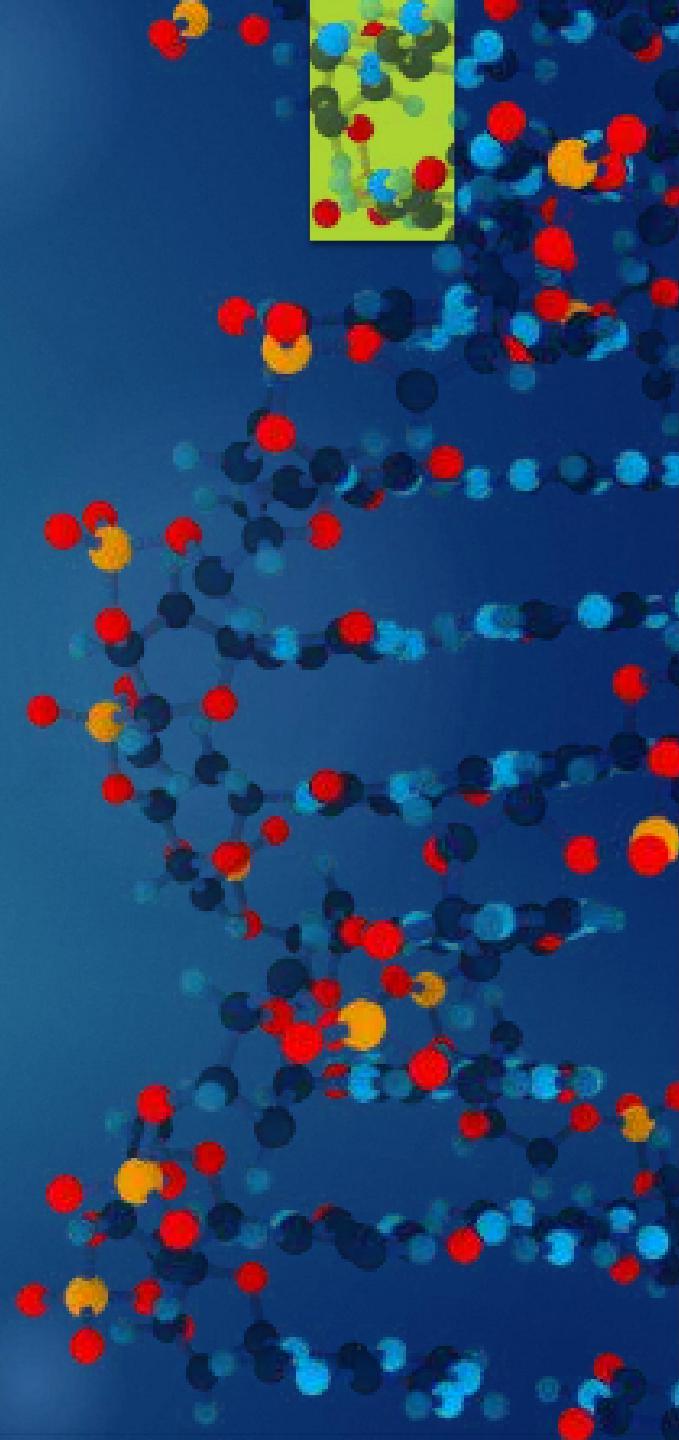


# Celiac Disease Triggers

MATTHIJS KNIGGE



# Overview

- ▶ Project goal
- ▶ Mendelian Randomization
- ▶ Methods
- ▶ Data
- ▶ Pipeline
- ▶ Preliminary results
- ▶ Future steps

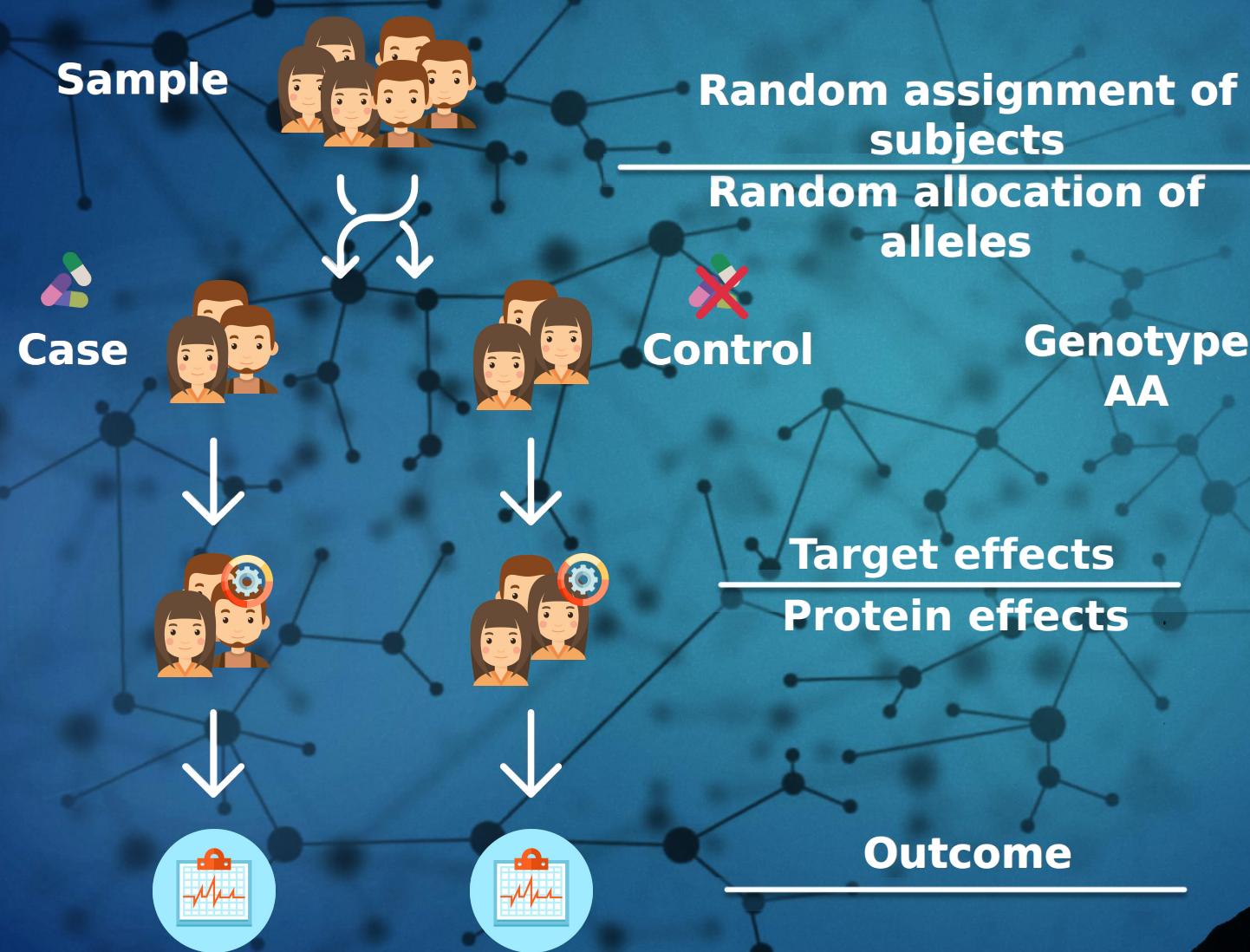


# Project Goal

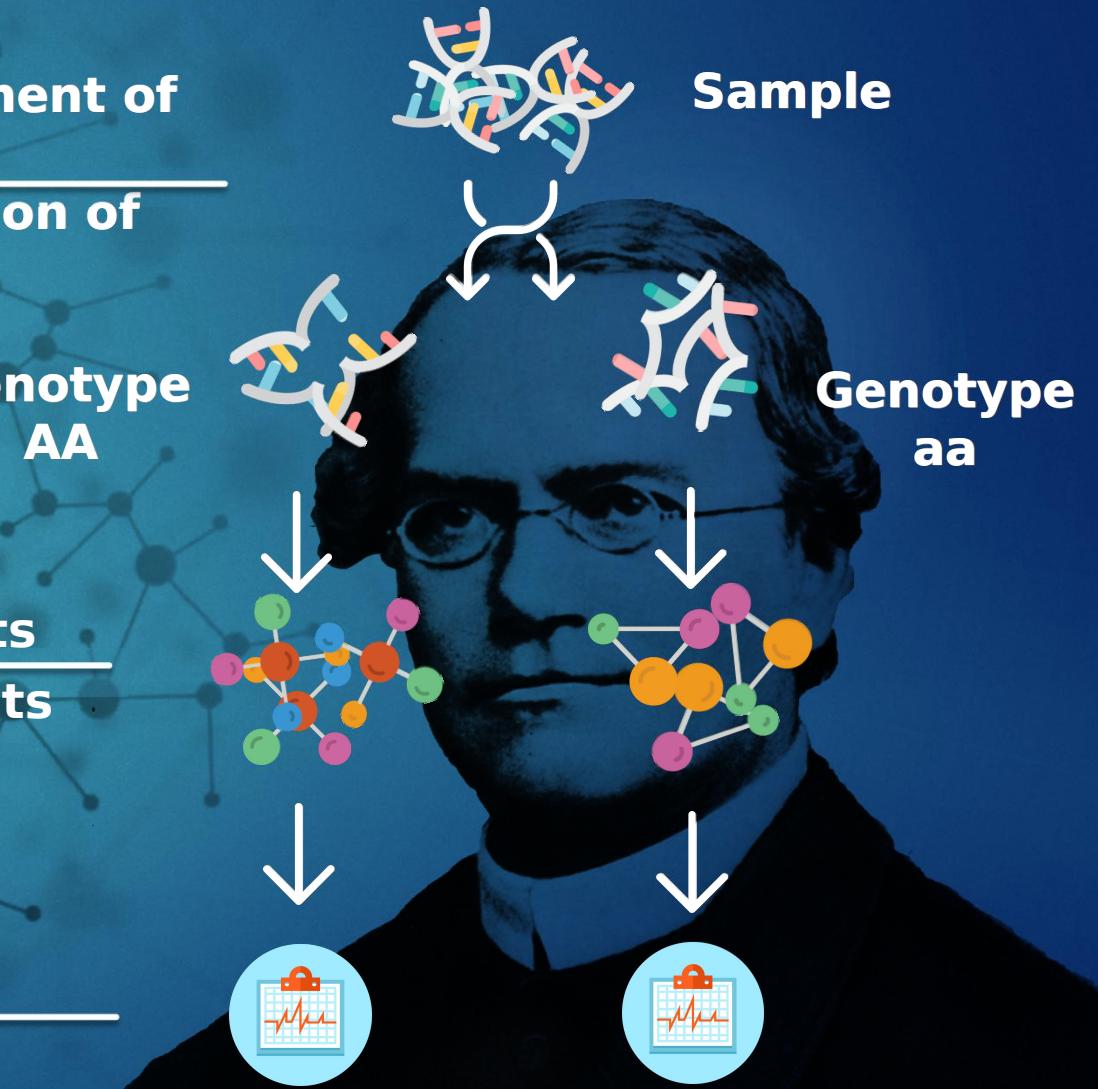
- ▶ Inspect > 440.000 clinical parameters and molecular mechanisms to;
- ▶ Identification of factors that cause or protect against Celiac Disease
- ▶ Quantify the impact of causal or protective factors



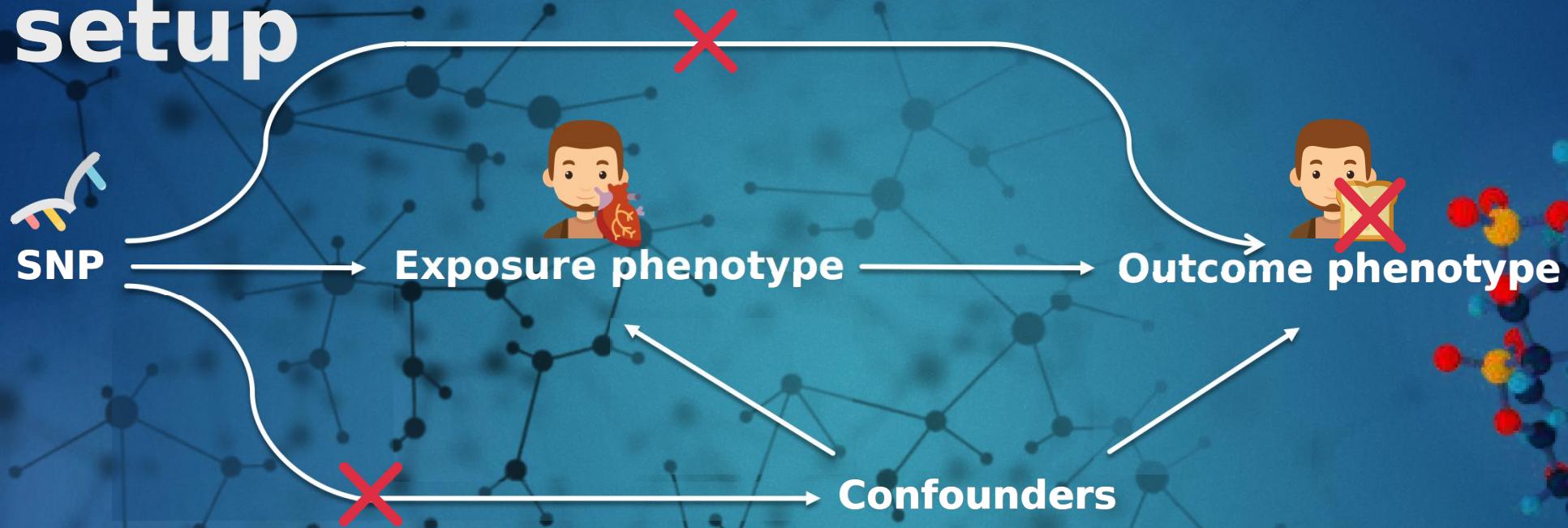
# Randomized Controlled Trials (RCT)



# Mendelian Randomization (MR)



# Mendelian Randomization setup



- ▶ Rules that need to be met before using a genetic variant in the MR framework
  - ▶ The genetic variant must be associated with the exposure of interest
  - ▶ The genetic variant must not be associated with confounders
  - ▶ The genetic variant may only affect the outcome through the exposure

# Methods

## Two-Sample MR



- Estimate causal effect between different samples
- Summary-level data from GWAS can be used



- Cannot test for confounding
- No overall causal estimate
- Assumes genetic variants are uncorrelated (not in linkage disequilibrium)



- Causal estimate between genetic variants

## Inverse-Variance Weighted method (IVW)

- Overall causal estimate between exposure and outcome
- Summary-level data from GWAS can be used

- Assumes causal estimates provide independent evidence (no correlation)
- Cannot test for confounding

- Overall causal estimate between exposure and outcome

## MR-egger method

- Can be deployed when the core assumptions do not hold.
- Can test for confounding (correlation between variants)
- Can test for a causal effect
- An estimate of the overall causal effect

- Needs 3 or more genetic variants
- Assumes genetic variants are uncorrelated
- Cannot distinguish between pleiotropy and a causal effect when genetic variants almost have equal estimates

- Overall causal estimate
- MR-egger causal test
- Overall pleiotropic effect

## Bidirectional MR

- Can determine when genetic variant exhibits primary effect on the exposure, or the effect is secondary to the outcome

- difficulty in the presence of genetic variants that influence each other

- MR analysis in both directions, that ascertains direction of causal relationship

# Data

	Type	Amount of Phenotypes	Direction
Celiac, Trynka 2011	GWAS Immunochip	1	Outcome
Celiac, Dubois 2010	GWAS Immunochip	1	Outcome
The NHGRI-EBI GWAS catalog	Published GWAS	2893	Exposure
MRbase Metabolite	GWAS on metabolites in whole blood	121	Exposure
MRbase Proteins	GWAS on protein levels whole blood	47	Exposure
MRbase Gene Expression levels (GTEX)	GWAS on gene identifiers in 44 different tissues	280380	Exposure
MRbase Methylation levels	GWAS on methylation levels in whole blood across 5 time points	151566	Exposure
additional downloaded GWAS	GWAS	1308	Exposure
UK.BIOBANK	GWAS	778	Exposure
Gene expression	GWAS	9744	Exposure
pQTL	GWAS	198	Exposure
<b>Total</b>		<b>447037</b>	

# Pipeline



**GWAS** →

SNP	effect_allele	beta	se	p
rs61733845	T	0.03536714	0.04432255	2.465e-06
rs1320571	A	0.01882175	0.04265126	6.590e-01
....	....	....	....	....

Celiac 2011. Gosia Trynka et al.



**GWAS** →

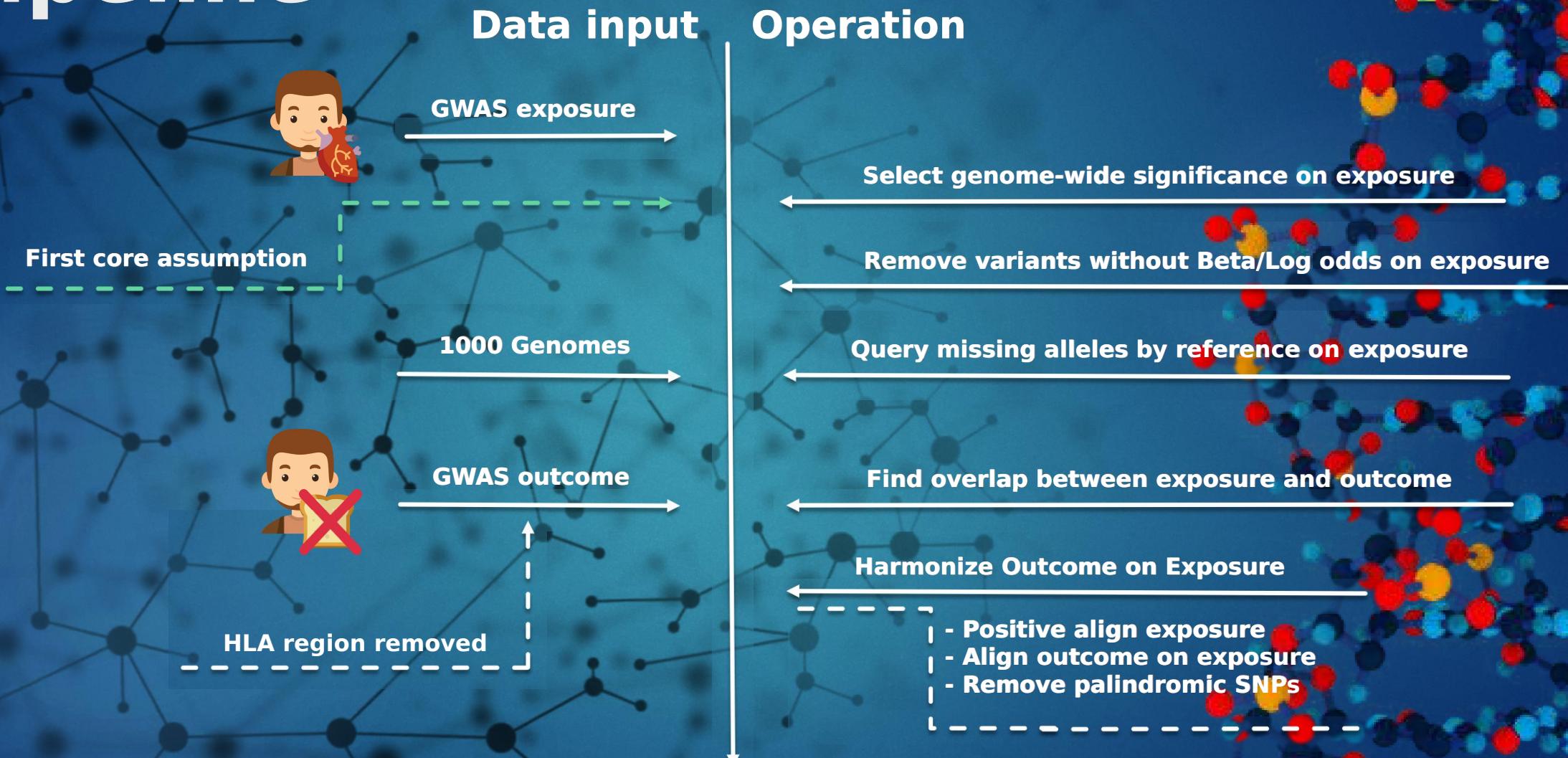
SNP	effect_allele	beta	se	p
rs314253	C	-0.0201900	0.002646	2.471e-14
rs7775397	G	-0.0369352	0.004845	2.721e-14
....	....	....	....	....

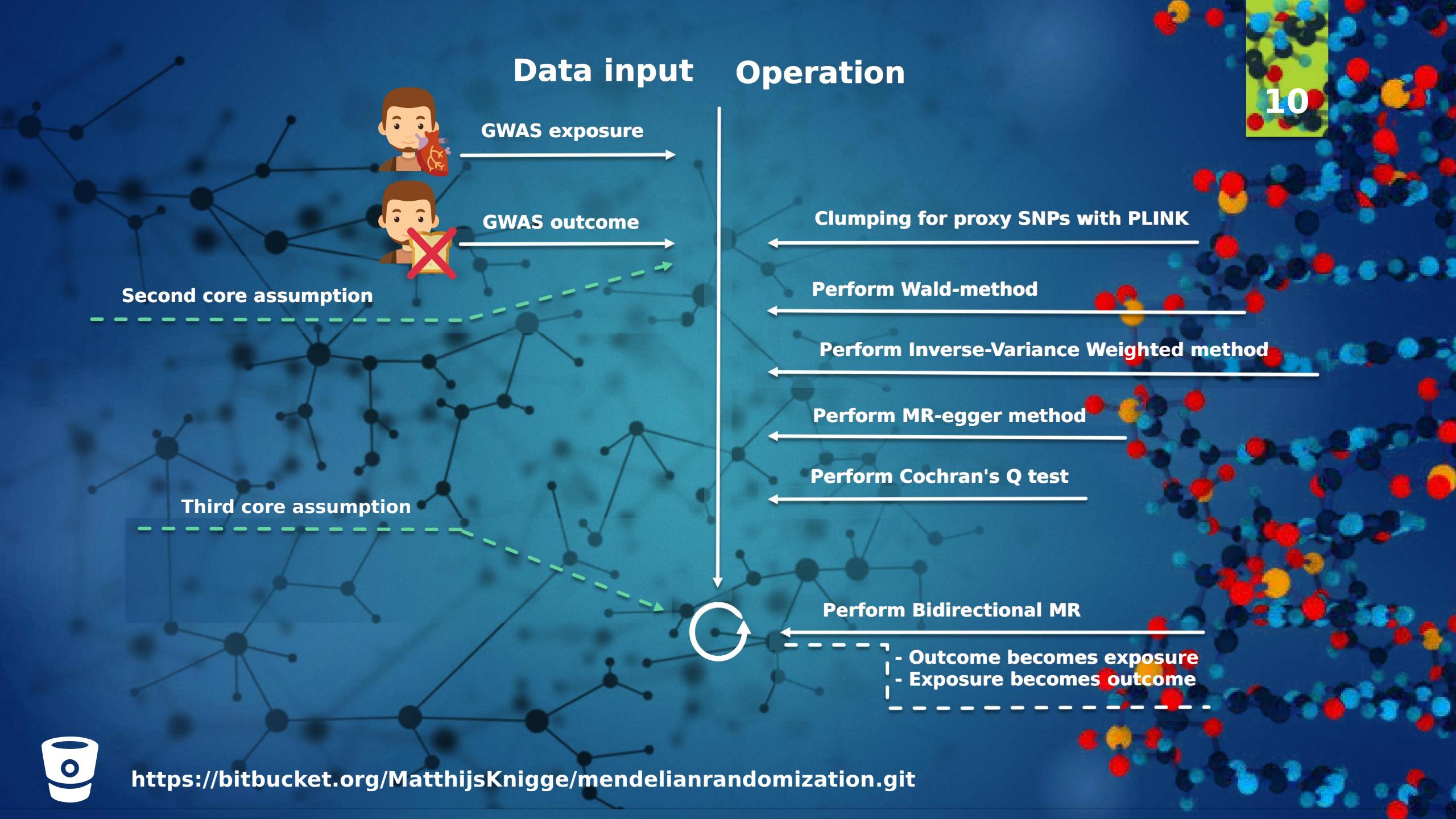
HDL, LDL, Triglycerides. Willer CJ et al. Discovery and refinement of loci associated with lipid levels. Nat. Genet. 2013. doi:10.1038/ng.2797

**Outcome = Celiac Disease, Exposure = HDL, LDL, Triglycerides**

# Pipeline

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# Preliminary results

## ► Before FDR correction

Category	Celiac 2011 ~ Exposure	Celiac 2010 ~ Exposure	Exposure ~ Celiac 2011, *	Exposure ~ Celiac 2010, *
Clinical Phenotype	1388	889	1990	1982
Metabolites	76	1	0	0
Proteins	3	3	1	1
Gene Expression	3461	4992	65	45
Methylation	3750	0	0	0

## ► After FDR correction

Category	Celiac 2011 ~ Exposure	Celiac 2010 ~ Exposure	Exposure ~ Celiac 2011, *	Exposure ~ Celiac 2010, *
Clinical Phenotype	166	32	208	145
Metabolites	0	0	0	0
Proteins	0	0	0	0
Gene Expression	25	14	1	0
Methylation	0	0	0	0

\* MRbase not analysed in reverse yet

# Preliminary results, Forward

- Celiac 2011, Gosia Trynka et al. ~ Exposure
  - 185 significant potential exposures

- Top 10

Exposure	IVW	Egger	nSNP
Type 1 Diabetes Meta Analysis	1.589504e-24	7.291180e-01	56
Type 1 Diabetes	2.052863e-20	7.348035e-01	50
Inflammatory Bowel Disease	8.241646e-12	6.301454e-01	59
Ulcerative colitis	1.149234e-10	7.566844e-02	36
Crohns disease	1.791261e-10	7.408463e-01	48
plateletcrit	3.435332e-10	7.110506e-08	67
IL18RAP (gene expression)	9.457623e-10	9.280580e-01	9
platelet count	2.839670e-09	7.039575e-06	77
Packed cell volume	4.735074e-09	3.384431e-01	5
Primary biliary cirrhosis	7.208134e-09	9.521848e-01	27

# Preliminary results, Reverse

- ▶ Exposure ~ Celiac 2011, Gosia Trynka et al.
- ▶ 209 significant potential exposures

- ▶ Top 10

Exposure	IVW	Egger	nSNP
Platelet crit	3.575738e-66	5.761514e-07	48
thyroid problem (not cancer)	8.414018e-60	3.193874e-01	48
Platelet count	2.033008e-51	4.996859e-01	48
Type 1 Diabetes Meta Analysis	7.136551e-50	6.925841e-01	48
Type 1 Diabetes	2.826052e-45	8.576514e-01	48
Eosinophil count	5.173730e-38	9.888424e-01	48
Lymphocyte percentage	8.730661e-36	2.770322e-01	48
Inflammatory Bowel Disease	3.094949e-34	5.491841e-01	48
E03 Other hypothyroidism	1.727432e-33	5.923195e-01	48
Eosinophil percentage	3.381566e-31	9.790395e-01	48

# Preliminary results, Forward

- Celiac 2010, Patrick Dubois et al. ~ Exposure
  - 32 significant potential exposures

- Top 10

Exposure	IVW	Egger	nSNP
Type 1 Diabetes Meta Analysis	4.859082e-22	1.487445e-01	33
Type 1 Diabetes	4.249713e-20	9.059520e-02	26
IL18RAP (gene expression)	1.339638e-11	1.274314e-02	9
CMTM7 (gene expression)	4.408545e-08	8.447033e-01	7
Inflammatory Bowel Disease	4.226268e-06	8.078122e-01	42
Ulcerative colitis	3.518295e-05	2.062526e-01	27
Primary biliary cirrhosis	3.768606e-05	9.328913e-01	20
Coronary artery disease mi additive	1.210912e-04	7.857370e-01	15
Crohns disease	1.678295e-04	8.598359e-01	36
Rheumatoid Arthritis	1.951175e-04	3.587785e-01	4

# Preliminary results, Reverse

- ▶ Exposure ~ Celiac 2010, Patrick Dubois et al.
- ▶ 145 significant potential exposures

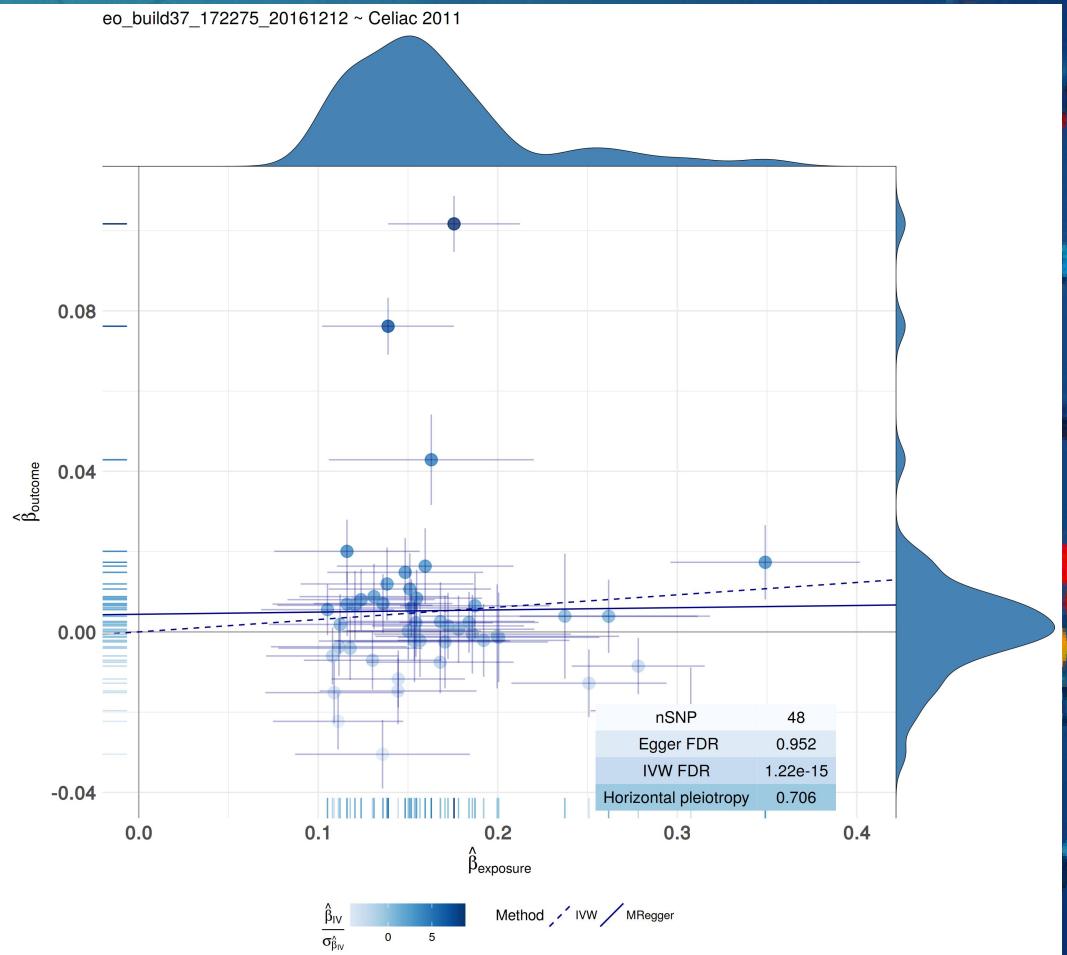
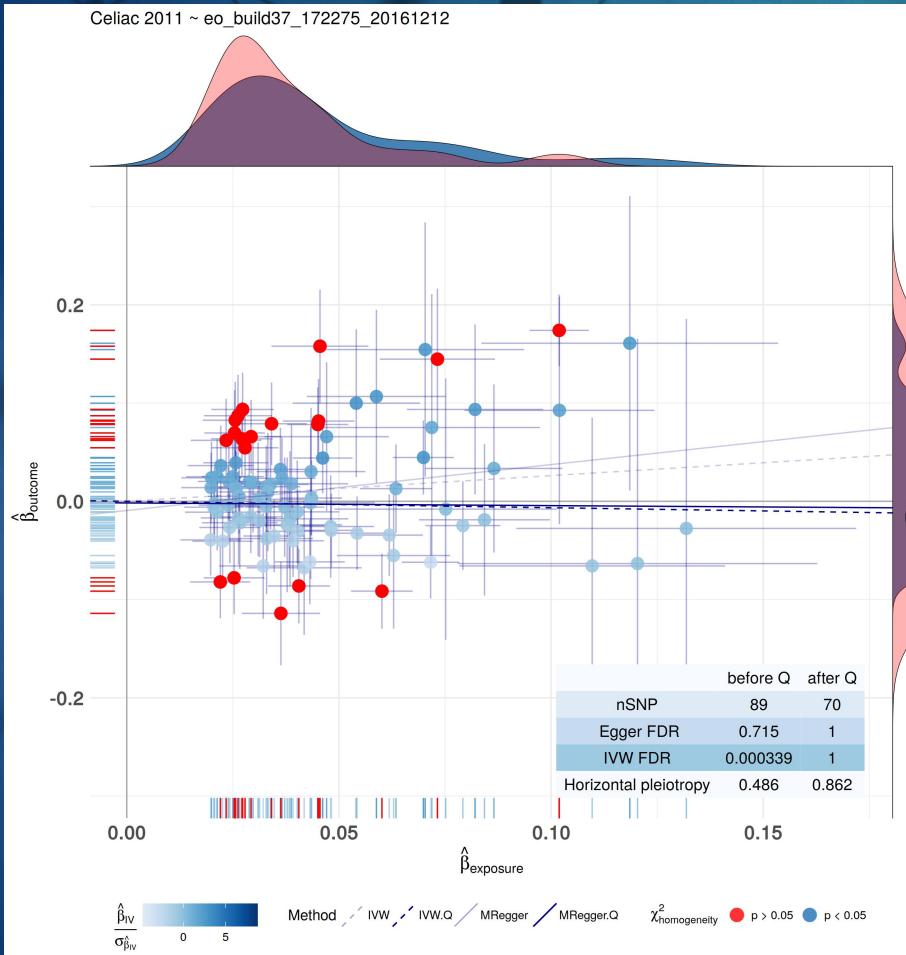
- ▶ Top 10

Exposure	IVW	Egger	nSNP
K90 Intestinal malabsorption	5.812490e-63	0.3689493413	10
Platelet crit	4.181290e-51	0.0001414908	10
thyroid problem (not cancer)	2.466238e-42	0.0976389945	10
Platelet count	9.184329e-40	0.0001414908	10
Eosinophil count	1.092300e-26	0.0004678592	10
E03 Other hypothyroidism	2.466970e-23	0.6321703636	10
E00-E07 Disorders of thyroid gland	2.754765e-20	0.6321703636	10
Eosinophil percentage	1.102760e-17	0.0011727342	10
K90-K93 Other diseases of the digestive system	2.370714e-17	0.9673294238	10
Inflammatory Bowel Disease	3.222523e-16	0.6983328248	10

# Preliminary results Bidirectional MR

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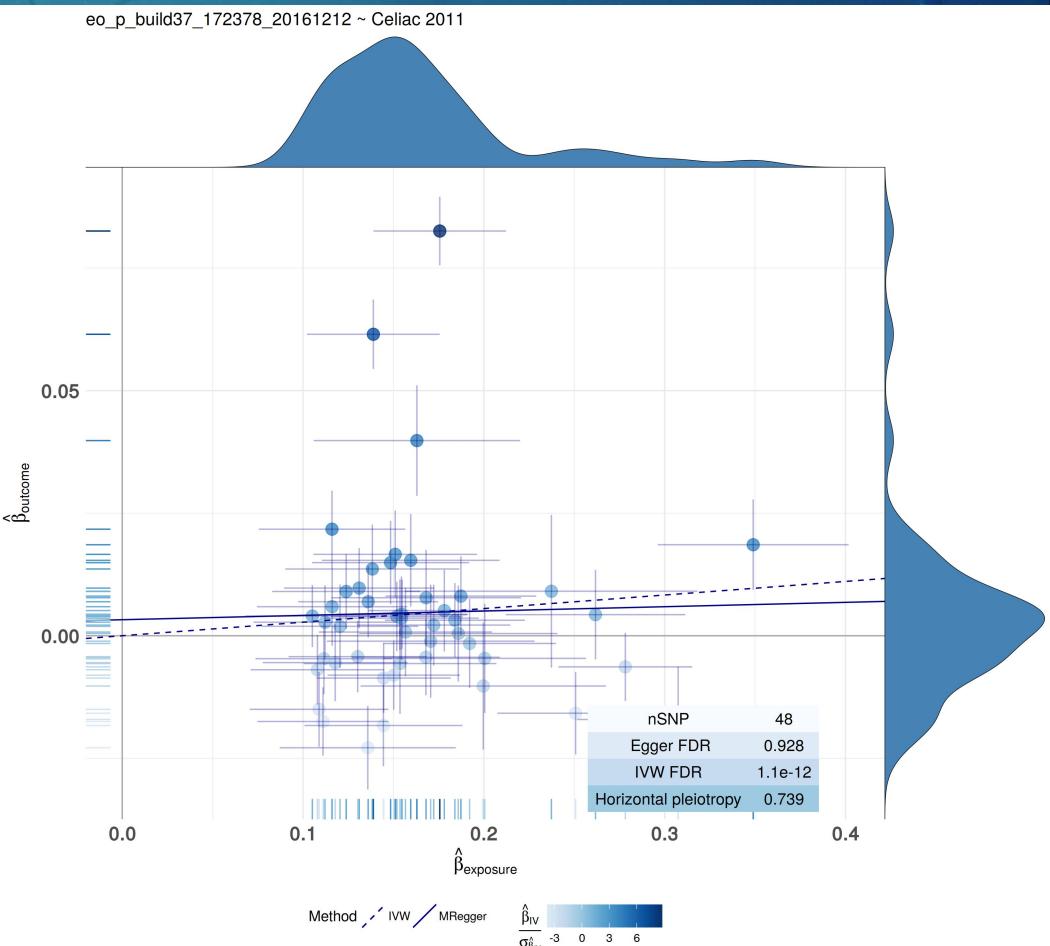
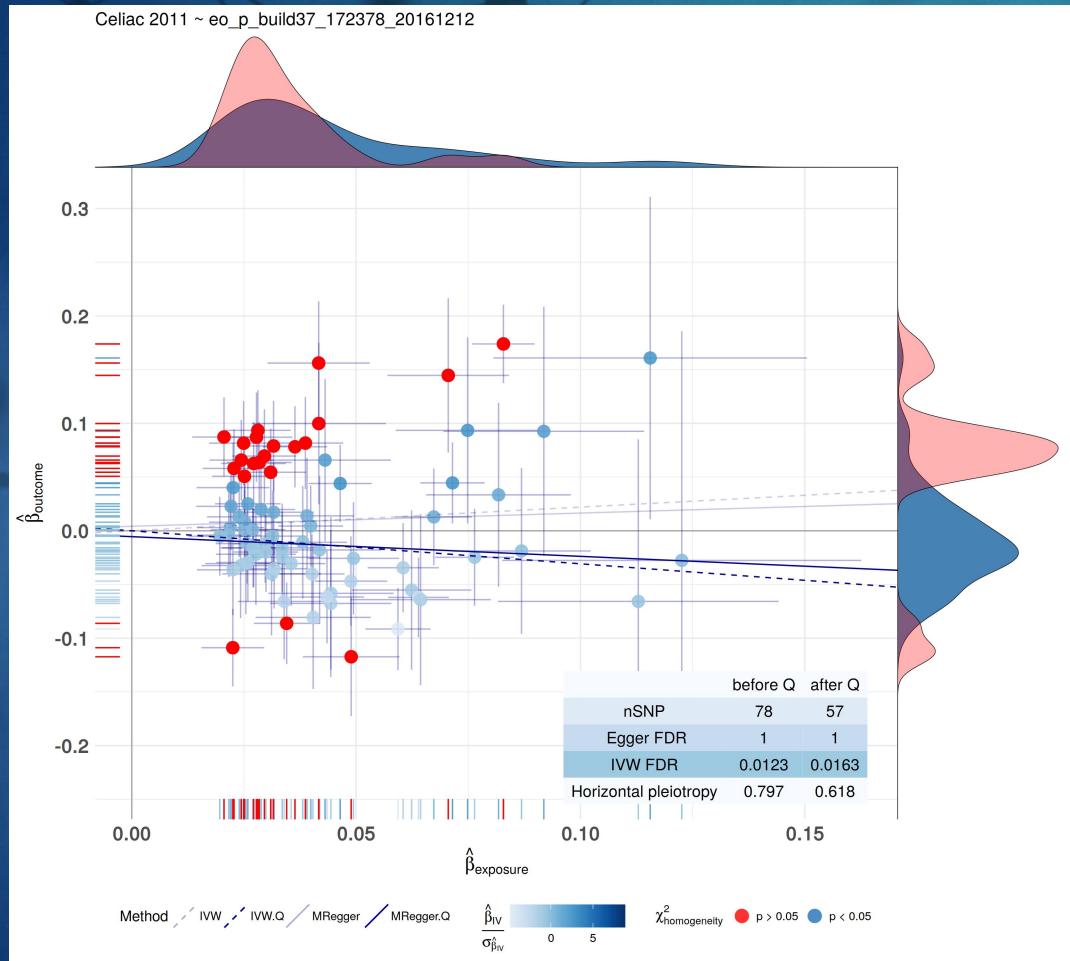
## Celiac 2011, Gosia Trynka et al.



# Preliminary results Bidirectional MR

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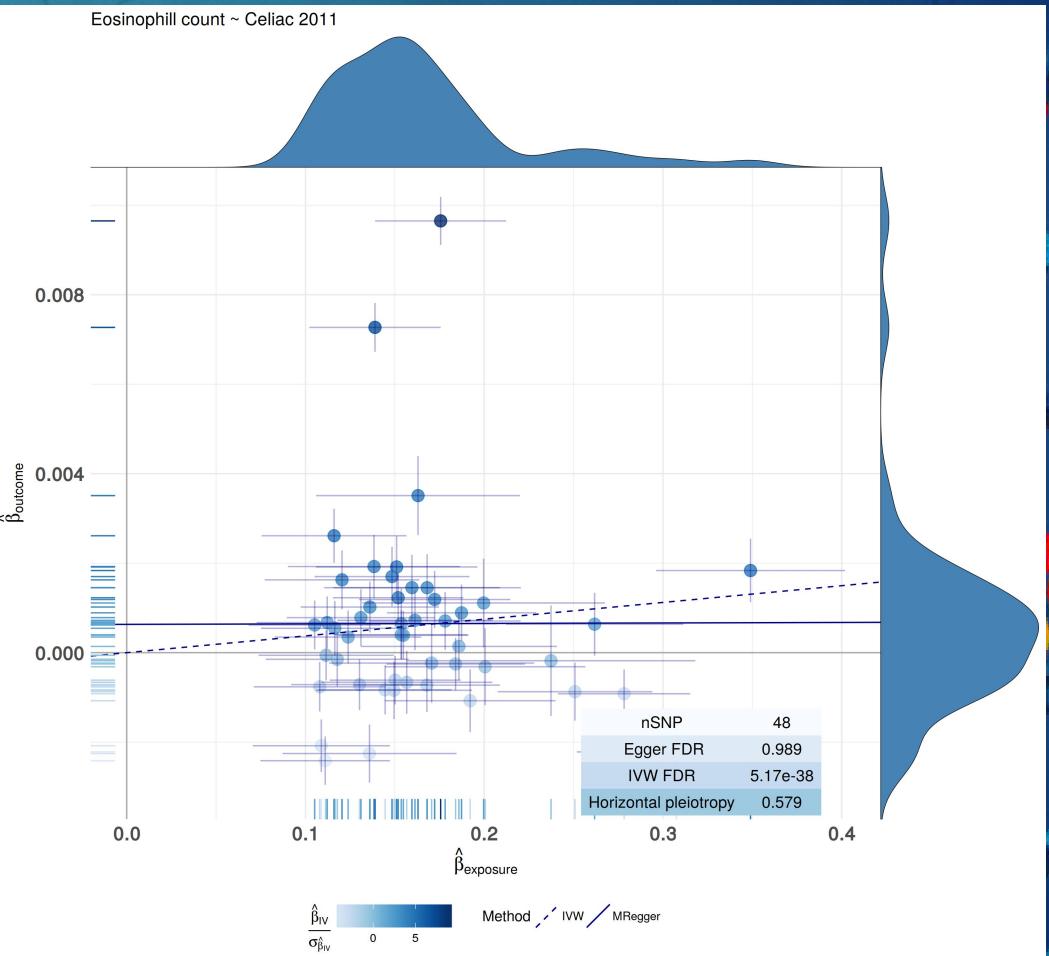
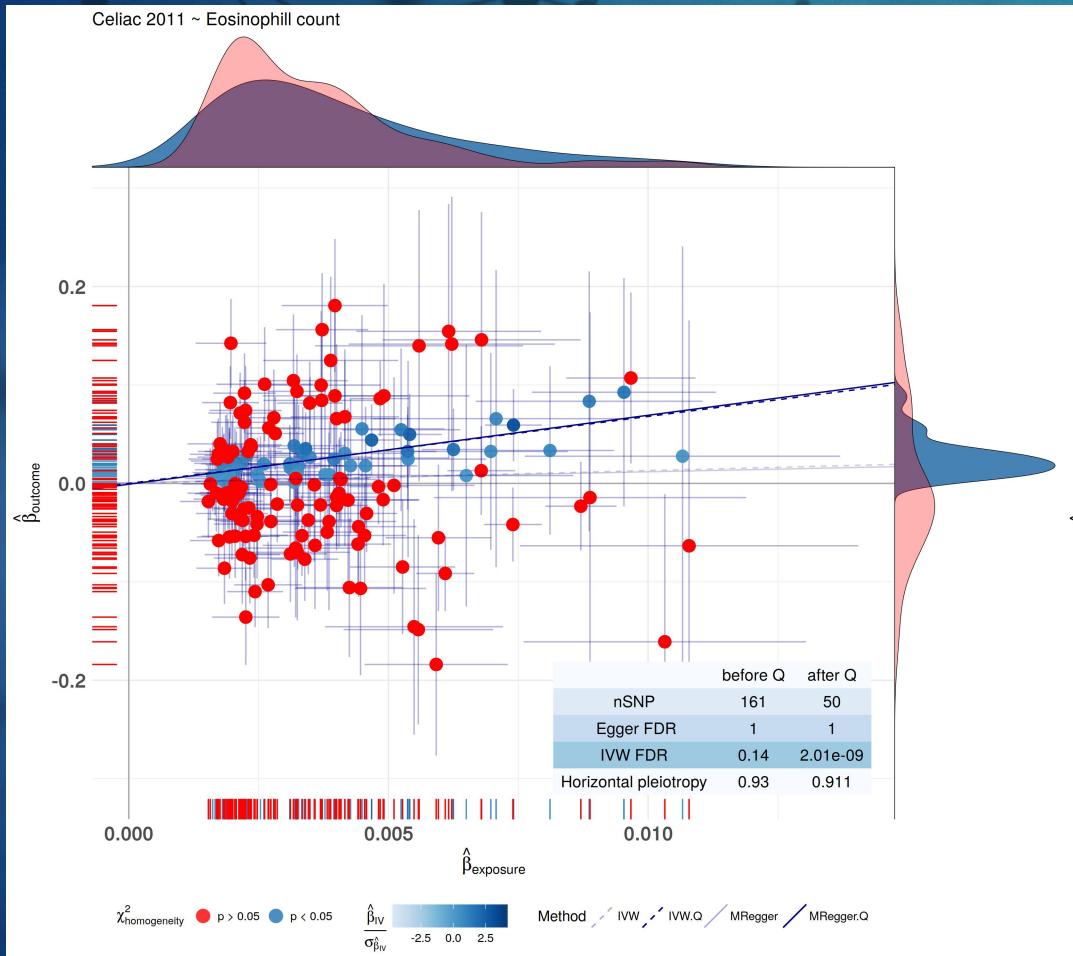
## Celiac 2011, Gosia Trynka et al.



# Preliminary results Bidirectional MR

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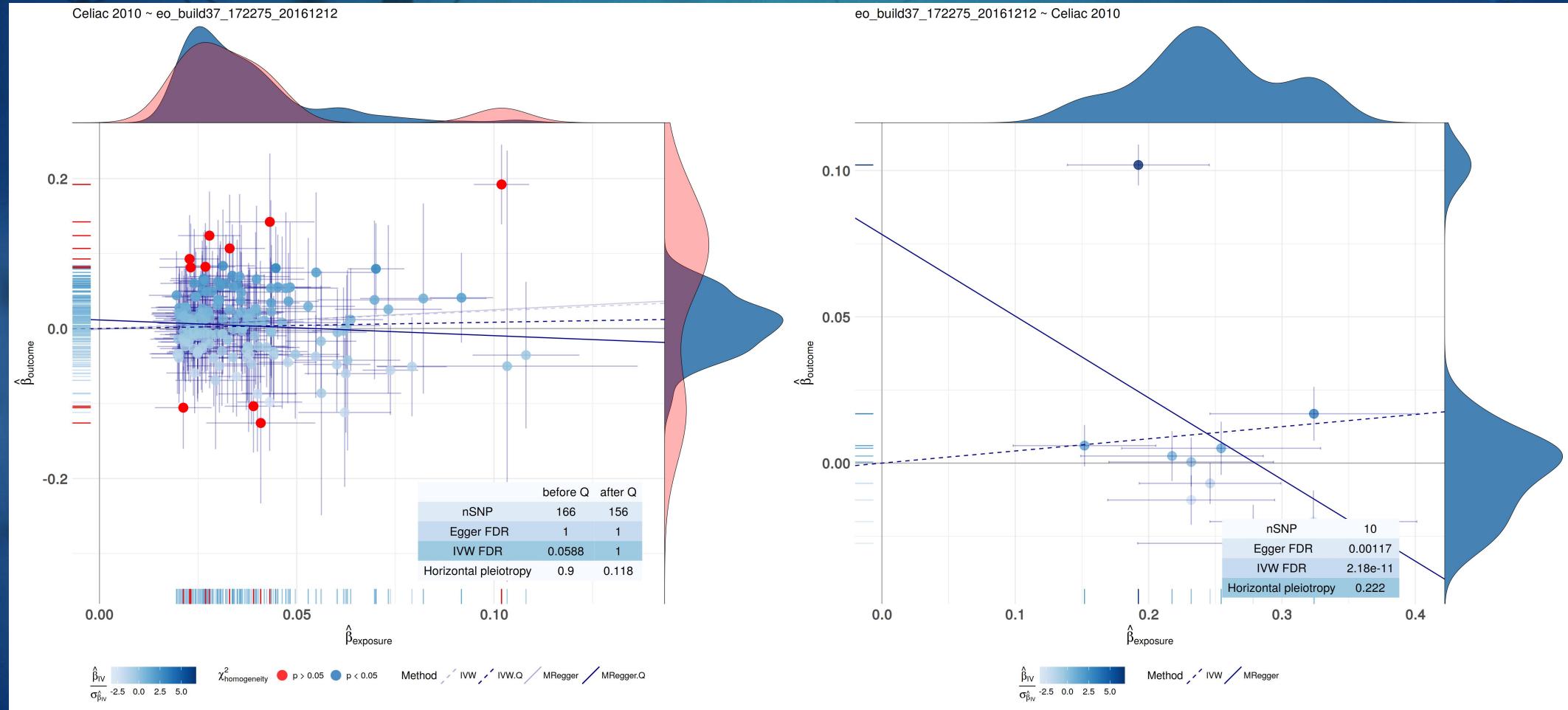
## Celiac 2011, Gosia Trynka et al.



# Preliminary results Bidirectional MR

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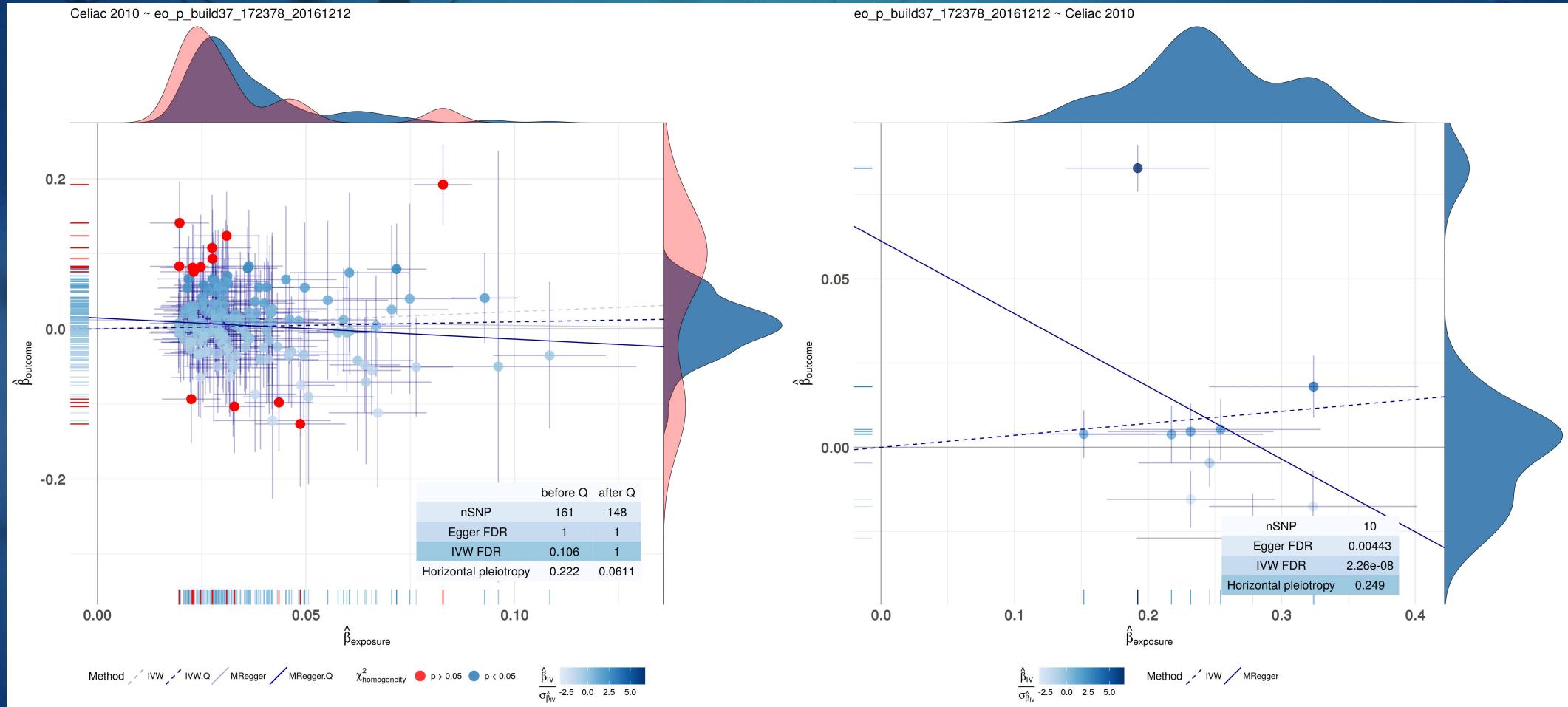
## Celiac 2010, Patrick Dubois et al.



# Preliminary results Bidirectional MR

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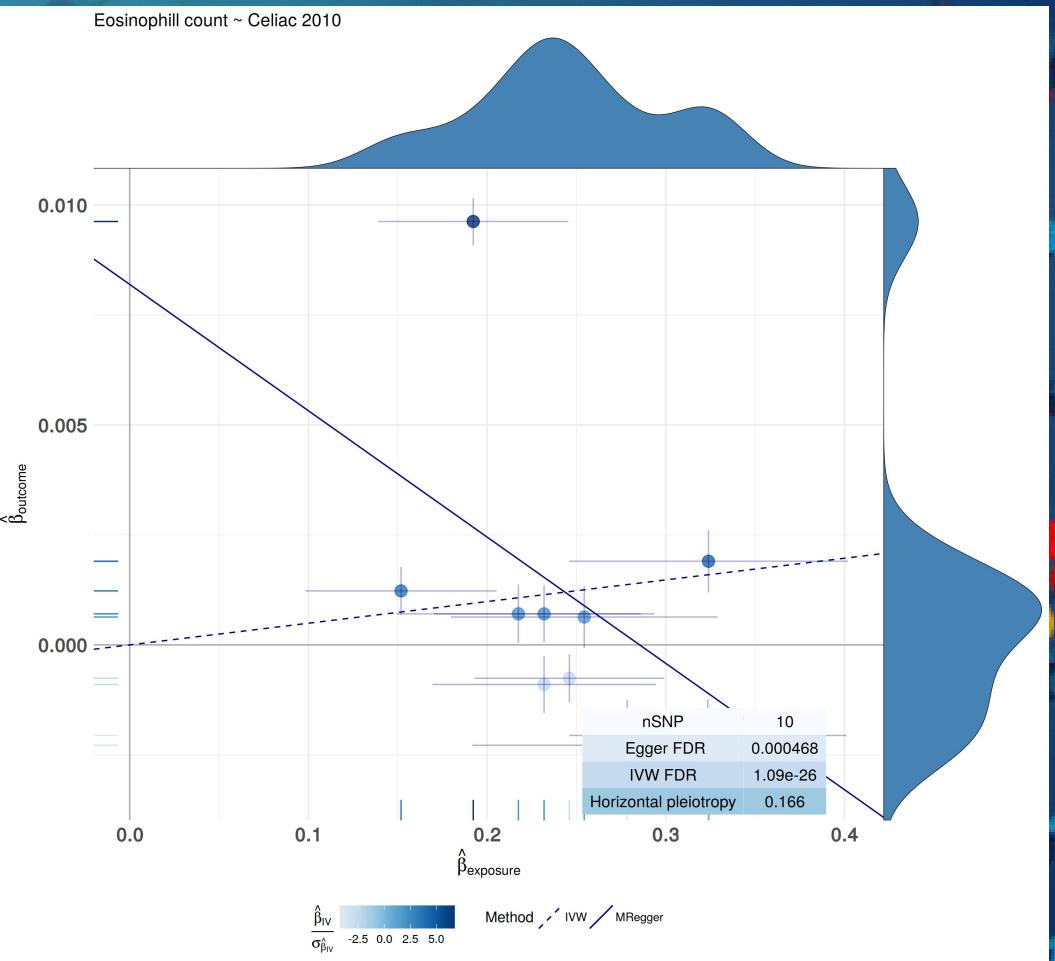
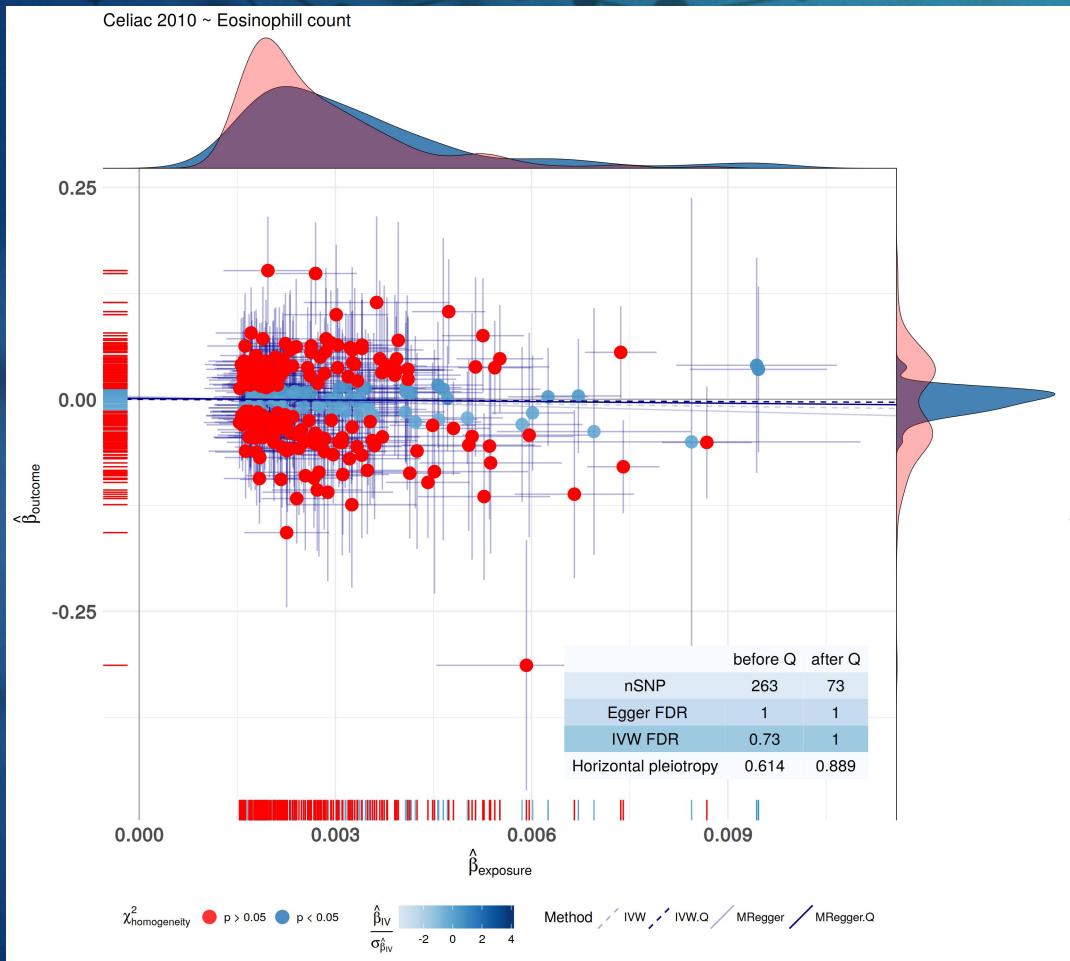
## Celiac 2010, Patrick Dubois et al.



# Preliminary results Bidirectional MR

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► Celiac 2010, Patrick Dubois et al.



# Conclusions

## ► Celiac 2011, Gosia Trynka et al. ~ Exposure, Forward

Phenotype	Conclusion
Type 1 Diabetes Meta Analysis	<b>Lower risk for Type 1 Diabetes protects for Celiac Disease</b>
Type 1 Diabetes	<b>Lower risk for Type 1 Diabetes protects for Celiac Disease</b>
Inflammatory Bowel Disease	<b>Lower risk for Inflammatory Bowel Disease protects for Celiac Disease</b>
Ulcerative colitis	<b>Lower risk for Ulcerative colitis protects for Celiac Disease</b>
Crohns disease	<b>Lower risk for Crohns disease protects for Celiac Disease</b>
plateletcrit	<b>Lower level of plateletcrit protects for Celiac Disease</b>
IL18RAP (gene expression)	<b>Higher level of IL18RAP expression protects for Celiac Disease</b>
platelet count	<b>Lower level of platelet count protects for Celiac Disease</b>
Packed cell volume	<b>Lower level of packed cell volume protects for Celiac Disease</b>
Primary biliary cirrhosis	<b>Lower risk for Primary biliary cirrhosis protects for Celiac Disease</b>

# Conclusions

## ► Exposure ~ Celiac 2011, Gosia Trynka et al., Reverse

Phenotype	Conclusion
Platelet crit	<b>Lower risk for Celiac Disease protects for higher levels of Platelet crit</b>
thyroid problem (not cancer)	<b>Lower risk for Celiac Disease protects for thyroid problem (not cancer)</b>
Platelet count	<b>Seemingly a higher risk for Celiac Disease protects for higher levels in Platelet count, but this is driver by a subset of SNPs</b>
Type 1 Diabetes Meta Analysis	<b>Lower risk for Celiac Disease protects for Type 1 Diabetes</b>
Type 1 Diabetes	<b>Lower risk for Celiac Disease protects for Type 1 Diabetes</b>
Eosinophil count	<b>Lower risk for Celiac Disease protects for higher Eosinophil count levels</b>
Lymphocyte percentage	<b>Higher risk for Celiac Disease protects for lower Lymphocyte percentage</b>
Inflammatory Bowel Disease	<b>Lower risk for Celiac Disease protects for Inflammatory Bowel Disease</b>
E03 Other hypothyroidism	<b>Lower risk for Celiac Disease protects for Other hypothyroidism</b>
Eosinophil percentage	<b>Low risk for Celiac Disease protects for lower Eosinophil percentage</b>

# Conclusions

## ► Celiac 2010, Patrick Dubois et al. ~ Exposure, Forward

Phenotype	Conclusion
Type 1 Diabetes Meta Analysis	
Type 1 Diabetes	
IL18RAP (gene expression)	
CMTM7 (gene expression)	
Inflammatory Bowel Disease	
Ulcerative colitis	
Primary biliary cirrhosis	
Coronary artery disease mi additive	
Crohns disease	
Rheumatoid Arthritis	

# Conclusions

## ► Exposure ~ Celiac 2010, Patrick Dubois et al., Reverse

Phenotype	Conclusion
K90 Intestinal malabsorption	
Platelet crit	
thyroid problem (not cancer)	
Platelet count	
Eosinophil count	
E03 Other hypothyroidism	
E00-E07 Disorders of thyroid gland	
Eosinophil percentage	
K90-K93 Other diseases of the digestive system	
Inflammatory Bowel Disease	

# Future steps

- ▶ Use PreventCD cohort to validate by predicting causal or protective factors identified
- ▶ Pathway analysis on SNPs in significant hits
- ▶ Network Mendelian Randomization