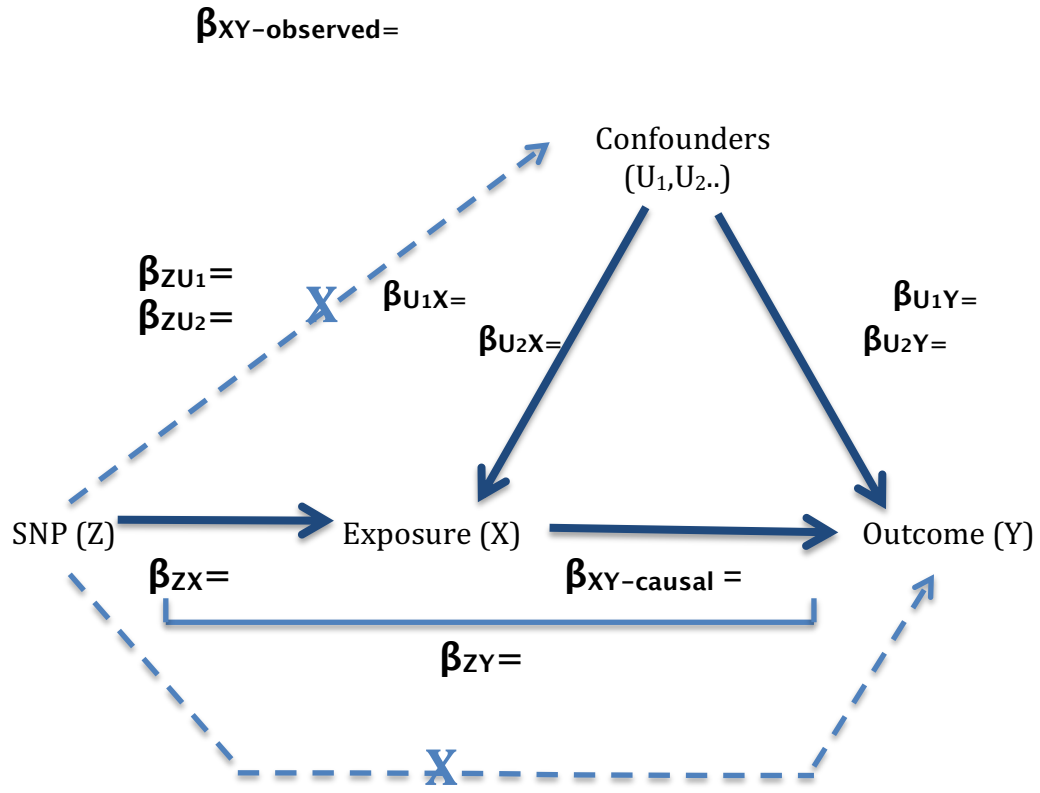
Mendelian Randomization Practical Exercise 1

Applied Research Question:
Does having higher proinflammatory CRP causally increase your blood pressure?

Graphical representation of a Mendelian Randomization IV analysis



Formulas for Wald Estimator
Where Z=SNP instrument, X=Exposure, Y=Outcome

$$\text{Causal } \beta_{IV} = \frac{\beta_{ZY}}{\beta_{ZX}}$$

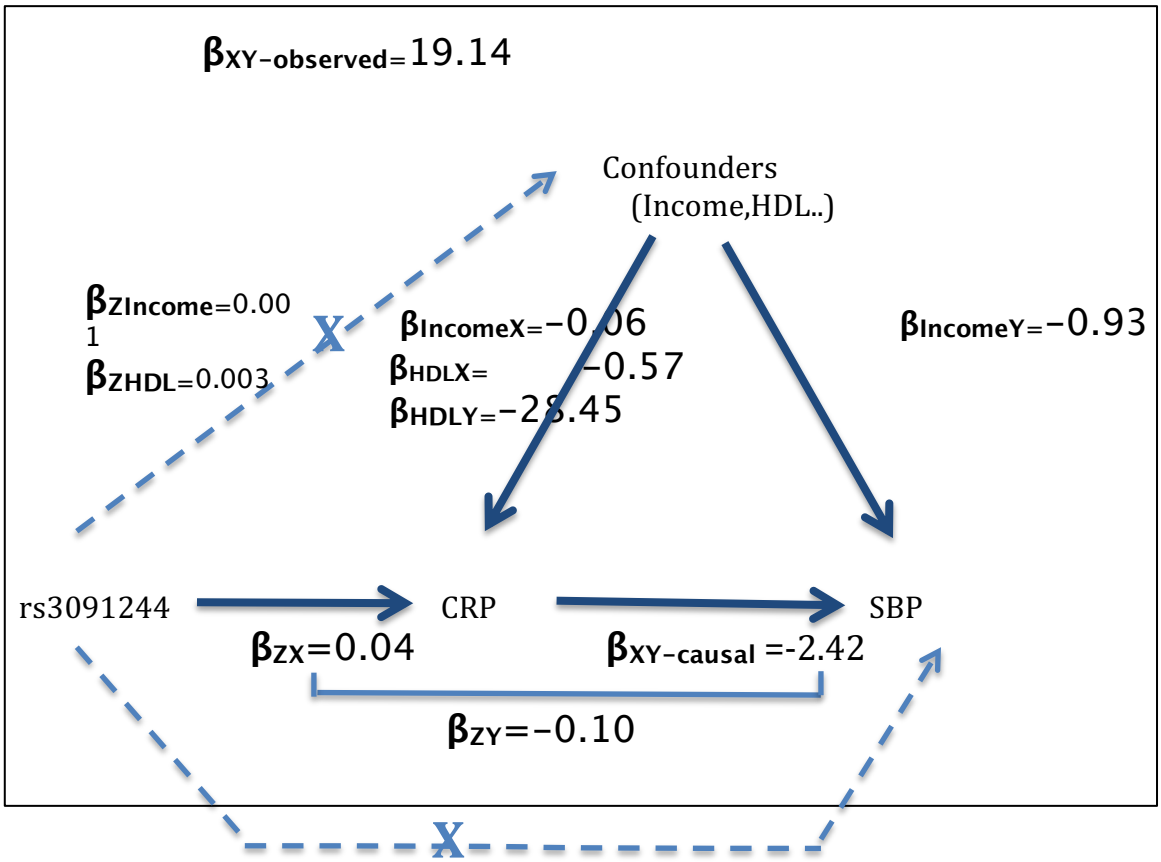
$$SE_{IV} = \frac{SE_{ZY}}{\beta_{ZX}}$$

$$95\%\ CI = \beta_{IV} \pm 1.96^{*}\ SE_{IV}$$

CRP AND BLOOD PRESSURE

Graphical Representation

Q1. As you're running the commands below, fill in the graphical representation of the IV analysis with the appropriate variables and beta-coefficients:



Observational analyses

Q2. What does the observational linear regression of SBP on CRP show?
A. Increased CRP predicts higher SBP. Every unit increase in CRP is associated with an increase in SBP of 19.1mmHg (0.45SE) $p<2x10^{-16}$

Q3. What does the OLS regression of the CRP SNP rs3091244 on CRP show?
A. The rs3091244 SNP is associated with higher CRP. Each copy of the effect allele is associated with an increase in CRP of 0.04 units (0.003 SE) $p<2x10^{-16}$

Q4. What do the OLS regressions of potential confounders (income, HDL) show?
A. That higher income is associated with lower BP and lower CRP, but income is not associated with the CRP-related *CRP* genotype. Same for HDL: that higher HDL associates with lower BP and lower CRP, but association of HDL with *CRP* genotype.

	Estimate	Std. Error	t value	Pr(>|t|)	
SBP~INCOME	-0.92975	0.09865	-9.425	<2e-16	***
HDL~INCOME	-0.056469	0.001956	-28.86	<2e-16	***
INCOME~rs3091244	0.001432	0.014091	0.102	0.919	

```
SBP~HDL          -28.4494    0.9566  -29.74   <2e-16 ***
CRP~HDL          -0.5695     0.0197   -28.9   <2e-16 ***
HDL~rs3091244    0.002531   0.001399   1.809   0.0704
```

CHECK
Add the observational-based association variables and parameters to your graphical representation.

Q5. What are the implications for these income and HDL associations for the observational CRP-SBP association?
A. The observational association between CRP and SBP could be due to confounding by HDL and Income

Q6. Compare the unadjusted and covariate-adjusted OLS observational regressions. What do they show?
A. The observational association for CRP and SBP reduces after covariate adjustment for HDL and Income in the regression model. However, an association still remains

Unadjusted
```
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  81.7846    0.8997   90.90   <2e-16 ***
CRP          19.1372    0.4475   42.77   <2e-16 ***
```

Adjusted
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Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  107.71279   1.87440  57.465   <2e-16 ***
CRP          16.83413    0.47291  35.597   <2e-16 ***
INCOME       0.20413    0.09293   2.196   0.0281 *
HDL          -19.06234    0.93616 -20.362   <2e-16 ***
```

Q7. What could explain this?
A. That there are other unmeasured confounding factors not being accounted for in the OLS regression model, **OR** that there is reverse causation of SBP effects on CRP, **OR** that CRP has some causal effects, over and above confounding. (Additionally, measurement error in the covariates can also lead to persisting associations after covariate adjustment has occurred).

MR/IV Analyses: Wald Estimator

Obtain the required estimates to compute the causal effect using the Wald Estimator from your dataset. Note, however, that an advantage of the Wald estimator is that you do not need individual level datasets to do the MR analysis. Reported SNP effects from published GWAS is sufficient and you can take the SNP effect on the exposure from one GWAS, and the SNP effect on outcome from a different GWAS sample ("Two sample MR")

Q8. Run the necessary OLS regressions to compute a Wald estimator

	Estimate	Std. Error	t value	Pr(>|t|)	
CRP~rs3091244	0.041937	0.002838	14.78	<2e-16	***
SBP~rs3091244	-0.1014	0.1396	-0.726	0.468	

Q9. From the above output, compute the causal effect using the Wald estimator, as well as it's SE and 95% CI. What do the results show and what do they mean?
A. Little strong evidence of causal effect of CRP on SBP

Wald estimator causal Beta = -0.1014/0.0419 = -2.42
SE = 0.1396/0.0419 = 3.33
Lower CI <- beta - (1.96*se)
Upper CI <- beta + (1.96*se)
95% CI = -8.95 to 4.11

Q10. Rerun the observational OLS of CRP and SBP and compare with the results from the Wald estimator. What do you notice about the Beta and SEs?
A. Much larger beta and much smaller SE.

	Estimate	Std. Error	t value	Pr(>|t|)	
CRP	19.1372	0.4475	42.77	<2e-16	***

MR/IV Analyses: TSLS
Two-stage least squares (TSLS) MR requires individual level data, and the exposure, SNP and outcome in the one sample ("Single sample MR").

Q11. What do the TSLS results show and did it differ to the Wald estimator?
A. No strong evidence of causal effect of CRP on SBP. No difference in the beta coefficients between Wald and TSLS, but SE slightly different

	Estimate	Std. Error	t value	Pr(>|t|)
CRP	-2.418	3.398	-0.712	0.477

Q12. Are they the same as 'ivreg' TSLS function?
A. Coefficient is the same but SE is slightly different

	Estimate	Std. Error	t value	Pr(>|t|)
Pred_CRP	-2.418	3.329	-0.726	0.468

CHECK
Are all the variables and parameters now complete in your graphical representation?

Weak instruments bias
Assessing instrument strength with the F-stat (looking for $\geq$10).
For Single SNP MR, the F-statistic is calculated as:

$$F_{stat} = \frac{R^2 * (N-1)}{(1-R^2)}$$

where **R^2** is the variance explained in exposure by the SNP, and **N** is number of individuals in the study. This statistic is available in the output for OLS and TSLS
Q14. Looking at the F-statistic, determine if weak instruments may be an issue
A. Fstat in both is 218. This is well over the threshold of 10, so no issues with weak instruments

Discuss:
Q14. How would having weak instruments change the causal estimate of CRP on SBP, in this study (single sample)?
A. For single-sample MR, weak instruments biases causal IV estimates towards the confounded observational association. So the null IV estimate would increase towards the observational association beta (observational beta = 19.1)