Using multiple independent combinations of genetic variants to strengthen causal inference in Mendelian randomization studies: height and lung function as an example

Tom Palmer David Evans George Davey Smith

MRC Centre for Causal Analyses in Translational Epidemiology,
School of Social and Community Medicine, University of Bristol, UK
tom.palmer@bristol.ac.uk

Summary

- We genotyped 15 SNPs associated with height in the ALSPAC cohort. We compared instrumental variable estimates of the effect of height on FVC lung function using all possible combinations of the genotypes as instruments.
- We found, as expected, using all possible combinations of $k = 1, \ldots, 15$ genotypes the IV estimates converged to the estimate using all 15 SNPs.
- Minimum distance tests of pairs of the IV estimates showed excess heterogeneity in agreement with the Sargan overidentification tests.

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 instrumental variables (IVs) to control for unmeasured confounding factors and reverse causation which can bias traditional epidemiological analyses (Davey Smith & Ebrahim, 2003).
- 20 SNPs were associated with height in genome-wide association studies (Frayling & et al., 2007; Loos & et al., 2008).
- Using multiple instruments can increase power and precision of IV estimates.
- We investigated the distribution of IV estimates of the effect of height on FVC lung function in children from all combinations of genotypes of the SNPs.

Methods

- We genotyped 20 SNPs in the Avon Longitudinal Study of Parents and Children (ALSPAC). We measured child height and FVC lung function at 8.5 years of age.

  The IV estimates using the combinations of genotypes were compared using the following algorithm:
- Given K SNPs, there are $2^K - 1$ combinations.
- The combinations from each set of $k = 1, \ldots, K$ SNPs was used as a set of multiple instruments in TSLS (Table 1).
- For each k there are $2^{k-1} - 1$ ways of dividing the $2^k - 1$ combinations into two (independent) groups.
- We perform IV estimation on each pair and performed minimum distance (MD) estimation on the two IV estimates (Figure 1) (Wooldridge, 2002).

Results

- Observational estimate of association between height and FVC 35.21 (95% CI 34.02, 36.40).
- The separate IV estimates of the 15 strongest SNPs ranged from 5 to 70, with mean 37.47 (Table 1) (we excluded 5 SNPs with very weak associations with height).
- The average IV estimates converge to the IV estimate using the 15 SNPs (Table 1).
- The average: standard error of the IV estimates, first stage F statistic, and first stage R2 statistic also converge to the model using 15 SNPs.
- For the model using 15 SNPs there was slight evidence against joint validity of the SNPs $P_{\text{Sargan}} = 0.183$.
- By combination size greater than 20% of Sargan tests gave $P < 0.2$ (Table 1).

k	$C(15,k)$	mean IV est.	mean SE	mean F	mean R^2	$P_{\text{Sargan}} < 0.2$ (%)
1	15	37.47	28.04	4.71	0.0011	NA
2	105	37.72	16.57	4.71	0.0022	21
3	455	37.26	12.62	4.70	0.0033	28
4	1365	36.92	10.58	4.69	0.0043	29
5	3003	36.67	9.29	4.69	0.0054	28
6	5005	36.50	8.39	4.68	0.0065	35
7	6435	36.36	7.71	4.67	0.0075	36
8	6435	36.26	7.17	4.67	0.0086	40
9	5005	36.17	6.74	4.66	0.0097	42
10	3003	36.09	6.37	4.65	0.0107	47
11	1365	36.03	6.06	4.64	0.0117	44
12	455	35.97	5.80	4.64	0.0128	50
13	105	35.92	5.56	4.63	0.0138	52
14	15	35.87	5.36	4.62	0.0148	60
15	1	35.83	5.17	4.62	0.0159	100

Table 1: *Average IV estimates by combination size (N=4311)*

- The quantiles of the MD test statistics deviate from the expected quantiles (Figure 1). This reflects the heterogeneity between the IV estimates as shown by the greater number of Sargan tests with $P < 0.20$ than expected (Table 1).

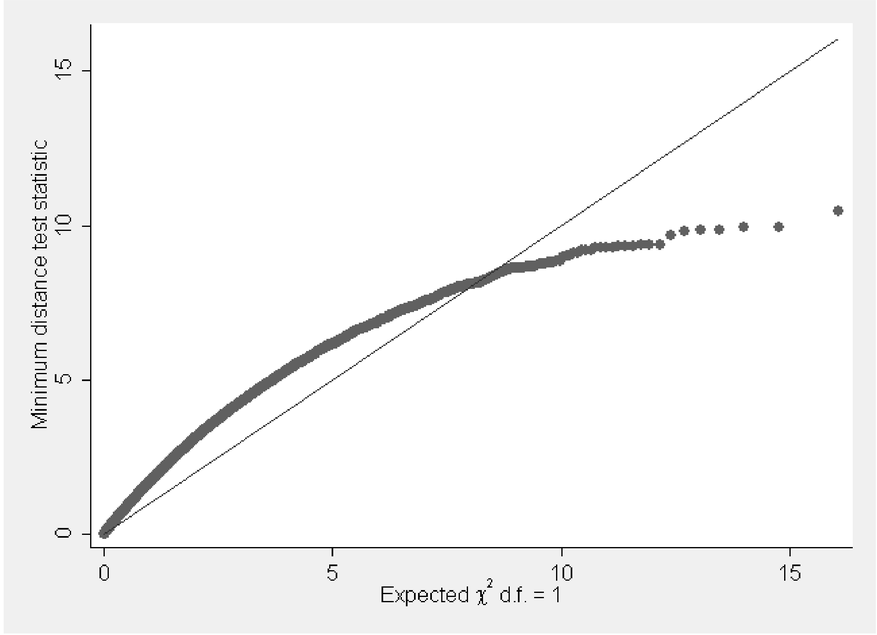

Figure 1: *QQ plot of minimum distance test statistics.*

Discussion

- If there is distortion in the causal effect by pleiotropy or linkage disequilibrium we expect to find heterogeneity between the IV estimates using combinations of genetic variants.
- Our approach has a close relationship to an over-identification test, which can be viewed as a heterogeneity test of the separate IV estimates.
- The IV estimates using the combinations of instruments converged to the IV estimate using all instruments.
- A greater proportion of Sargan tests than expected gave $P < 0.2$. This was reflected by the MD test statistics deviating from their expected distribution under the null.
- We note that Cochran's Q test (Cochran, 1954), commonly used in meta-analysis, is a MD test assuming zero covariance between the estimates.

Acknowledgements: TP was supported by MRC Collaborative Project Grant G0601625. TP, DE, and GDS work in the MRC CAiTE Centre (MRC Grant G0600705). Thanks to Frank Windmeijer (CMPO, University of Bristol) for very helpful comments.

References

Batty, G. D., Gunnell, D., Langenberg, C., Davey Smith, G., Marmot, M. G., & Shipley, M. J. 2006. Adult height and lung function as markers of life course exposures: associations with risk factors and cause-specific mortality. *European Journal of Epidemiology*, **21**(11), 795–801.

Cochran, W. G. 1954. The combination of estimates from different experiments. *Biometrics*, **10**(1), 101–129.

Davey Smith, G., & Ebrahim, S. 2003. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease. *International Journal of Epidemiology*, **32**, 1–22.

Frayling, T. M., & et al. 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science*, **316**(5826), 889–894.

Loos, Ruth J F, & et al. 2008. Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nature Genetics*, **40**(6), 768–775.

Wooldridge, J. M. 2002. *Econometric Analysis of Cross Section and Panel Data*. MIT Press.