but perhaps more importantly, we find that a genetic locus known to influence adiposity likely has *direct* effects on phobic anxiety. In other words, adiposity and phobic anxiety share common genetic determinants. It is critical to extend these findings in larger and more diverse samples, especially given the mixed pattern of results from different cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Causal diagram representing the assumptions for genetic IV analyses to estimate the effect of BMI on anxiety

The causal diagram follows the rules for directed acyclic graphs (DAG) and describes the assumed causal structure motivating the IV analyses. Key assumptions represented in this diagram are: 1) the genotype affects BMI; 2) the genetic instrumental variables do not influence anxiety except via BMI; and 3) there are no common causes of genotype and anxiety. Under these assumptions, the genotype can be used as an instrumental variable to estimate the effect of BMI on phobic anxiety, even when there are unmeasured confounders of BMI and anxiety.

Table 1

	Men (HPFS)	Women (NHS)
N	3697	5911
Age at Anxiety Assessment (mean, SD)*	67.85 (8.54)	70.44 (6.78)
Anxiety Score (mean, SD)**	1.92 (1.90)	2.74 (2.32)
Anxiety Score ≥ 4 (%)	18 %	31 %
Body Mass Index (kg/m ²) (mean, SD)***	25.74 (3.28)	25.59 (4.54)
Coronary Heart Disease (%)	19 %	11%
Type 2 Diabetes (%)	11 %	13%
Cancer (%)	6 %	7%
FTO (Frequency of risk allele A)	43.3%	40.4%
MC4R (Frequency of risk allele A)	23.6%	22.8%
Speliotes Risk Score (mean, SD)****	3.99 (0.52)	3.98 (0.53)
Speliotes Risk Score excluding FTO (mean, SD)	3.65 (0.47)	3.67 (0.46)

* Anxiety assessed in men (2000), women (2004).

** Crown Crisp Experimental Index (phobic anxiety) measured in 2000 (HPFS) and 2004 (NHS). Items from eight questions related to fear of crowds, heights, enclosed spaces, going out alone, and worrying were summed resulting in an overall score ranging from 0 to 16. Higher scores indicate a higher level of phobic anxiety.

*** Body Mass Index was assessed as mean of all measurements available prior to the assessment of anxiety. NHS: 1976–2002 (14 measurements), HPFS: 1988 – 1998 (6 measurements).

**** Weighted sum of the number of alleles (0,1, or 2) with its published effect estimate for all 32 SNPs that were significantly associated with obesity published by Speliotes et al.

Table 2

First Stage and Genetic IV estimates of the effect of BMI on phobic anxiety, comparing alternative genetic IVs.

First Stage of IV Estimates*				Split Sample IV Estimates*				Estimated Direct Genetic Effects*			
Estimated effect of genetic factor on average BMI				Estimated Effect of Average BMI on Anxiety				Estimated Effect of genetic factor on Anxiety adjusting for Average BMI			
	Beta	95%-CI	p-value	Partial R2	Beta	95%-CI	p-value	Wu**	Beta	95%-CI	p-value
Men											
FTO	1.13	0.54 – 1.71	<0.001	0.85%	0.32	0.00 – 0.64	0.048	0.08	0.28	−0.034 – 0.59	0.081
MC4R	1.00	0.00 – 2.00	0.050	0.17%	0.05	−0.51 – 0.62	0.851	0.87	0.04	−0.53 – 0.61	0.888
GRS	0.66	0.42 – 0.91	<0.001	1.07%	0.04	−0.11 – 0.18	0.623	0.94	0.01	−0.13 – 0.15	0.883
GRSexFTO	0.45	0.15 – 0.76	0.004	0.39%	−0.07	−0.25 – 0.12	0.486	0.36	−0.08	−0.26 – 0.10	0.384
Women											
FTO	1.47	0.90 – 2.04	<0.001	0.75%	0.31	0.01 – 0.62	0.045	0.12	0.24	−0.06 – 0.55	0.118
MC4R	1.01	−0.07 – 2.09	0.066	0.09%	−0.06	−0.61 – 0.49	0.839	0.69	−0.11	−0.67 – 0.44	0.691
GRS	1.42	1.13 – 1.71	<0.001	2.73%	0.09	−0.06 – 0.23	0.258	0.82	0.02	−0.14 – 0.17	0.830
GRSexFTO	1.40	1.06 – 1.73	<0.001	1.96%	0.01	−0.116 – 0.18	0.919	0.49	−0.06	−0.24 – 0.11	0.491
ALL***											
FTO	1.30	0.89 – 1.71	<0.001	0.79%	0.32	0.10 – 0.54	0.005	0.02	0.26	0.04 – 0.48	0.019
MC4R	1.01	0.27 – 1.74	0.007	0.13%	−0.00	−0.40 – 0.39	0.987	0.74	−0.04	−0.43 – 0.36	0.852
GRS	0.97	0.79 – 1.16	<0.001	2.06%	0.06	−0.04 – 0.16	0.256	0.81	0.01	−0.09 – 0.12	0.799
GRSexFTO	0.89	0.66 – 1.11	<0.001	1.39%	−0.03	−0.15 – 0.10	0.688	0.38	−0.07	−0.20 – 0.06	0.272

* additionally adjusted of age, age², and the top 3 eigenvectors to increase precision.

** p-value from Wu-Hausman Test for equality between OLS and IV estimates

*** results from inverse-variance weighted meta-analysis.

Table 3

First Stage and Genetic IV estimates of the effect of BMI on phobic anxiety, comparing mechanism specific genetic IVs.

	First Stage of IV Estimates*				Split Sample IV Estimates*				Estimated Direct Genetic Effects*			
	Estimated effect of genetic factor on average BMI		Estimated effect of genetic factor on Anxiety		Estimated effect of Average BMI on Anxiety		Estimated Effect of genetic factor on Anxiety adjusting for Average BMI		Estimated Effect of genetic factor on Anxiety adjusting for Average BMI			
	beta	95% CI	p-value	beta	95% CI	p-value	beta	95% CI	beta	95% CI	p-value	p-value
Men												
Appetite	0.70	0.42 – 1.00	<0.001	0.05	-0.13 – 0.23	0.56	0.03	-0.15 – 0.21	0.745			
Adipogenesis	1.01	0.29 – 1.72	0.006	0.21	-0.23 – 0.65	0.35	0.17	-0.26 – 0.61	0.435			
Cardio-pulmonary	0.79	-0.03 – 1.60	0.058	0.23	-0.25 – 0.72	0.34	0.18	-0.29 – 0.67	0.442			
Other/unknown	0.17	-0.36 – 0.69	0.531	-0.21	-0.55 – 0.14	0.25	-0.22	-0.57 – 0.13	0.223			
Women												
Appetite	1.37	1.02 – 1.72	<0.001	0.19	0.01 – 0.38	0.040	0.13	-0.06 – 0.32	0.174			
Adipogenesis	1.86	0.84 – 2.88	<0.001	-0.13	-0.62 – 0.36	0.603	-0.23	-0.72 – 0.27	0.370			
Cardio-pulmonary	1.38	0.30 – 2.46	0.013	-0.04	-0.54 – 0.47	0.888	-0.11	-0.61 – 0.40	0.676			
Other/unknown	1.54	0.88 – 2.18	<0.001	-0.10	-0.45 – 0.26	0.597	-0.17	-0.53 – 0.18	0.335			
All**												
Appetite	0.98	0.75 – 1.21	<0.001	0.13	-0.01 – 0.25	0.064	0.08	-0.05 – 0.21	0.238			
Adipogenesis	1.29	0.70 – 1.87	<0.001	0.06	-0.27 – 0.39	0.727	-0.00	-0.33 – 0.33	0.995			
Cardio-pulmonary	1.00	0.35 – 1.65	0.003	0.10	-0.25 – 0.46	0.558	0.05	-0.30 – 0.40	0.790			
Other/unknown	0.71	0.30 – 1.12	<0.001	-0.15	-0.40 – 0.10	0.234	-0.20	-0.44 – 0.06	0.123			

* additionally adjusted of age, age², and the top 3 eigenvectors to increase precision

** results from inverse-variance weighted meta-analysis

Appetite rs10150332 (NRXN3), rs10767664 (BDNF), rs1558902 (FTO), rs2815752 (NEGR1), rs2867125 (TMEM18), rs543874 (SEC16B), rs571312 (MC4R), rs7359397 (SH2B1), rs9816226 (ETV5);
Adiposity rs12444979 (GPRC5B), rs1555543 (PTBP2), rs2890652 (LRPIB), rs3817334 (MTCH2), rs4929949 (RPL27A), rs7138803 (FAIM2), rs987237 (TFAP2B); **Cardiopulmonary Factors**
 rs10938397 (GNPDA2), rs11847697 (PRKD1), rs13107325 (SLC39A8), rs1514175 (TNNI3K); **Others** rs10968576 (LRRN6C), rs13078807 (CADM2), rs206936 (NUDT3), rs2112347 (FLJ35779),
 rs2241423 (MAP2K5), rs2287019 (QPCTL), rs29941 (KCTD15), rs3810291 (TMEM160), rs4771122 (MTIF3), rs4836133 (ZNF608), rs713586 (RBJ), rs887912 (FANCL)