

SUPPLEMENT

The use of two-sample methods for Mendelian randomization analyses on single large datasets

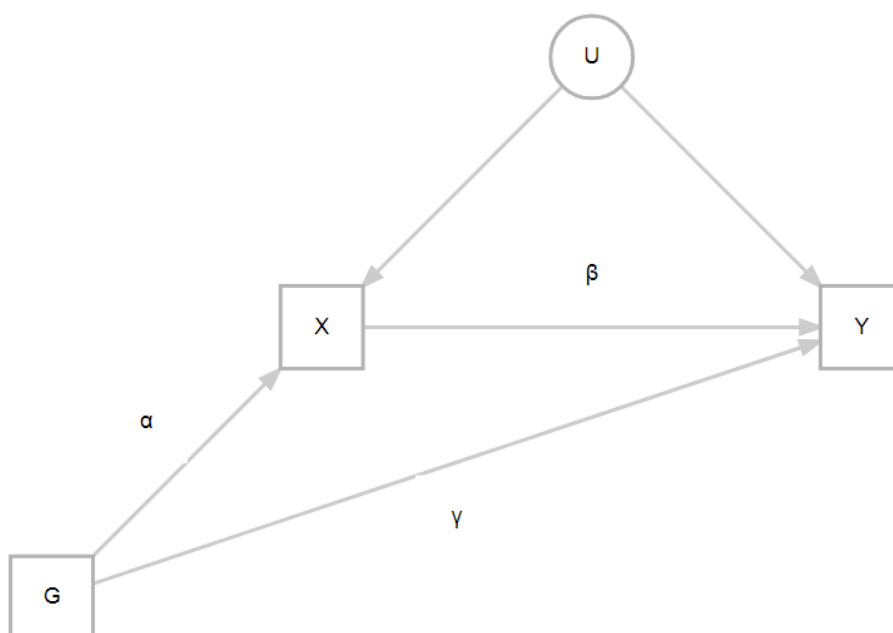
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Supplementary Methods

Here we provide details on our simulation work, in particular on the parameters used to generate the simulated data, the analyses applied, and the performance measures used.

The simulations compare two-sample methods using single sample data and two-sample methods using two-sample data. To do this, we simulate a single sample containing X, Y and G and then using exactly the same parameters we simulate a second sample containing X and G. The first dataset then becomes the Y and G data for the genuine two-sample analysis.

Below is the causal diagram describing the situation our simulations refer to, with G = genetic variant; X = exposure; Y = outcome; U = unmeasured confounders; α = effect of G on X; β = causal effect of X on Y; γ = pleiotropic effect of G on Y. The confounding effect of U was simulated by generating a correlation in the errors of X and Y.



Methods of MR Analysis

We consider 5 two-sample methods of analysis:

- Fixed-effect IVW (IVW FE): The G-X and G-Y estimates are first obtained for each SNP; SNP-specific MR estimates are derived using the Wald estimator (ratio of G-Y over G-X), with standard error obtained using the Delta method ¹; the causal effect is then obtained by pooling SNP-specific MR estimates using a fixed-effect or a random-effects model, respectively. IVW FE is the most powerful two-sample MR method, but it assumes no pleiotropy ².
- Random-effects IVW (IVW RE): It is performed in the same way as IVW FE, but a *multiplicative* random-effects meta-analysis model is used instead of a fixed-effect model to allow for pleiotropy. This method assumes that pleiotropic effects across SNPs are random (balanced pleiotropy), and that their magnitude is independent of the magnitude of the corresponding G-X effects (InSIDE assumption) ². Compared with an additive RE model, the multiplicative RE model is more robust to weak instrument bias since it downweights variants with a weaker G-X association, and to the presence of outlying estimates, which are more likely to represent pleiotropic variants ². For these reasons, a multiplicative RE IVW has been recommended ³.
- Weighted median estimator: It assumes that more than 50% of the information contributing to the analysis comes from genetic variants that are valid (i.e. not pleiotropic) ⁴.
- Weighted mode estimator: It assumes that the largest weighted contribution of similar (identical in infinite samples) SNP-specific MR estimates comes from valid instruments ⁵.
- MR-Egger regression: G-Y estimates for the individual SNPs are regressed on their G-X estimates, with the intercept representing the overall pleiotropy and the slope the adjusted MR estimate ⁶. MR-Egger makes the assumption of overall directional pleiotropy as well as the InSIDE assumption, and it works well only in the presence of a large spread of strengths, represented by the heterogeneity in G-X estimates across SNPs, I^2_{GX} , with a recommended I^2_{GX} exceeding 90% ⁷.

In the one-sample MR, we also consider the 2SLS using individual-level data since this is a gold standard in the absence of pleiotropy.

Parameters monitored

For each method of analysis, for all scenarios and for both one-sample and two-sample data, we monitored the MR estimate and its standard error, as well as coverage and root mean square error (RMSE).

We also monitored:

- The ordinary least squares (OLS) estimate
- R^2 for the regression of X on all SNPs
- R^2 for the regression of Y on all SNPs
- I^2 for the G-X estimates
- An estimate of the residual correlation under the assumption of no pleiotropy
- Average SNP-specific F statistic
- Number of SNP-specific F-statistics below 10
- I^2 and Q for the FE IVW meta-analysis of SNP-specific MR estimates

The **residual correlation**, that is the correlation due to confounding rather than to the causal effect of X on Y, was estimated using the following approach: X is regressed on G, and fitted values (f_x) and residuals (r_x) are calculated; Y is then regressed on f_x and the residuals are calculated (r_y); the residual correlation due to confounding is estimated as the correlation between r_x and ($r_y - \beta \cdot r_x$), where β is the MR estimate. This approach provided estimates very close to the true values in our simulations (data not shown), and can be used in practice in a MR study to estimate the correlation due to confounding.

The **F statistic**, a function of magnitude and precision of the G-X estimate that represents the strength of the SNP as an instrument for X ⁸, was calculated using the formula:
$$F = GX^2/SE_{GX}^2.$$

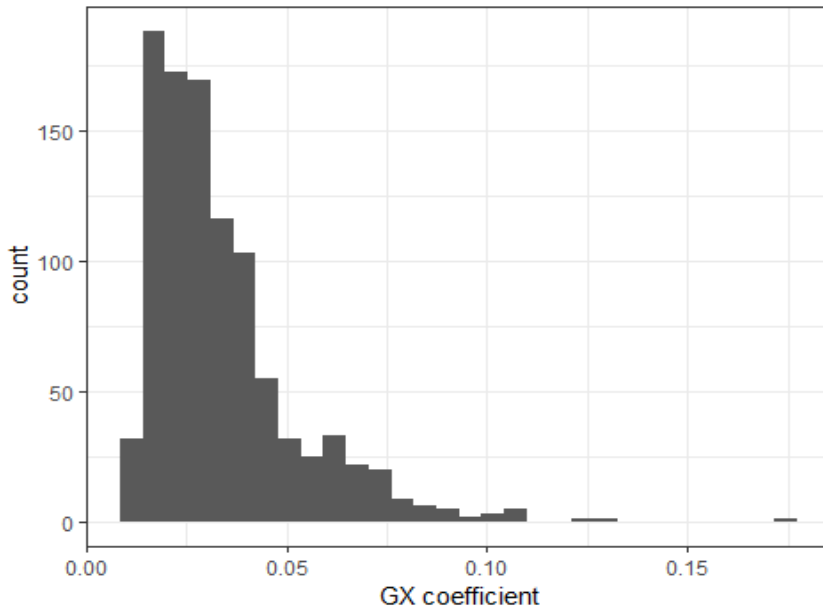
Simulation parameters

The simulation is based on a sample size of 300,000 and 100 independent SNPs with random allele frequency uniformly distributed between 1% and 99%.

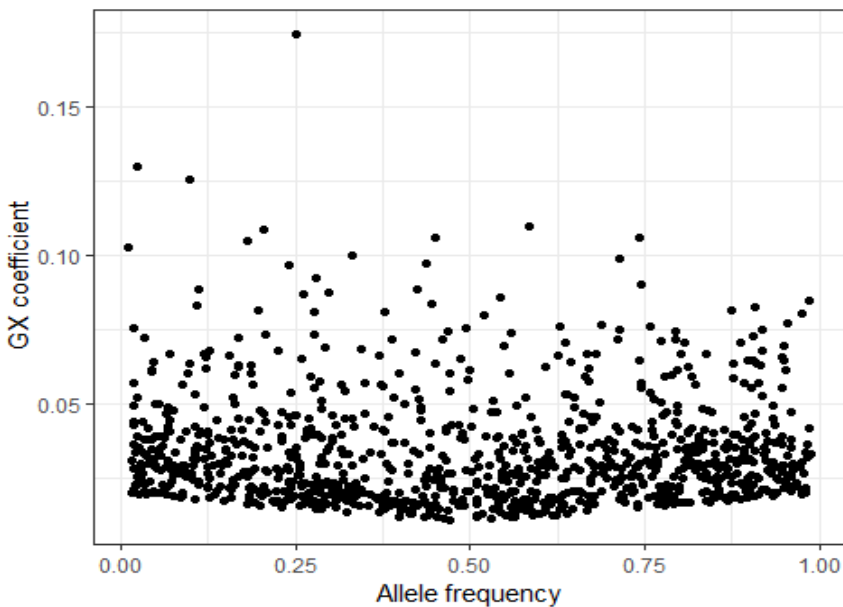
The five correlations between the error components in X and Y (-0.4, -0.2, 0, 0.2, 0.4) represent different degrees of negative and positive confounding.

The true causal effect is either 0 (null) or 1.

SNP coefficients for the strength of association with X are exponential, so that there are many SNPs with small effects and a few with large effects. The figure below shows a histogram of the SNP coefficients for 1,000 simulated SNPs.



The SNP coefficients are adjusted so that rare SNPs cannot have very small coefficients, as otherwise the simulation would include large numbers of weak SNPs. The figure below shows the relationship between allele frequency and SNP coefficient for 1,000 simulated SNPs.



The individual SNPs have an average F-statistic of 67.9; out of each set of 100 SNPs, on average 5 SNPs had an F-statistic below 10 and would generally be considered as weak. SNP strength does not vary across the simulation experiment.

The average SNP explains 2.26% of the variance in X, and 0.22% of the variance in Y. The average percent of variance explained for G-X is the same for all scenarios, while for G-Y is

larger when there is a causal relationship between X and Y and when there is a direct pleiotropic effect of G on Y.

Finally, the average I^2 is 90.8%, and this does not vary across the simulation experiment.

Pleiotropy scenarios

We generated data under three scenarios; no pleiotropy, directional pleiotropy in 20% of the SNPs, and balanced pleiotropy in 20% of the SNPs.

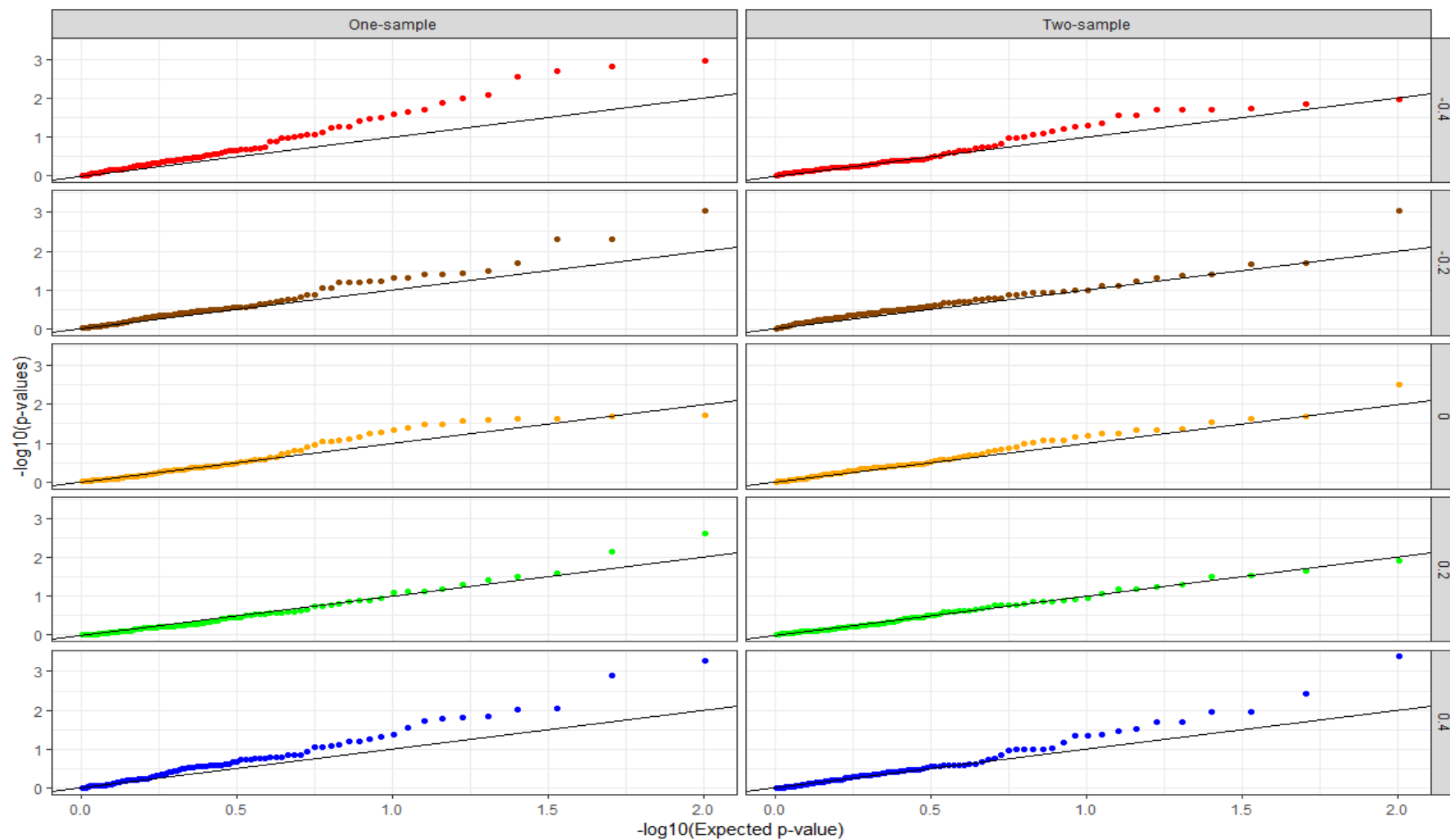
In the **directional pleiotropy** simulations, 20 of the 100 SNPs were pleiotropic in the sense of having a direct association between G and Y that did not pass through X. The coefficients for the pleiotropic effects were randomly chosen from the same distribution as was used for the G-X coefficients. Since both the SNP GX coefficients and the pleiotropic GY coefficients are positive, the pleiotropy will act to make the MR estimates larger. The bias of 0.15 to 0.19 across all scenarios with directional pleiotropy (with and without a causal effect, and with different degrees of correlation) observed for 2SLS, IVW FE and IVW RE (Supplementary table 4) gives an indication of the amount of pleiotropy that we have introduced by our choice of parameter values.

In the **balanced pleiotropy** simulations, 20 of the 100 SNPs were pleiotropic in the sense of having a direct association between G and Y that did not pass through X. The coefficients for the pleiotropic effects were randomly chosen from the same distribution as was used for the G-X coefficients, but then given a random sign so that on average the pleiotropy was zero.

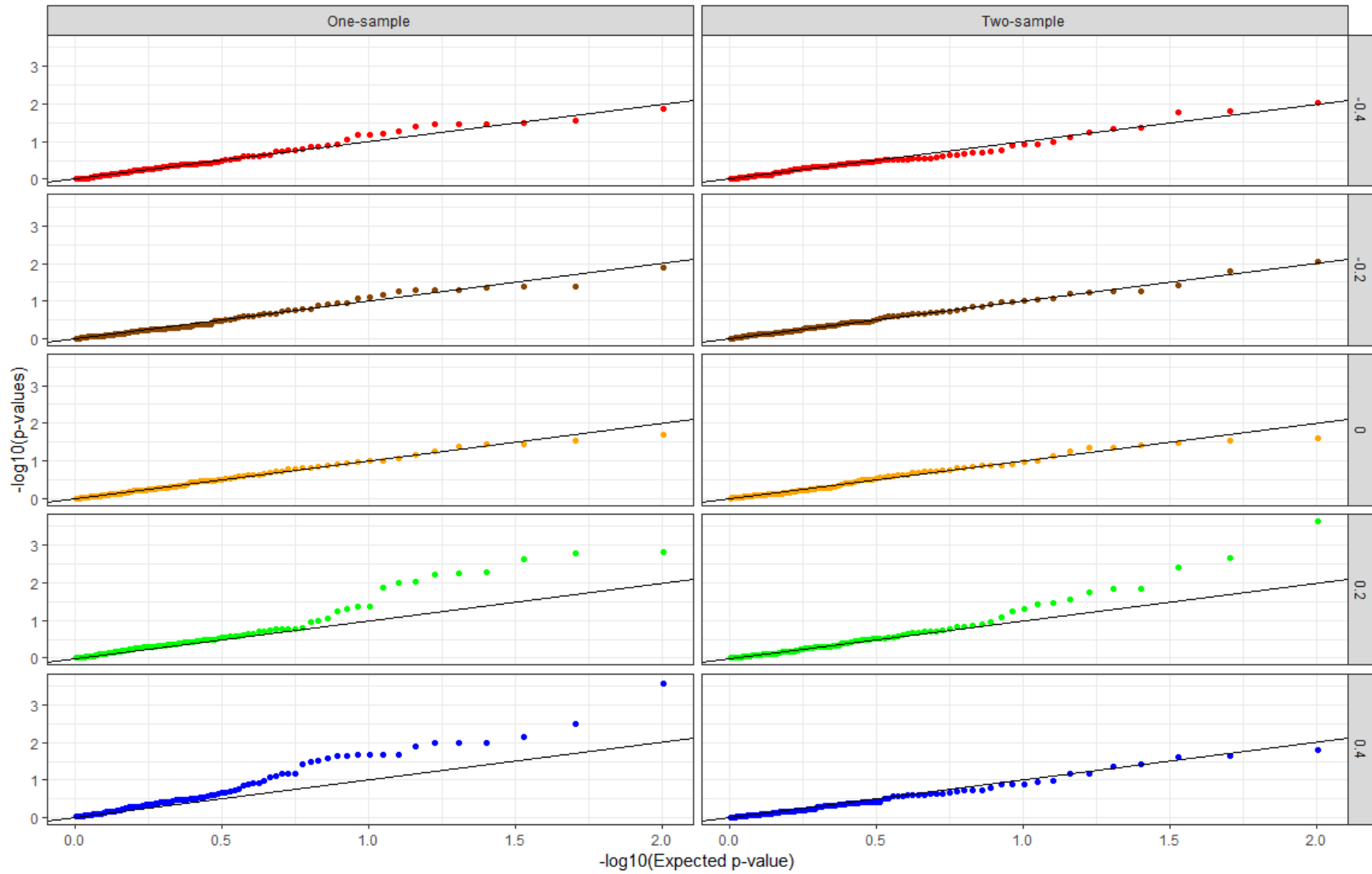
Supplementary Figures & Tables

Supplementary Figure 1. Simulations with no causal effect in the presence of pleiotropy: Q-Q plot of MR-Egger null p-values for different levels of correlation between X and Y due to confounding, in one-sample and two-sample MR. a) Balanced pleiotropy; b) Directional pleiotropy.

a) Balanced pleiotropy

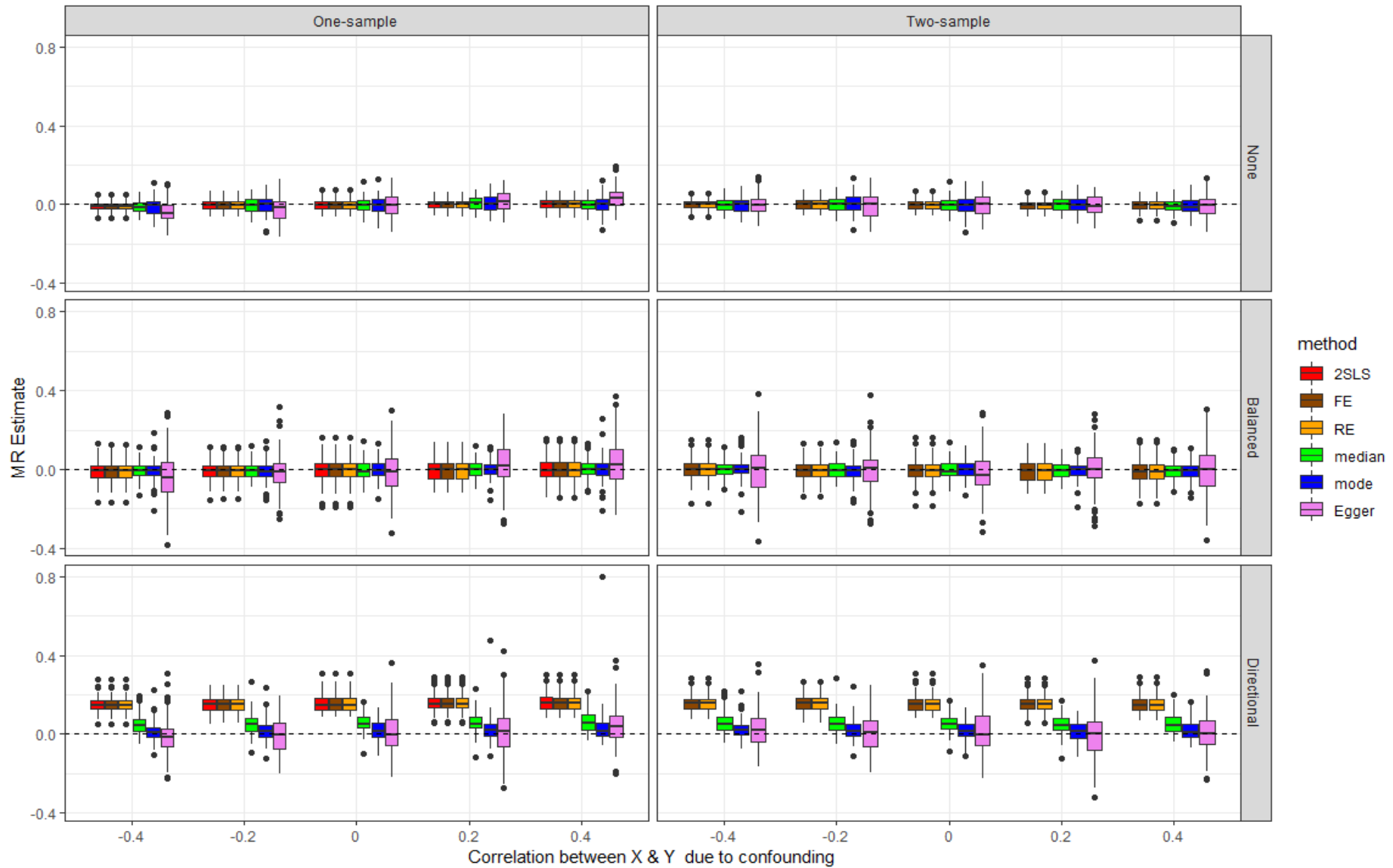


b) Directional pleiotropy

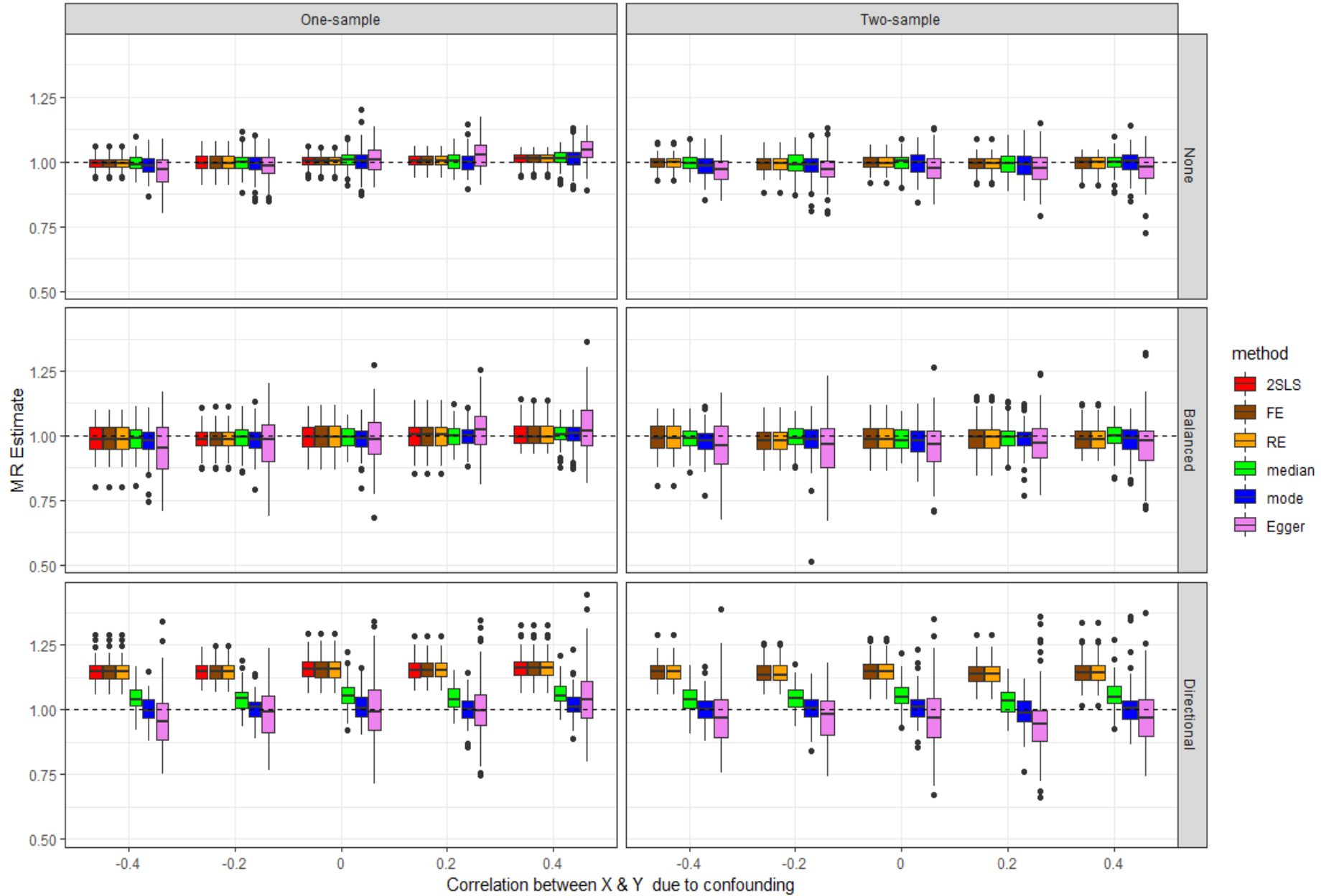


Supplementary Figure 2. Results of secondary analyses where the simulated G-X effect sizes have larger variability (I^2_{GX} of 0.97). Outliers have been excluded to improve readability of the plots. a) No causal effect; b) Causal effect of 1.

a) No causal effect



b) Causal effect of 1



Supplementary Table 1: Characteristics and methods used in 27 MR investigations reported in the 10 papers reviewed. We only included one-sample MR analyses performed within UKB; when MR investigations were performed for multiple outcomes, we only considered one outcome per paper. IVW: inverse-variance weighted method; in brackets is specified whether a fixed-effect (“Fixed”) or a random-effects (“Random”) meta-analysis model was used, whenever this was specified. GRS: Genetic risk score. NR: Not reported. ^a Multiple exposures; ^b Overall F statistic for the GRS; ^c Binary exposures, for which % variance explained cannot be meaningfully estimated; ^d Multiple exposures and different population subgroups (males/females).

Study	Sample size	N. SNPs	% Variance explained	F statistic	MR methods	
					Main analysis	Secondary analyses
Hindy, 2019 ⁹	376,435	76	7.3	NR	IVW	MR-Egger, Weighted median estimator, Multivariable MR
Tyrrell, 2019 ¹⁰	287,503	14; 73 ^a	0.2; 1.7 ^a	86.5; 4,705 ^b	2SLS using a GRS	IVW, MR-Egger, Median estimator, Penalized weighted median estimator
Taylor, 2019 ¹¹	335,937	97	NR	NR	IVW (Random)	MR-Egger, Weighted median estimator, Weighted mode estimator
Sun, 2019 ¹²	366,385	73	1.6	5,964 ^b	Ratio method using a GRS	MR-Egger, Weighted median estimator
Sun (2), 2019 ¹³	318,664	134; 233 ^a	/ ^c	NR	IVW, MR-Egger, Simple median estimator, Weighted median estimator, MR-RAPS, MR-PRESSO	Leave-one-out
Khandaker, 2019 ¹⁴	367,703	2 to 185 ^a	NR	NR	IVW	MR-Egger, Weighted median estimator
Gharakhani, 2019 ¹⁵	310,793	520	7	NR	IVW (Fixed)	MR-Egger
Doherty, 2018 ¹⁶	278,374	24 to 68 ^a	NR	NR	Maximum likelihood MR	MR-Egger, Weighted median estimator, Weighted mode estimator, Leave-one-out, MR Steiger filtering
Wade, 2018 ¹⁷	335,308	77	1.82	NR	Ratio method using a GRS	IVW, MR-Egger, Weighed median estimator, Weighted mode estimator
Minelli, 2018 ¹⁸	180,957 to 202,567 ^d	24 to 206 ^d	/ ^c	10.1 to 382.5 ^d	IVW (Fixed)	IVW (Random), MR-Egger, Weighted median estimator, 2SLS

Supplementary Table 2. Simulations with no pleiotropy. Beta: causal effect estimate; SE: standard error; Cover.: coverage; RMSE: root mean square error.

Data	Causal Effect	Confounding	2SLS		IVW FE		IVW RE		Median		Mode		Egger	
			Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE
One-sample	0.0	-0.4	-0.015	92.000	-0.015	93.000	-0.015	93.000	-0.025	93.000	-0.025	96.000	-0.115	75.000
			0.036	0.040	0.036	0.040	0.037	0.040	0.056	0.059	0.131	0.083	0.092	0.161
		-0.2	-0.014	93.000	-0.015	93.000	-0.015	94.000	-0.017	97.000	-0.019	97.000	-0.055	86.000
			0.037	0.037	0.037	0.037	0.038	0.037	0.057	0.051	0.088	0.072	0.096	0.121
		0.0	-0.000	95.000	-0.001	95.000	-0.001	95.000	-0.002	98.000	-0.002	100.000	0.002	96.000
	0.036		0.034	0.036	0.034	0.037	0.034	0.056	0.048	0.089	0.064	0.093	0.087	
	0.2	0.015	88.000	0.015	88.000	0.015	89.000	0.019	95.000	0.019	98.000	0.062	87.000	
		0.036	0.044	0.036	0.044	0.037	0.044	0.056	0.052	0.087	0.069	0.092	0.115	
	1.0	-0.4	0.986	95.000	0.986	92.000	0.986	96.000	0.979	95.000	0.965	95.000	0.889	80.000
			0.036	0.038	0.033	0.038	0.036	0.038	0.056	0.052	0.124	0.072	0.088	0.147
-0.2		0.994	90.000	0.994	90.000	0.994	91.000	0.988	96.000	0.974	98.000	0.940	88.000	
		0.036	0.040	0.036	0.040	0.037	0.040	0.058	0.054	0.087	0.077	0.091	0.112	
0.0		0.999	92.000	0.999	95.000	0.999	95.000	1.000	100.000	0.990	99.000	1.007	98.000	
	0.036	0.036	0.038	0.036	0.039	0.036	0.061	0.043	0.132	0.063	0.096	0.084		
0.2	1.008	96.000	1.008	98.000	1.008	98.000	1.011	98.000	1.000	99.000	1.052	92.000		
	0.037	0.036	0.041	0.036	0.041	0.036	0.065	0.054	0.120	0.081	0.102	0.105		
0.4	1.013	94.000	1.013	97.000	1.013	97.000	1.017	100.000	1.010	99.000	1.107	84.000		
	0.036	0.038	0.042	0.038	0.042	0.038	0.066	0.054	0.096	0.075	0.104	0.139		

Data	Causal Effect	Confounding	2SLS		IVW FE		IVW RE		Median		Mode		Egger	
			Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE
Two-sample	0.0	-0.4			0.002	93.000	0.002	93.000	-0.005	97.000	-0.010	98.000	-0.009	96.000
					0.036	0.036	0.037	0.036	0.056	0.050	0.085	0.073	0.093	0.094
		-0.2			-0.005	96.000	-0.005	96.000	-0.004	100.000	-0.005	99.000	0.003	90.000
					0.037	0.036	0.038	0.036	0.057	0.052	0.157	0.070	0.097	0.108
		0.0			0.000	94.000	0.000	94.000	0.001	98.000	0.002	99.000	0.008	96.000
				0.036	0.034	0.037	0.034	0.056	0.048	0.088	0.066	0.093	0.090	
	0.2			0.006	90.000	0.006	90.000	0.005	96.000	0.004	97.000	0.006	96.000	
				0.036	0.040	0.037	0.040	0.056	0.048	0.083	0.069	0.091	0.090	
	0.4			-0.000	95.000	-0.000	96.000	0.000	100.000	0.003	100.000	0.005	96.000	
				0.036	0.035	0.037	0.035	0.057	0.044	0.124	0.083	0.092	0.084	
1.0	-0.4			0.986	94.000	0.986	98.000	0.978	97.000	0.966	98.000	0.904	82.000	
				0.033	0.038	0.036	0.038	0.055	0.051	0.081	0.071	0.087	0.133	
	-0.2			0.989	90.000	0.989	93.000	0.978	99.000	0.957	95.000	0.903	80.000	
				0.036	0.043	0.038	0.043	0.059	0.056	0.128	0.086	0.093	0.137	
	0.0			0.983	92.000	0.983	94.000	0.981	98.000	0.966	99.000	0.906	89.000	
			0.038	0.043	0.041	0.043	0.062	0.051	0.099	0.072	0.101	0.131		
0.2			0.985	96.000	0.985	97.000	0.984	98.000	0.974	95.000	0.902	92.000		
			0.041	0.044	0.043	0.044	0.066	0.060	0.102	0.084	0.107	0.144		
0.4			0.981	90.000	0.981	93.000	0.980	98.000	0.968	98.000	0.904	86.000		
			0.042	0.049	0.045	0.049	0.069	0.055	0.101	0.084	0.111	0.144		

Supplementary Table 3. Simulations with *balanced pleiotropy*. Beta: causal effect estimate; SE: standard error; Cover.: coverage; RMSE: root mean square error.

Data	Causal Effect	Confounding	2SLS		IVW FE		IVW RE		Median		Mode		Egger	
			Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE
One-sample	0.0	-0.4	-0.017	75.500	-0.017	75.600	-0.017	93.000	-0.022	94.000	-0.025	96.400	-0.107	86.700
			0.036	0.061	0.036	0.061	0.057	0.061	0.060	0.063	0.104	0.080	0.141	0.182
		-0.2	-0.010	77.100	-0.010	76.800	-0.010	94.900	-0.012	93.900	-0.012	96.200	-0.055	91.400
			0.036	0.058	0.036	0.058	0.057	0.058	0.060	0.063	0.126	0.085	0.141	0.162
		0.0	-0.002	76.000	-0.002	76.400	-0.002	92.500	-0.001	93.800	-0.003	96.700	-0.001	94.600
	0.036		0.061	0.036	0.061	0.057	0.061	0.060	0.064	0.114	0.085	0.142	0.145	
	0.2	0.008	74.700	0.008	74.900	0.008	94.000	0.011	93.700	0.013	96.700	0.057	91.800	
		0.036	0.061	0.036	0.061	0.057	0.061	0.060	0.063	0.115	0.081	0.140	0.156	
	0.4	0.018	74.000	0.018	74.200	0.018	93.400	0.021	92.000	0.022	95.000	0.100	88.700	
		0.036	0.061	0.036	0.061	0.057	0.061	0.060	0.065	0.109	0.083	0.141	0.177	
1.0	-0.4	0.983	73.200	0.983	69.500	0.983	93.400	0.976	92.400	0.956	92.000	0.890	87.400	
		0.036	0.062	0.033	0.062	0.057	0.062	0.060	0.067	0.104	0.094	0.142	0.185	
	-0.2	0.991	76.400	0.991	75.600	0.991	92.600	0.989	94.100	0.973	95.500	0.952	92.100	
		0.036	0.061	0.036	0.061	0.057	0.061	0.063	0.063	0.120	0.121	0.141	0.157	
	0.0	0.999	76.700	0.999	79.300	0.999	94.400	1.000	96.800	0.985	97.700	1.003	94.600	
0.036		0.059	0.038	0.059	0.057	0.059	0.065	0.060	0.125	0.081	0.142	0.147		
0.2	1.008	75.700	1.008	80.300	1.008	92.000	1.013	97.600	1.001	98.200	1.057	95.000		
	0.036	0.062	0.040	0.062	0.057	0.062	0.068	0.063	0.111	0.083	0.142	0.150		
0.4	1.017	76.200	1.017	83.700	1.017	92.900	1.023	97.900	1.013	99.100	1.108	86.900		
	0.036	0.061	0.042	0.061	0.057	0.061	0.071	0.062	0.142	0.087	0.141	0.183		

Data	Causal Effect	Confounding	2SLS		IVW FE		IVW RE		Median		Mode		Egger	
			Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE
Two-sample	0.0	-0.4			-0.000	75.900	-0.000	95.100	0.001	95.400	-0.000	97.000	-0.006	94.100
					0.036	0.059	0.057	0.059	0.060	0.060	0.105	0.077	0.142	0.146
		-0.2			-0.002	77.700	-0.002	94.900	0.001	94.300	0.004	96.600	-0.002	93.200
					0.036	0.057	0.057	0.057	0.060	0.062	0.105	0.119	0.141	0.153
		0.0			-0.001	75.400	-0.001	92.200	-0.000	93.700	-0.001	96.500	0.003	93.000
				0.036	0.062	0.057	0.062	0.060	0.064	0.111	0.085	0.142	0.151	
	1.0	-0.4			0.986	70.200	0.986	92.600	0.980	92.900	0.959	93.900	0.906	88.400
					0.033	0.061	0.057	0.061	0.059	0.064	0.091	0.088	0.141	0.175
		-0.2			0.985	73.300	0.985	92.600	0.979	93.800	0.960	94.700	0.915	89.300
					0.036	0.062	0.058	0.062	0.063	0.066	0.104	0.091	0.144	0.175
0.0				0.985	75.800	0.985	93.600	0.981	93.900	0.961	95.400	0.917	90.800	
			0.038	0.063	0.059	0.063	0.066	0.069	0.123	0.095	0.148	0.175		
1.0	0.2			0.986	77.500	0.986	92.200	0.983	94.700	0.965	95.000	0.916	91.900	
				0.040	0.067	0.061	0.067	0.070	0.072	0.130	0.105	0.151	0.166	
	0.4			0.985	80.300	0.985	95.000	0.983	94.600	0.962	94.000	0.911	91.600	
				0.042	0.065	0.062	0.065	0.073	0.073	0.144	0.109	0.154	0.181	

Supplementary Table 4. Simulations with *directional pleiotropy*. Beta: causal effect estimate; SE: standard error; Cover.: coverage; RMSE: root mean square error.

Data	Causal Effect	Confounding	2SLS		IVW FE		IVW RE		Median		Mode		Egger	
			Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE
One-sample	0.0	-0.4	0.150	3.700	0.150	3.600	0.150	15.700	0.056	85.600	0.005	97.800	-0.094	89.800
			0.037	0.157	0.036	0.157	0.055	0.157	0.061	0.082	0.106	0.075	0.134	0.163
		-0.2	0.161	2.500	0.161	2.500	0.161	10.700	0.073	77.300	0.021	96.100	-0.030	92.900
			0.037	0.168	0.036	0.168	0.055	0.168	0.061	0.096	0.109	0.083	0.134	0.143
		0.0	0.169	0.600	0.169	0.600	0.169	5.200	0.078	76.300	0.029	95.400	0.012	94.400
	0.036		0.174	0.036	0.174	0.055	0.174	0.060	0.098	0.096	0.084	0.135	0.141	
	0.2	0.180	0.900	0.180	0.900	0.180	4.800	0.090	71.000	0.044	94.700	0.066	91.800	
		0.036	0.185	0.036	0.185	0.055	0.185	0.061	0.109	0.104	0.101	0.137	0.158	
	0.4	0.185	0.100	0.185	0.100	0.185	2.500	0.104	61.300	0.057	92.100	0.115	84.100	
		0.035	0.191	0.036	0.191	0.054	0.191	0.060	0.121	0.119	0.098	0.134	0.182	
1.0	-0.4	1.153	3.700	1.153	2.900	1.153	16.700	1.058	84.000	0.987	96.100	0.903	88.400	
		0.037	0.161	0.033	0.161	0.055	0.161	0.060	0.085	0.107	0.078	0.135	0.170	
	-0.2	1.160	2.800	1.160	2.700	1.160	11.600	1.068	84.100	1.004	97.200	0.960	93.500	
		0.037	0.167	0.036	0.167	0.055	0.167	0.063	0.092	0.106	0.091	0.134	0.145	
	0.0	1.167	0.700	1.167	1.300	1.167	7.800	1.079	80.300	1.012	98.400	1.005	94.100	
0.036		0.173	0.038	0.173	0.055	0.173	0.066	0.100	0.111	0.131	0.136	0.138		
0.2	1.175	0.400	1.175	1.200	1.175	5.500	1.090	77.500	1.028	97.300	1.062	90.400		
	0.036	0.181	0.040	0.181	0.054	0.181	0.069	0.109	0.114	0.085	0.134	0.154		
0.4	1.187	0.400	1.187	0.900	1.187	2.600	1.105	72.200	1.042	98.000	1.123	84.200		
	0.036	0.192	0.043	0.192	0.054	0.192	0.072	0.120	0.116	0.086	0.136	0.186		

Data	Causal Effect	Confounding	2SLS		IVW FE		IVW RE		Median		Mode		Egger	
			Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE	Beta SE	Cover. RMSE
Two-sample	0.0	-0.4			0.167	0.900	0.167	7.900	0.078	76.600	0.030	96.300	0.009	95.800
					0.036	0.173	0.054	0.173	0.061	0.098	0.124	0.083	0.134	0.131
		-0.2			0.169	1.200	0.169	7.600	0.084	71.000	0.035	94.600	0.021	94.200
					0.036	0.176	0.054	0.176	0.061	0.104	0.096	0.086	0.134	0.138
		0.0			0.168	0.700	0.168	5.900	0.078	75.700	0.032	95.500	0.011	94.400
				0.036	0.174	0.055	0.174	0.060	0.098	0.120	0.146	0.135	0.141	
	1.0	-0.4			1.155	2.800	1.155	14.700	1.058	84.700	0.991	96.300	0.918	88.400
					0.033	0.162	0.055	0.162	0.060	0.086	0.105	0.078	0.134	0.164
		-0.2			1.154	4.100	1.154	16.600	1.063	84.600	0.997	95.900	0.922	91.400
					0.036	0.161	0.056	0.161	0.064	0.090	0.113	0.086	0.137	0.160
0.0				1.153	4.700	1.153	19.700	1.067	83.500	0.999	98.200	0.916	89.300	
			0.038	0.161	0.058	0.161	0.067	0.094	0.135	0.085	0.142	0.172		
Two-sample	-0.4			1.151	7.000	1.151	24.600	1.067	84.900	1.003	96.700	0.918	90.600	
				0.040	0.159	0.059	0.159	0.070	0.098	0.126	0.112	0.144	0.169	
	-0.2			1.156	7.500	1.156	22.700	1.073	84.700	1.006	97.400	0.923	91.300	
				0.043	0.164	0.061	0.164	0.074	0.102	0.136	0.094	0.150	0.173	

Supplementary Table 5. Ordinary least squares results for the regression analysis of Y on X in the one-sample data. OLS: ordinary least squares; SE: standard error; RMSE: root mean square error.

Pleiotropy	Causal Effect	Confounding	OLS Estimate	OLS SE	Bias	Coverage	RMSE
None	0.0	-0.4	-1.172	0.0050	-1.172	0.0	1.172
		-0.2	-0.586	0.0053	-0.586	0.0	0.586
		0.0	-0.000	0.0054	-0.000	97.0	0.005
		0.2	0.587	0.0053	0.587	0.0	0.587
		0.4	1.173	0.0050	1.173	0.0	1.173
	1.0	-0.4	-0.174	0.0050	-1.174	0.0	1.174
		-0.2	0.414	0.0053	-0.586	0.0	0.586
		0.0	0.999	0.0054	-0.001	100.0	0.005
		0.2	1.586	0.0053	0.586	0.0	0.586
		0.4	2.174	0.0050	1.174	0.0	1.174
Balanced	0.0	-0.4	-1.174	0.0050	-1.174	0.0	1.174
		-0.2	-0.588	0.0053	-0.588	0.0	0.588
		0.0	0.001	0.0054	0.001	93.0	0.006
		0.2	0.585	0.0053	0.585	0.0	0.585
		0.4	1.173	0.0050	1.173	0.0	1.173
	1.0	-0.4	-0.172	0.0050	-1.172	0.0	1.172
		-0.2	0.414	0.0053	-0.586	0.0	0.586
		0.0	1.001	0.0054	0.001	98.0	0.005
		0.2	1.587	0.0053	0.587	0.0	0.587
		0.4	2.174	0.0050	1.174	0.0	1.174
Directional	0.0	-0.4	-1.170	0.0050	-1.170	0.0	1.170
		-0.2	-0.582	0.0053	-0.582	0.0	0.582
		0.0	0.004	0.0054	0.004	86.0	0.007
		0.2	0.591	0.0053	0.591	0.0	0.591
		0.4	1.176	0.0050	1.176	0.0	1.176
	1.0	-0.4	-0.169	0.0050	-1.169	0.0	1.169
		-0.2	0.417	0.0053	-0.583	0.0	0.583
		0.0	1.004	0.0054	0.004	85.0	0.007
		0.2	1.590	0.0053	0.590	0.0	0.590
		0.4	2.177	0.0050	1.177	0.0	1.177

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