Including multiple instrumental variables in Mendelian randomization analyses

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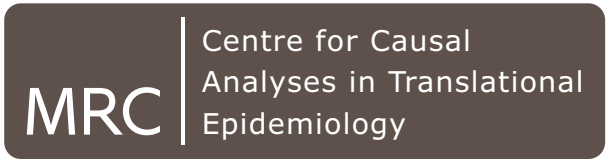
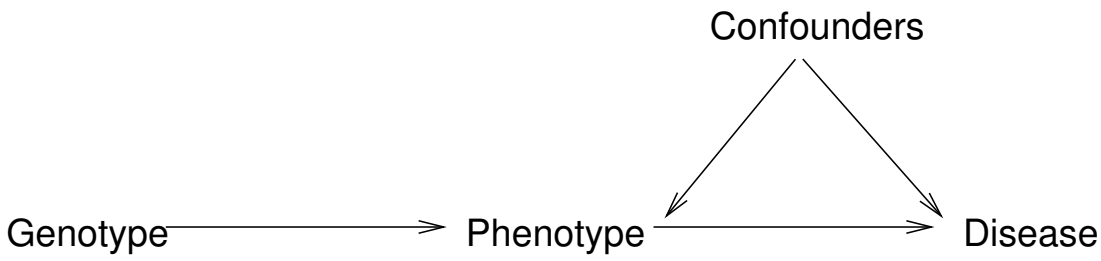


Outline

- Introduction:
  - the Mendelian randomization approach
  - rationale for multiple instruments
- Multiple instruments example:
  - increase precision
  - instrument strength
  - over-identification
  - use of allele score as IV
- Multiple instruments discussion

Introduction to the Mendelian randomization approach

- Use of genotypes as instrumental variables (Davey Smith & Ebrahim, 2003)
- Epi analyses - potential for unmeasured confounding, reverse causation
- Infer causal phenotype-disease association



IV assumptions; the genotype should be:

(i) independent of confounders

(ii) associated with phenotype

(iii) independent of disease given phenotype and confounders

Rationale for using multiple instruments

Problem: MR analyses - low power

- Weak instruments - bias IV estimate
- $F < 10$: $0.015 < p < 0.05$ GP coefficient stat. sig. & weak

  (Lawlor, Harbord, Sterne, Timpson, & Davey Smith, 2008)
- Genotypes - small effects wrt phenotype st.dev. - wide IV CIs

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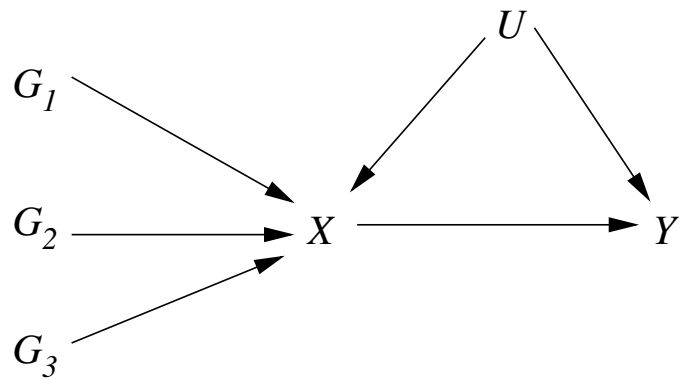
- Ideal situation (Didelez & Sheehan, 2007):



Possible solutions:

- Increase study sample size
- Stronger instrument
- Multiple instruments
- (Meta-analysis)

Estimation

- Single instrument - ratio of coefficients: $PD = GD/GP$
- Multiple instruments - motivation for TSLS
  (Theil, 1953; Basmann, 1957)
- TSLS, LIML, GMM estimators (`ivregress`, `ivreg2`, `condivreg`)
  - equivalent with 1 instrument & 1 phenotype
  - differ slightly with multiple instruments
  - LIML - smallest finite sample bias (Ullah, 2004)
  - Other types of CIs: AR (LIML), LM, CLR (`condivreg`)
  (Mikusheva & Poi, 2006)
  - Bayesian approaches (Kleibergen & Zivot, 2003)

Multiple instruments

- Multiple instruments: Cragg-Donald F-statistic
  - inflates first stage F-statistic

  (Cragg & Donald, 1993; Stock, Wright, & Yogo, 2002)
- Over-identification: Sargan & Hansen tests
  - if significant 1 or more instruments not valid
- Practical issue: increase in precision about IV estimate due to use of multiple IVs
  - countered by loss of precision due to extra missing data

Is fat mass causally related to bone mineral density?

- Eligible sample: 5509 children, age 7-9yrs, ALSPAC cohort
- Outcome: bone mineral density
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  (Frayling et al., 2007; Loos et al., 2008; Willer et al., 2009)

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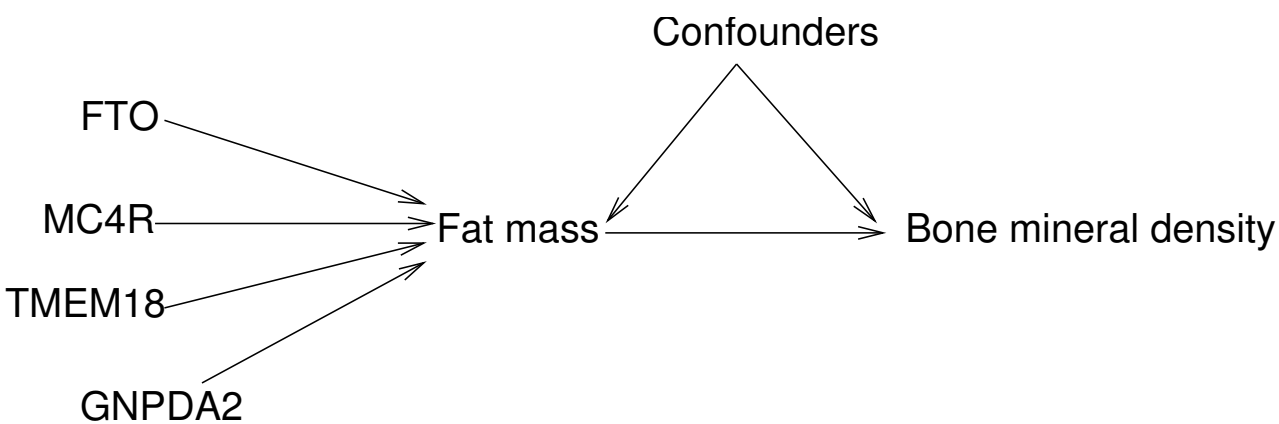
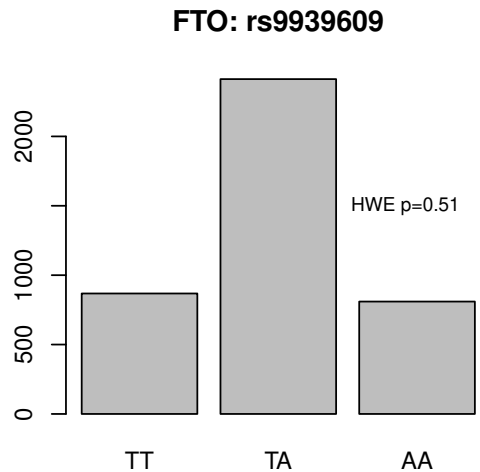
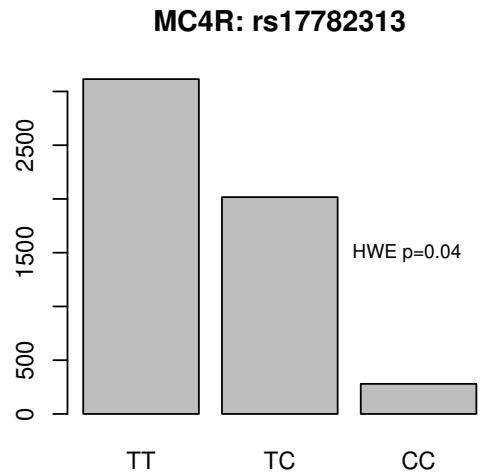
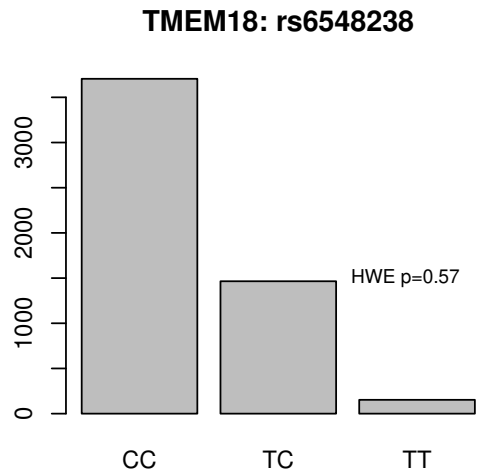
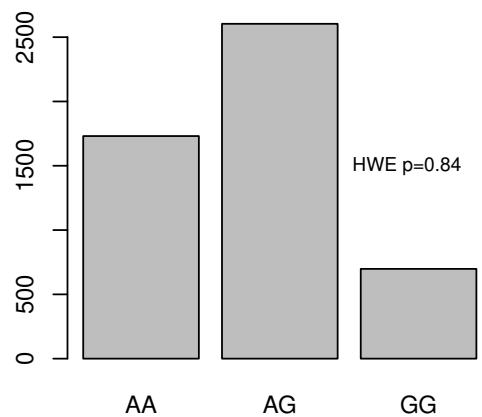


Distribution of SNPs in sample





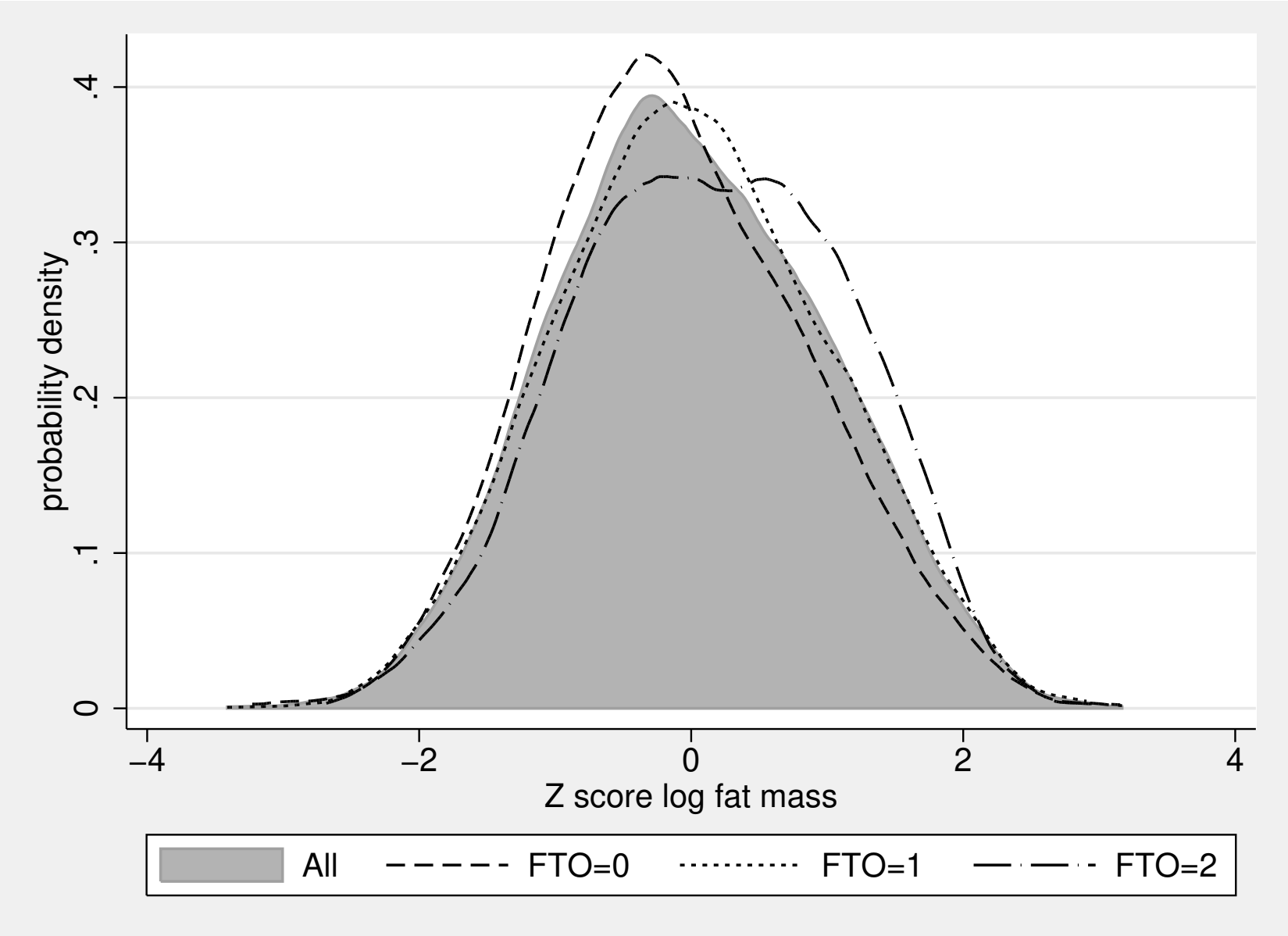




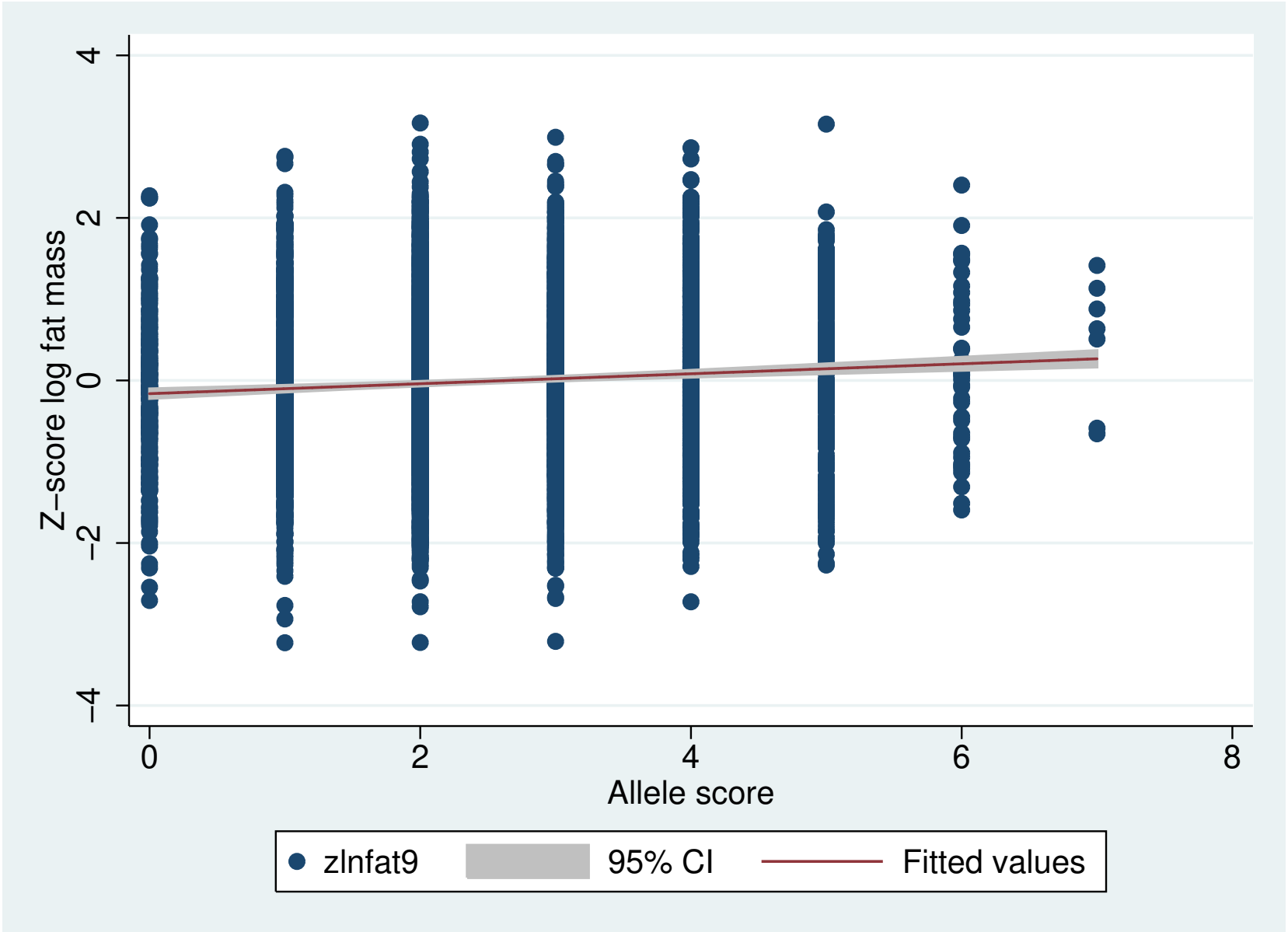
Example continued

- IV estimation:
  - each SNP separately
  - all four SNPs
  - allele score as IV - sum of risk alleles (Weedon et al., 2008)
- Fat mass & bone mineral density:
  - $+$vely skewed; logged & z-scored
  - exp(β); ratio of geometric means (RGM)

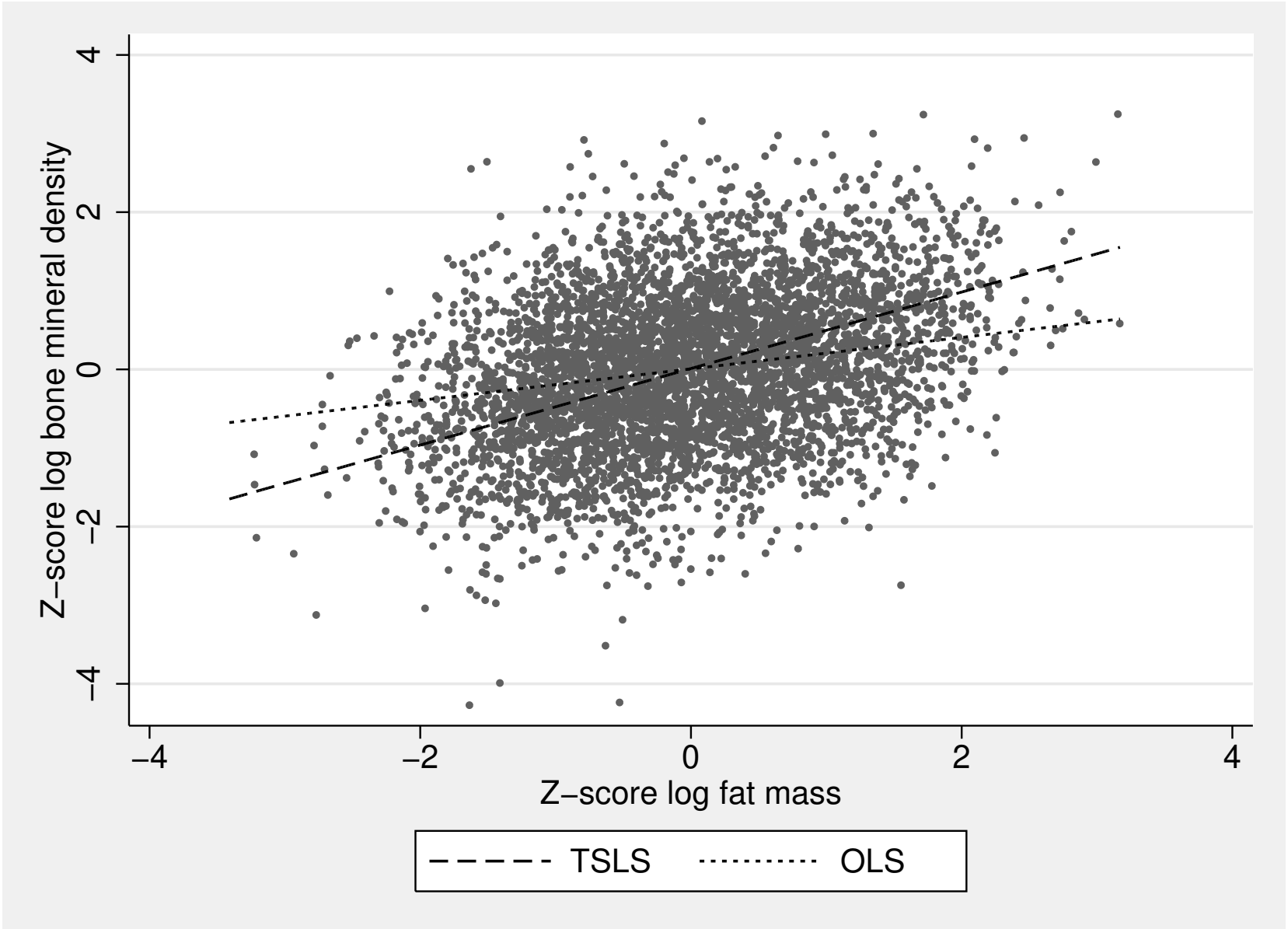
Distribution of log fat mass by FTO genotypes



First stage regression



Second stage regression



OLS: 1.22 (1.19, 1.26); IV allele score: 1.40 (0.99, 1.98)

IV estimates

Model	RGM (95% CI)	F
FTO	1.44 (1.05, 1.97)	39.8
MC4R	2.33 (1.34, 4.05)	17.9
TMEM18	2.27 (0.98, 5.28)	7.5
GNPDA2	0.98 (0.47, 2.03)	7.6
4 SNPs: TSLS	1.63 (1.28, 2.06)	$18.6_{16.9}$
AR(LIML)	1.66 (1.29, 2.23)	
LM	(1.30, 2.21)	
CLR	(1.30, 2.20)	
Allele sc.	1.40 (0.99, 1.98)	33.2

- precision
- strength
- missing data: $N = 4796$

FTO: 5091; MC4R: 5412; TMEM18: 5323; GNPDA2: 5303

- over-id: 4 SNPs Sargan test, P=0.16

Multiple instruments discussion

- One way to increase precision of IV estimates
  - given each instrument meets IV assumptions
- Investigate:
  - joint strength - Cragg-Donald F-statistic
  - over-identification - Sargan test

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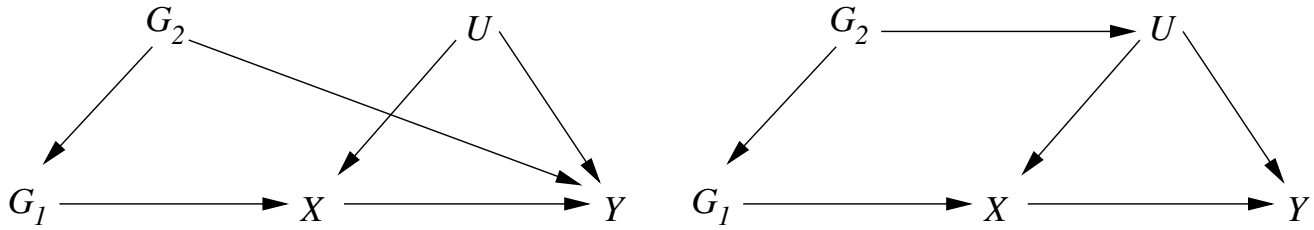
Possible practical problems

- Population stratification
  - mixture of genetic popns in sample
  - could confound IV estimates
- Linkage disequilibrium
  - problematic if a gene in LD with instrument is associated with the outcome and/or confounders



- Pleiotropy
  - SNP has multiple functions
  - problematic if one of these functions is associated with the outcome

Missing data: multiple imputation & IV estimation

- Misleading to think about causal model in context of MI
  - more important to think about missing data mechanism - MNAR/MAR/MAR-CD/MCAR
- Although including all available variables in all chained equations would appear to violate IV DAG:
  - this doesn't matter if MAR
- SNPs: Maternal genotypes available in ALSPAC - impute offspring genotypes
  - HapMap type imputations (`impute`/`mach`)