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Strengthening Mendelian randomization through utilizing multiple independent paired combinations of genetic variants to evaluate potential pleiotropy

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Summary

- We compared instrumental variable (IV) estimates of the effect of height on forced vital capacity (FVC) lung function using 20 genotypes as multiple instruments in the Avon Longitudinal Study of Parents and Children (ALSPAC).
- We compared I^2 statistics from a fixed effects meta-analysis using two-stage least squares (TSLS) weights with *P*-values from Sargan over-identification tests from TSLS models. These measures showed some degree of agreement, but the Sargan test is preferable as it's properties are better known.

Introduction

- FVC is strongly associated with height because of the close dependence of lung volume on height (Batty *et al.* , 2006).
- Mendelian randomization uses genotypes as IVs to control for unmeasured confounding factors which can bias epidemiological analyses (Davey Smith & Ebrahim, 2003).
- A model with more instruments than exposures is called over-identified.
- We aim to formally compare over-identification tests and methods for assessing heterogeneity in multiple instrument IV estimates.

Methods

- We genotyped 20 SNPs in ALSPAC. We measured child height and FVC at 8.5 years.
- We added SNPs as instruments into the model in order of instrument strength. We compared TSLS over-identification tests with heterogeneity tests from a fixed effects meta-analysis model (using TSLS weights, i.e. each instrument is given the same weighting that it receives in TSLS).
- We also split the 20 instruments into 524,287 mutually exclusive pairs from the 1,048,575 possible combinations of instrument sets. We derived IV estimates using each combination and compared the difference between the IV estimate from each pair (see bottom left figure for a schematic for 4 instruments).

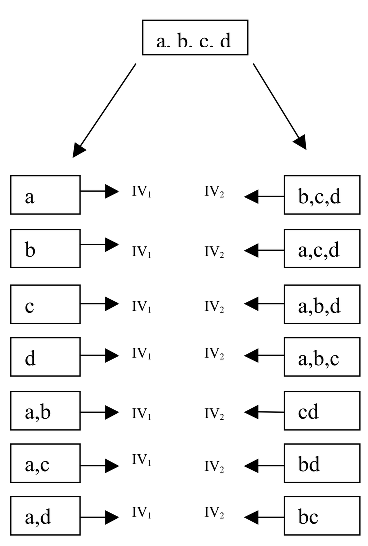
Results

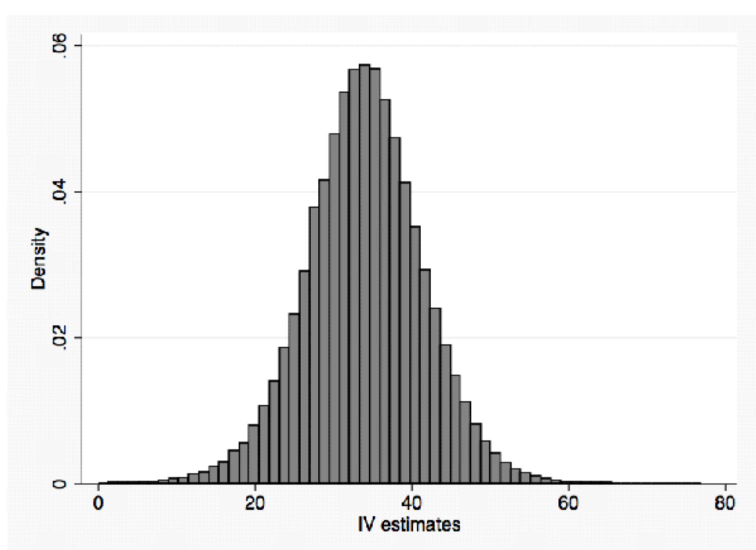
- We find an association between height and FVC 35.21 ml/cm (95% CI 34.0, 36.4).
- The Sargan test and meta-analysis (using TSLS weights) heterogeneity tests gave a similar trend in *P*-values upto 15 instruments (see Table).
- The I^2 statistics from the meta-analysis (using TSLS weights) gave a similar opposite trend to the Sargan test *P*-values (see Table).
- The histogram of IV estimates from the all possible combinations of the instruments was centred on the IV estimate using all 20 instruments (see bottom middle figure). And the histogram of the difference between the pairs was centred on 0 (see bottom right figure).

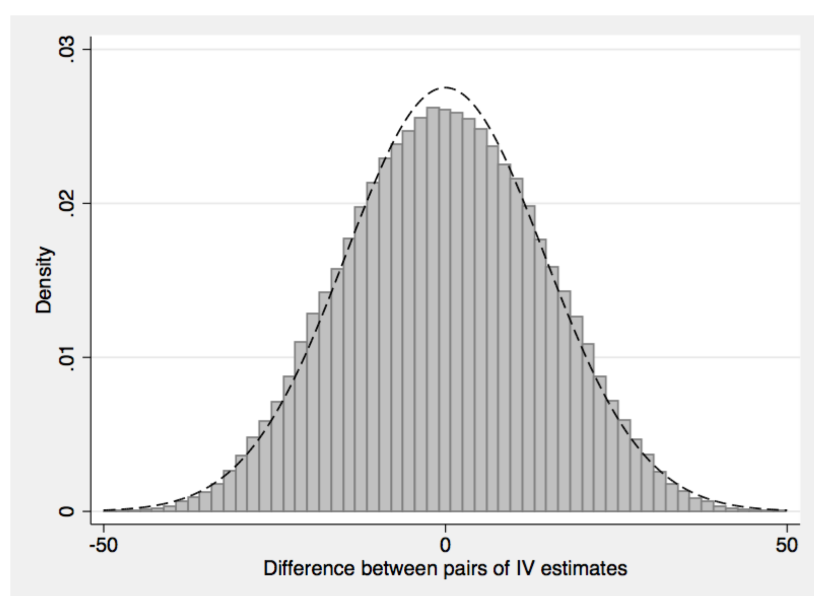
IV estimates using multiple instruments (including SNPs in order of instrument strength).

Number of instruments	SNPs used as instruments	First stage F	First stage R^2	IV estimate (95% CI)	SE of IV estimate	Sargan test P-value	I^2 (%, TSLS weights)	Het P-value (TSLS weights)
1	rs3791675	10.0	0.0024	12.7 (-17.3, 42.7)	15.3	-	-	
2	& rs2055059	8.0	0.0038	30.1 (9.0, 51.2)	10.8	0.037	70.4	0.066
3	& rs6440003	7.4	0.0053	30.3 (12.4, 48.3)	9.1	0.114	41.0	0.184
4	& rs1390401	7.0	0.0066	29.4 (13.4, 45.5)	8.2	0.223	12.4	0.331
5	& rs3116602	6.6	0.0078	29.0 (14.3, 43.8)	7.5	0.357	0.0	0.488
6	& rs10906982	6.8	0.0096	31.2 (18.0, 44.4)	6.7	0.431	0.0	0.570
7	& rs4549631	6.4	0.0106	30.4 (17.7, 43.1)	6.5	0.542	0.0	0.668
8	& rs1042725	6.3	0.0118	35.5 (23.6, 47.4)	6.1	0.138	3.0	0.407
9	& rs16896068	5.9	0.0124	37.5 (25.9, 49.1)	5.9	0.106	7.5	0.373
10	& rs12735613	5.7	0.0133	35.2 (24.1, 46.4)	5.7	0.078	8.7	0.362
11	& rs6686842	5.5	0.0141	37.4 (26.5, 48.3)	5.6	0.053	13.3	0.317
12	& rs11107116	5.1	0.0143	37.2 (26.4, 48.0)	5.5	0.076	5.6	0.390
13	& rs42046	4.8	0.0147	36.8 (26.1, 47.4)	5.4	0.103	0.0	0.463
14	& rs10935120	4.5	0.0149	36.5 (25.9, 47.0)	5.4	0.134	0.0	0.535
15	& rs8041863	4.3	0.0153	36.8 (26.3, 47.2)	5.3	0.174	0.0	0.603
16	& rs6724465	4.1	0.0153	36.5 (26.1, 47.0)	5.3	0.136	0.0	0.676
17	& rs2814993	3.9	0.0154	35.7 (25.3, 46.2)	5.3	0.081	0.0	0.723
18	& rs6060373	3.7	0.0156	34.0 (23.6, 44.3)	5.3	0.015	0.0	0.729
19	& rs8099594	3.5	0.0156	33.9 (23.5, 44.2)	5.3	0.017	0.0	0.783
20	& rs10512248	3.3	0.0156	33.9 (23.6, 44.2)	5.3	0.011		

N=4,216 for all models







Discussion

- We found heterogeneity tests gave generally the same trend in *P*-values but with quantitatively different values. And there were some instances (e.g. for more than 15 instruments) where the Sargan test *P*-values were small but the heterogeneity test *P*-values were large.
- The I^2 statistics from the meta-analysis (using TSLS weights) were difficult to interpret because the different instruments were fitted on the same observations.
- The distribution of IV estimates using all possible combinations of multiple instruments could indicate potential pleiotropy especially if the difference in mutually exclusive instrument set pairs was not centred on zero.

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