Celiac Disease Triggers

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Overview

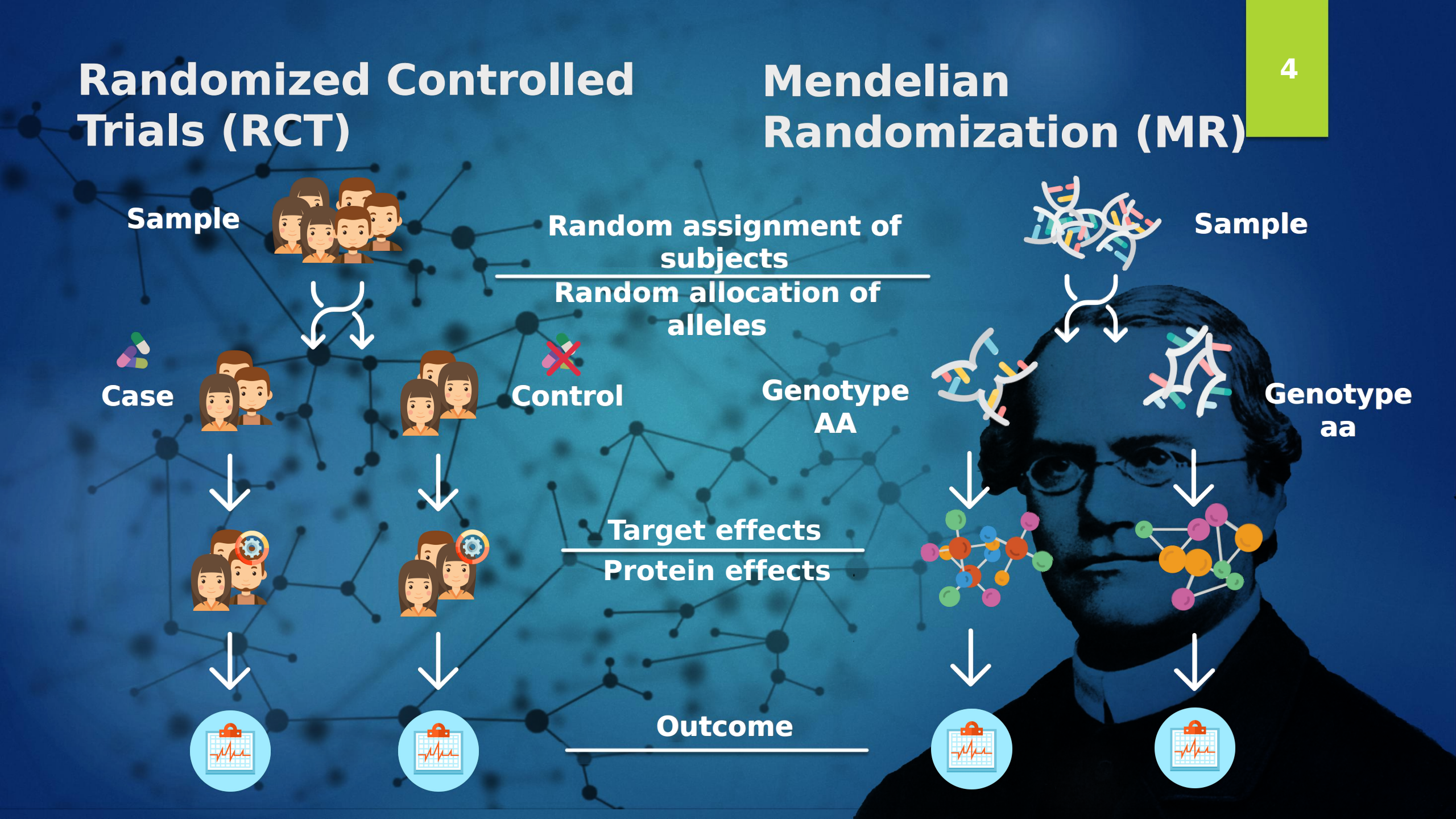
- Project goal
- Mendelian Randomization
- Methods
- Data
- Pipeline
- Preliminary results
- Future steps

Project Goal

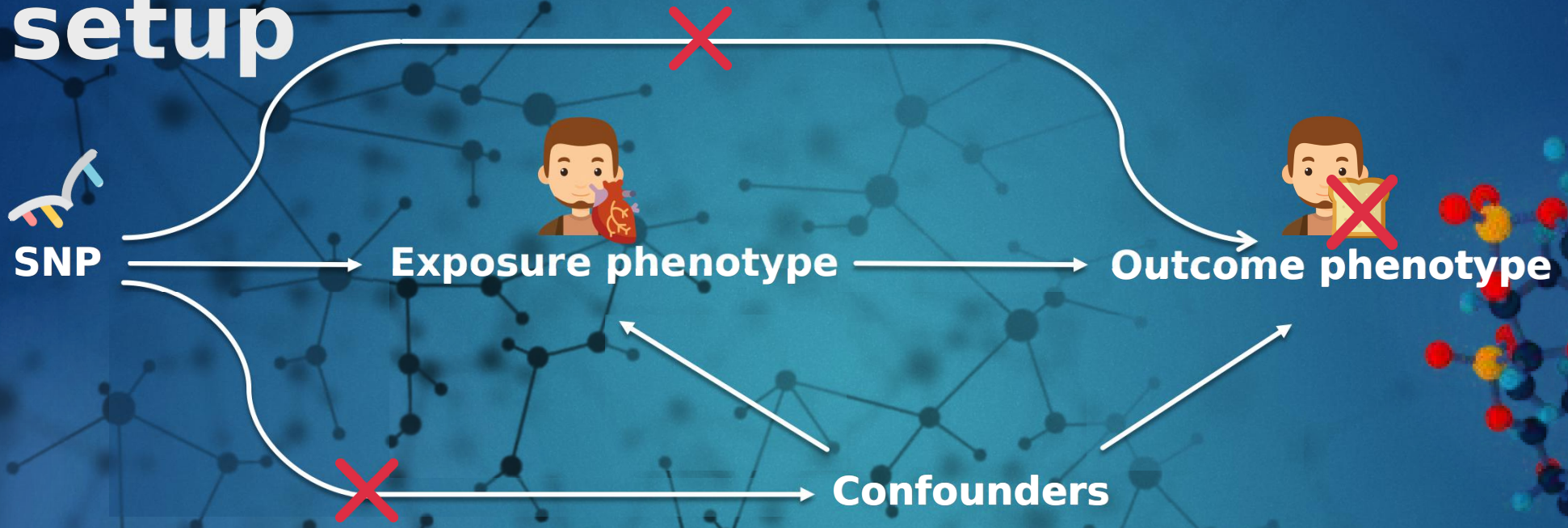
- Inspect > 80.000 clinical parameters and molecular mechanisms to;
  - Identification of factors that cause or protect against Celiac Disease
  - Quantify the impact of causal or protective factors

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	Publicated 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	32432	Exposure
MRbase Methylation levels	GWAS on methylation levels in whole blood across 5 time points	33256	Exposure
additional downloaded GWAS	GWAS	1308	Exposure
UK.BIOBANK	GWAS	778	Exposure
Gene expression	GWAS	9744	Exposure
pQTL	GWAS	198	Exposure
Total		**80779**	

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.

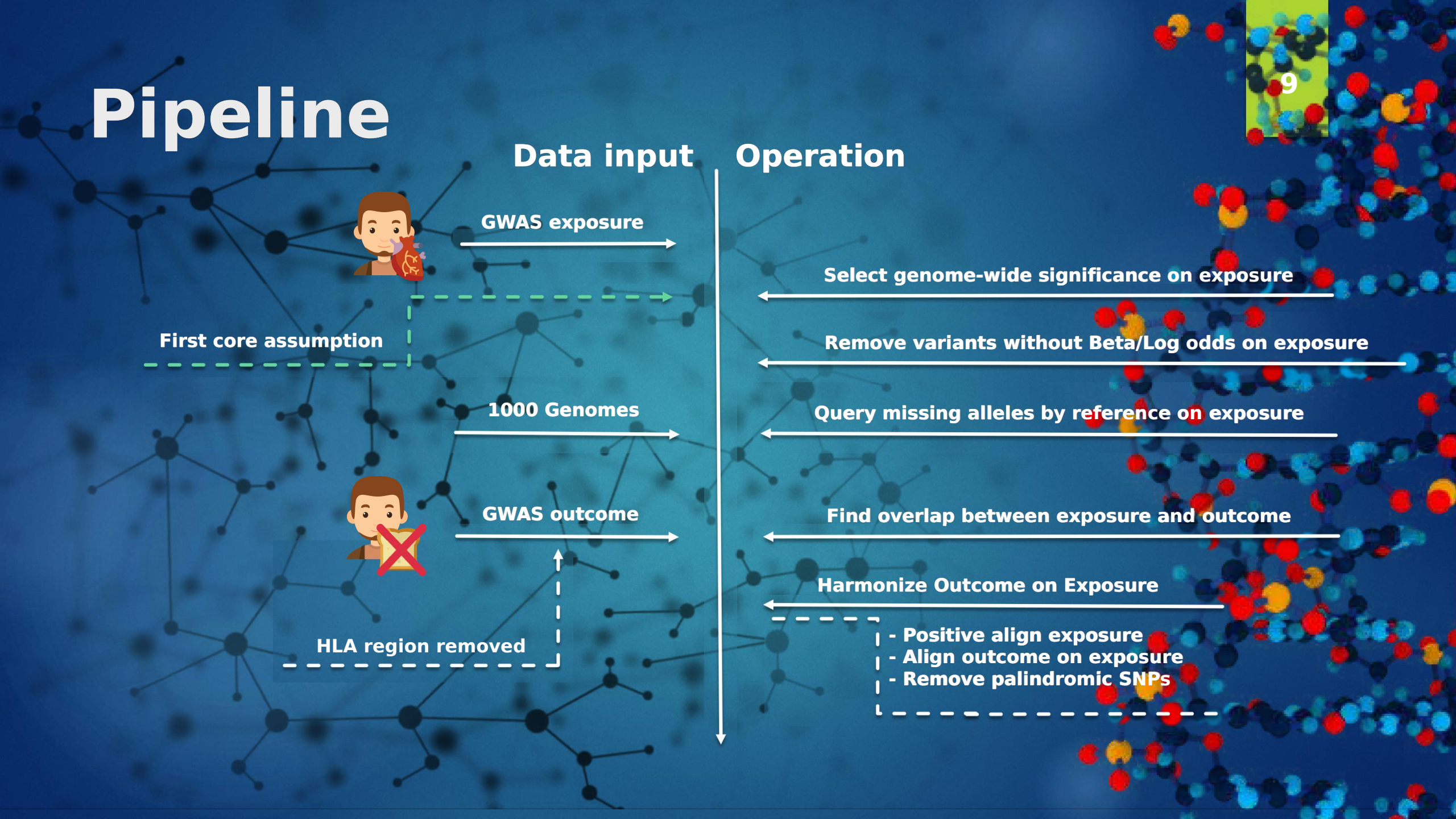
GWAS

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


Data input
Operation
GWAS exposure
Select genome-wide significance on exposure
First core assumption
Remove variants without Beta/Log odds on exposure
1000 Genomes
Query missing alleles by reference on exposure
GWAS outcome
Find overlap between exposure and outcome
Harmonize Outcome on Exposure
- Positive align exposure
- Align outcome on exposure
- Remove palindromic SNPs
HLA region removed

Data input

Operation

GWAS exposure

GWAS outcome

Second core assumption

Third core assumption

Clumping for proxy SNPs with PLINK

Perform Wald-method

Perform Inverse-Variance Weighted method

Perform MR-egger method

Perform Cochran's Q test

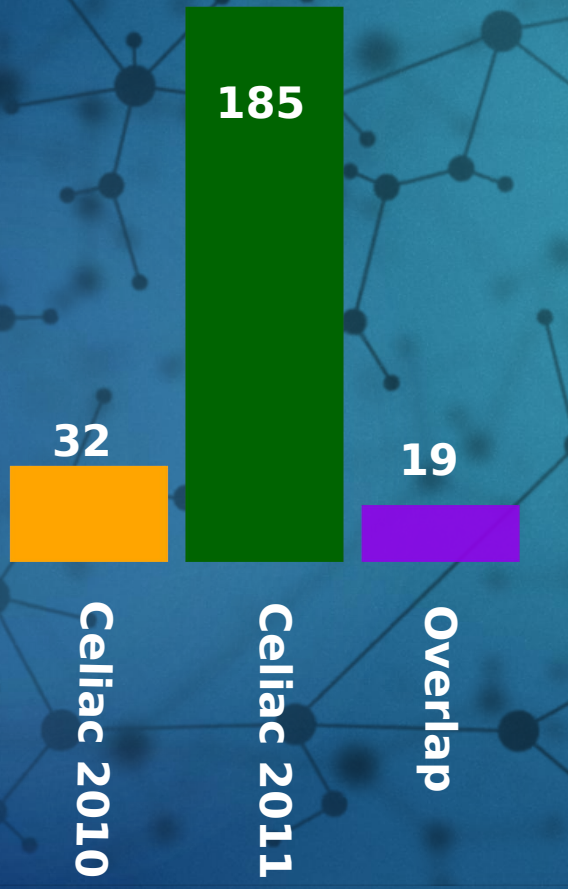
Perform Bidirectional MR

- Outcome becomes exposure
- Exposure becomes outcome

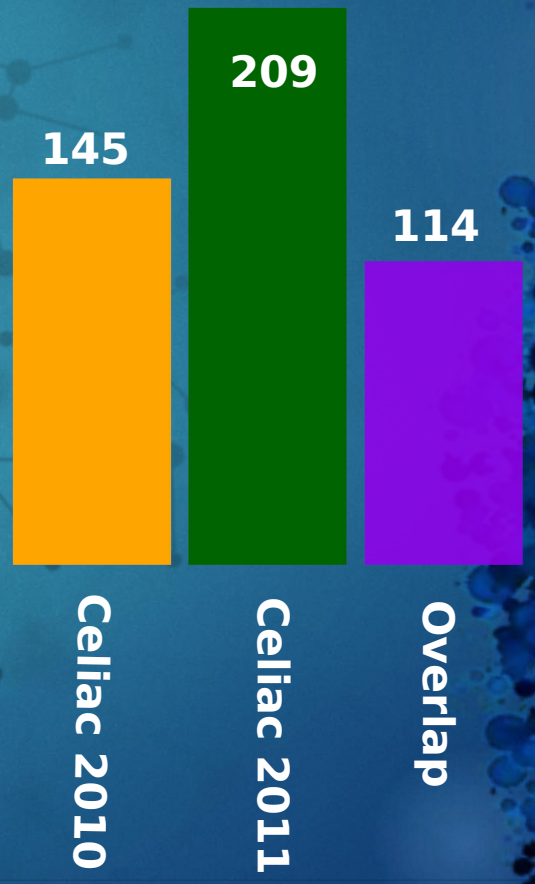
https://bitbucket.org/MatthijsKnigge/mendelianrandomization.git

Preliminary results

- Direction: Celiac ~ Exposure
- Considerd FDR < .05 for MR-egger | IVW



- Direction: Exposure ~ Celiac
- Considerd FDR < .05 for MR-egger | IVW



Preliminary results

- Celiac 2011, Gosia Trynka et al. ~ Exposure
  - 185 significant potential exposures
- Top 10

Exposure	IVW	Egger	nSNP
T1D_meta_2015_25751624_hg19	1.455825e-24	6.978655e-01	56
Type1 Diabetes	2.467783e-20	6.982018e-01	50
Inflammatory Bowel Disease	1.698417e-11	6.096369e-01	59
Ulcerative colitis	2.142757e-10	5.890897e-02	36
Crohns disease	3.229960e-10	7.074762e-01	48
plateletcrit	5.768434e-10	4.884381e-08	67
IL18RAP	9.457623e-10	9.280580e-01	9
lymphocytes count	1.914990e-09	6.584747e-01	61
platelet count	4.433270e-09	4.835657e-06	77
Packed cell volume	7.589489e-09	3.143014e-01	5

Preliminary results

- Exposure ~ Celiac 2011, Gosia Trynka et al.
  - 209 significant potential exposures
- Top 10

Exposure	IVW	Egger	nSNP
Platelet crit	3.575738e-66	9.174225e-01	48
thyroid problem (not cancer)	8.414018e-60	3.193874e-01	48
Platelet count	2.033008e-51	4.996859e-01	48
T1D_meta_2015_25751624_hg19	7.136551e-50	6.925841e-01	48
Type1 Diabetes	2.826052e-45	8.576514e-01	48
Eosinophill count	5.173730e-38	9.888424e-01	48
Ease of skin tanning	4.506061e-36	6.760805e-15	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

Preliminary results

- Celiac 2010, Patrick Dubois et al. ~ Exposure
  - 32 significant potential exposures
- Top 10

Exposure	IVW	Egger	nSNP
T1D_meta_2015_25751624_hg19	8.154147e-21	6.682035e-01	33
Type1 Diabetes	7.131549e-19	5.441627e-01	26
Inflammatory Bowel Disease	7.092205e-05	9.985827e-01	42
Ulcerative colitis	5.904139e-04	7.836626e-01	27
Primary biliary cirrhosis	6.324192e-04	9.985827e-01	20
Coronary artery disease mi additive	2.032062e-03	9.869497e-01	15
Crohns disease	2.816389e-03	9.985827e-01	36
eosinophils+basophils count	9.680962e-03	6.728569e-01	161
eosinophils count	1.797706e-02	7.925704e-01	166
plateletcrit	1.558333e-01	2.015023e-03	198

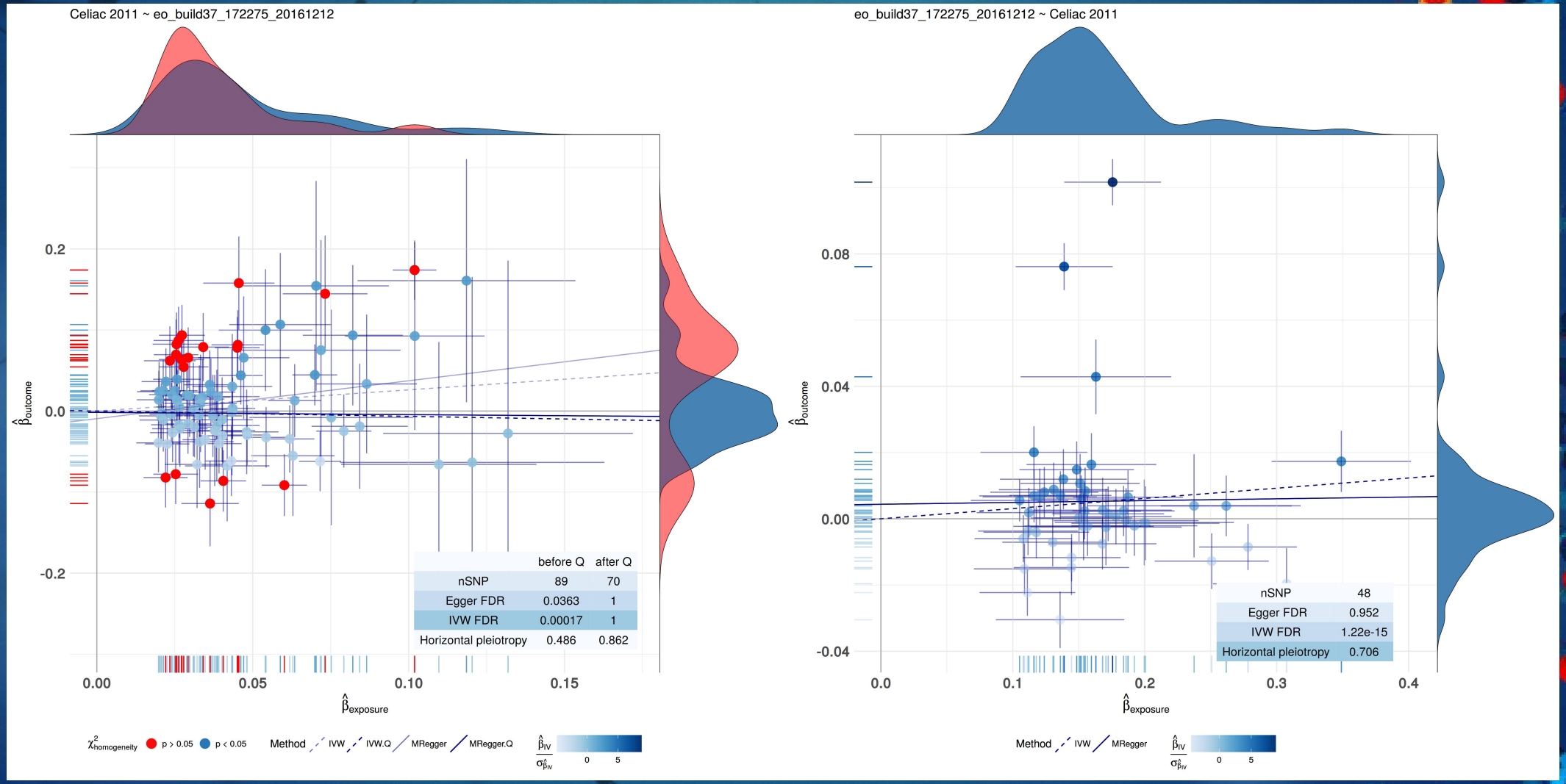
Preliminary results

- 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
Eosinophill 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
Eosinophill 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

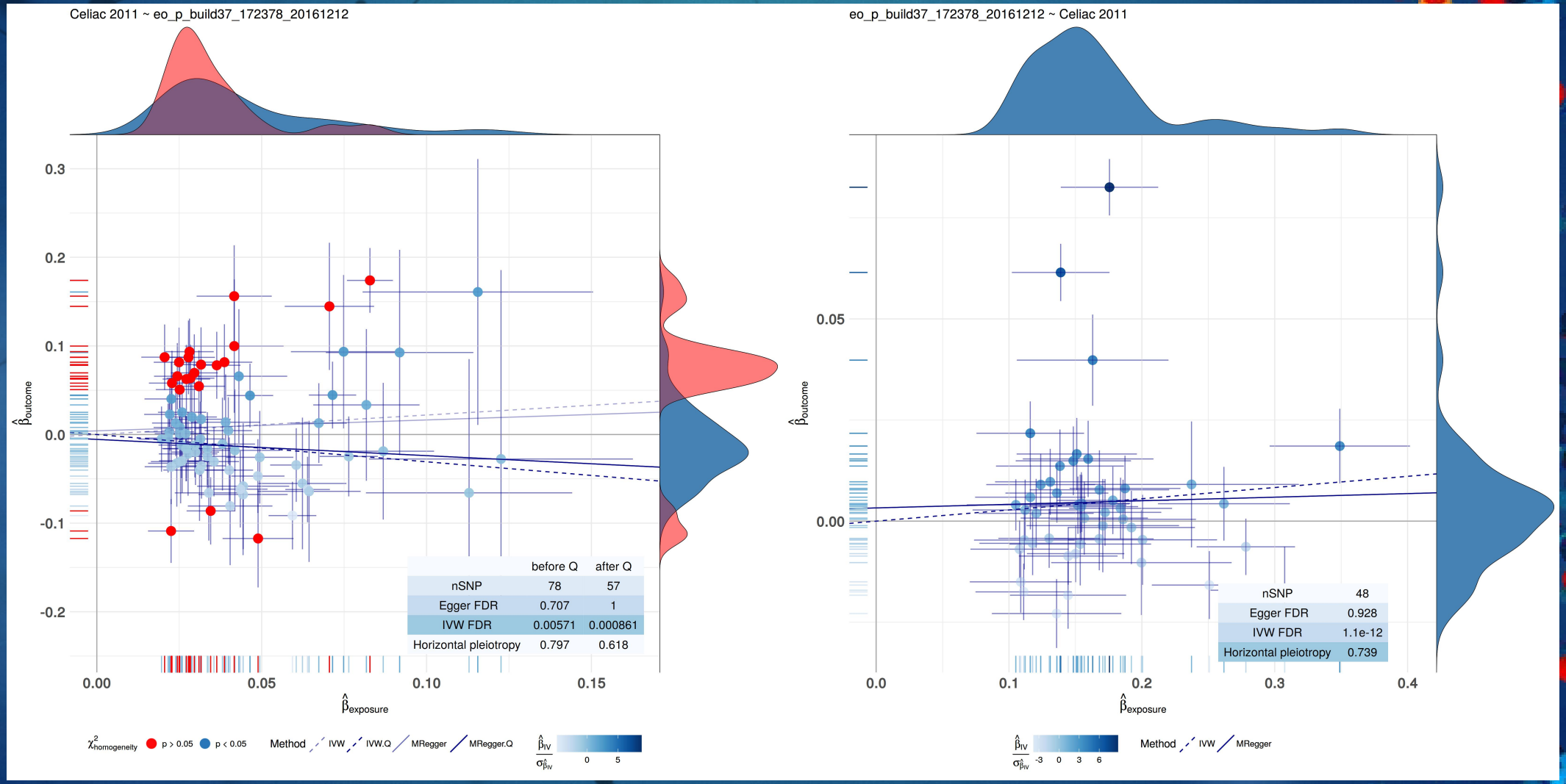
Preliminary results Bidirectional MR

- Celiac 2011, Gosia Trynka et al.



Preliminary results Bidirectional MR

- Celiac 2011, Gosia Trynka et al.



Preliminary results Bidirectional MR

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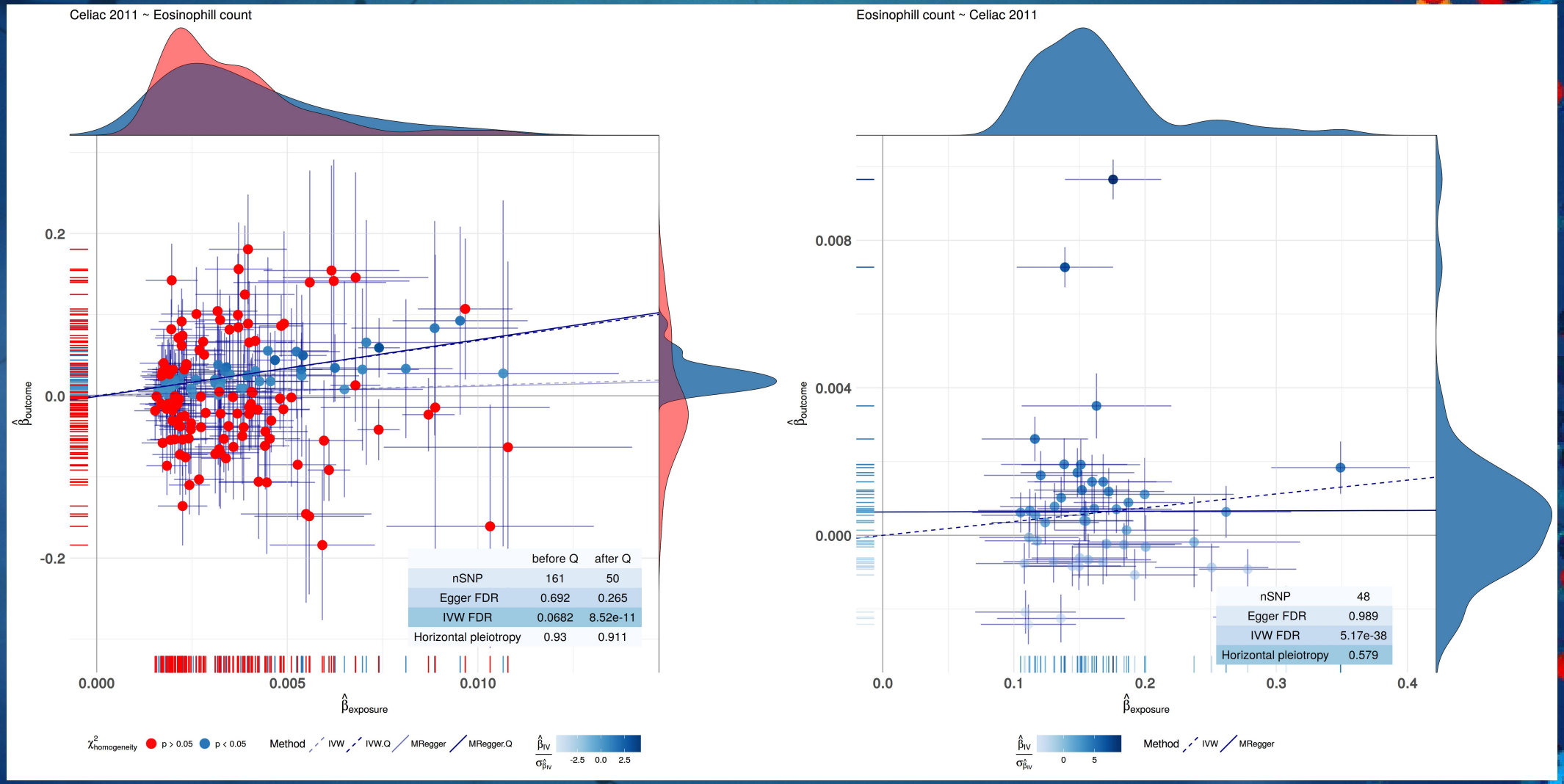

Preliminary results Bidirectional MR

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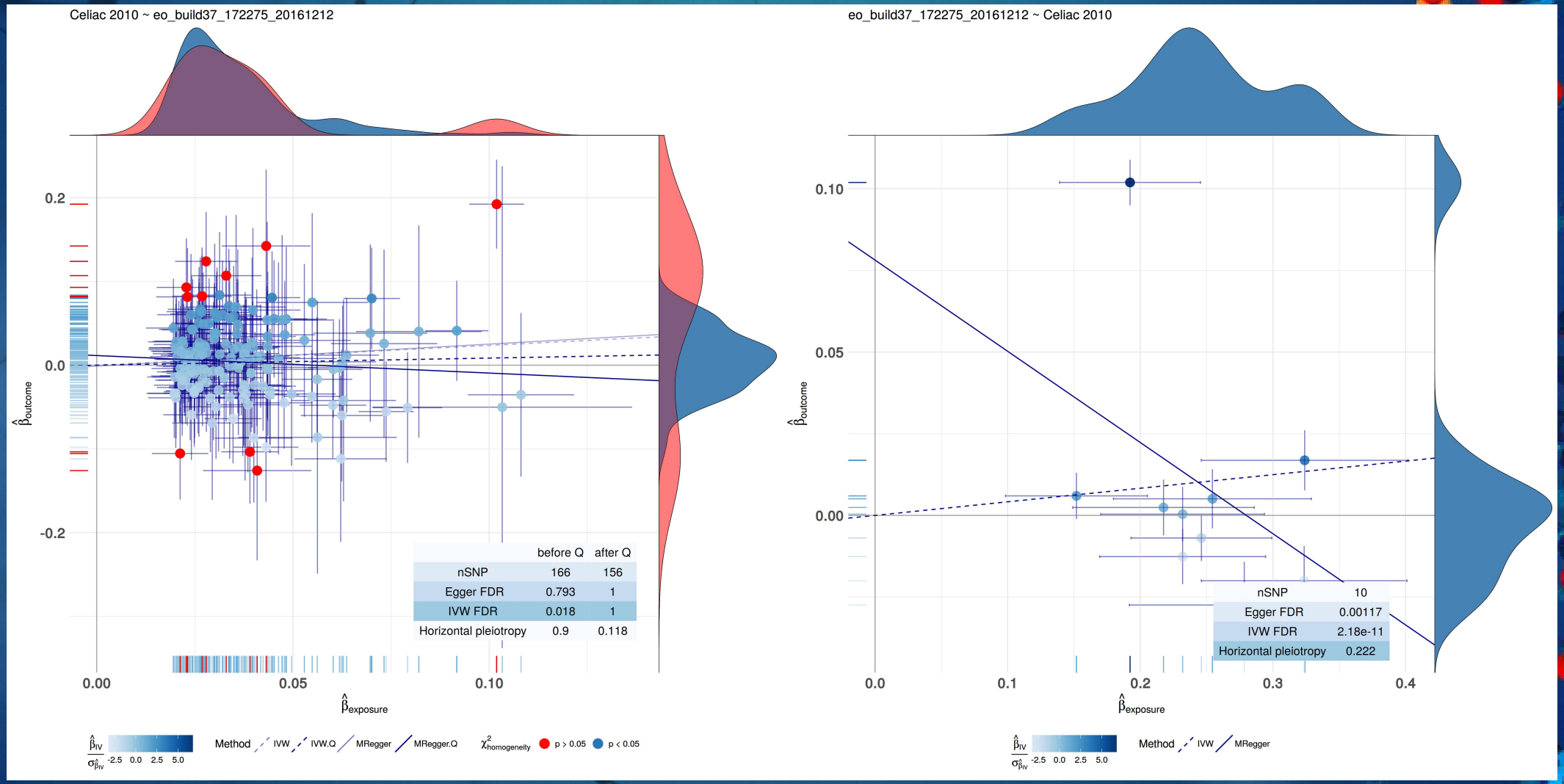

Preliminary results Bidirectional MR

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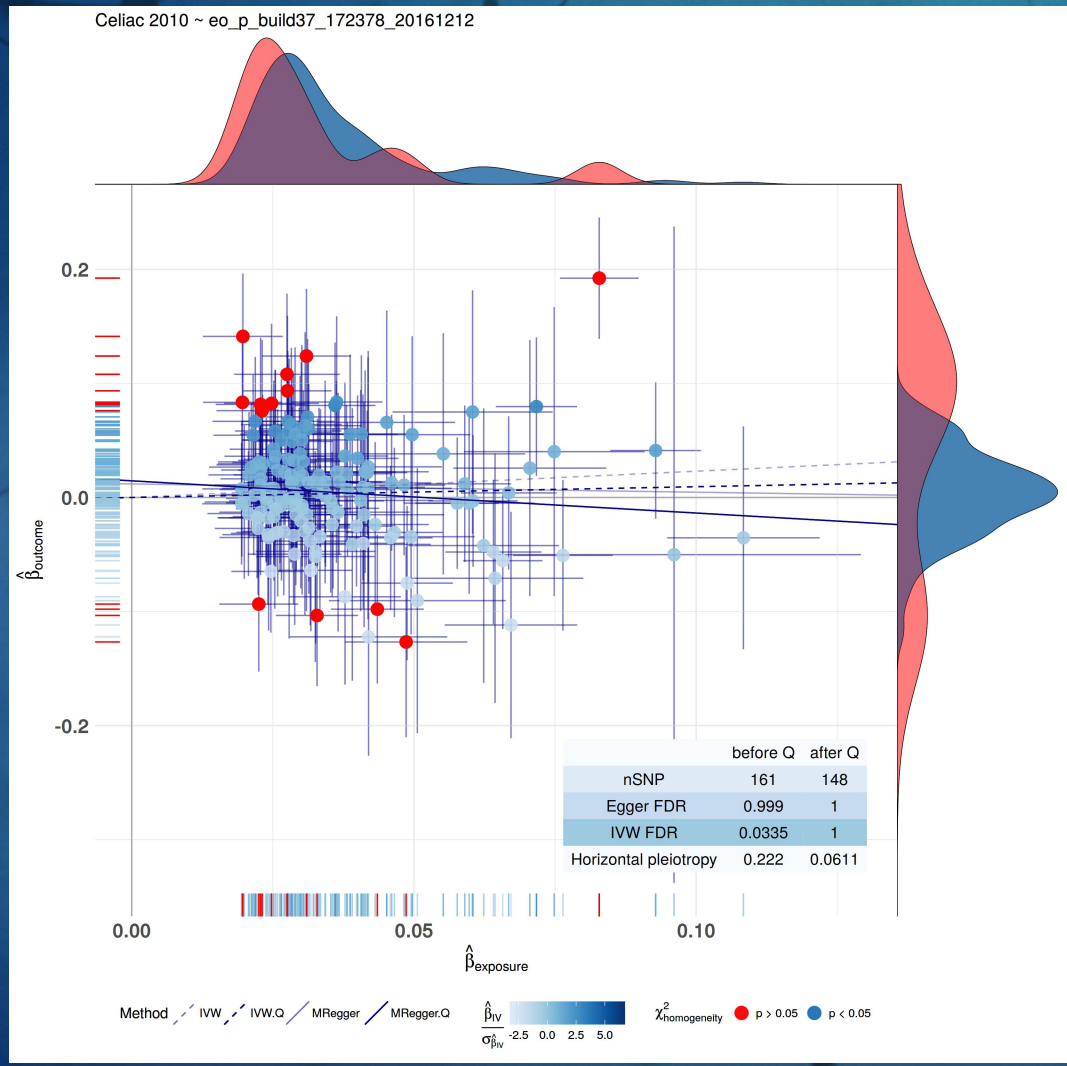
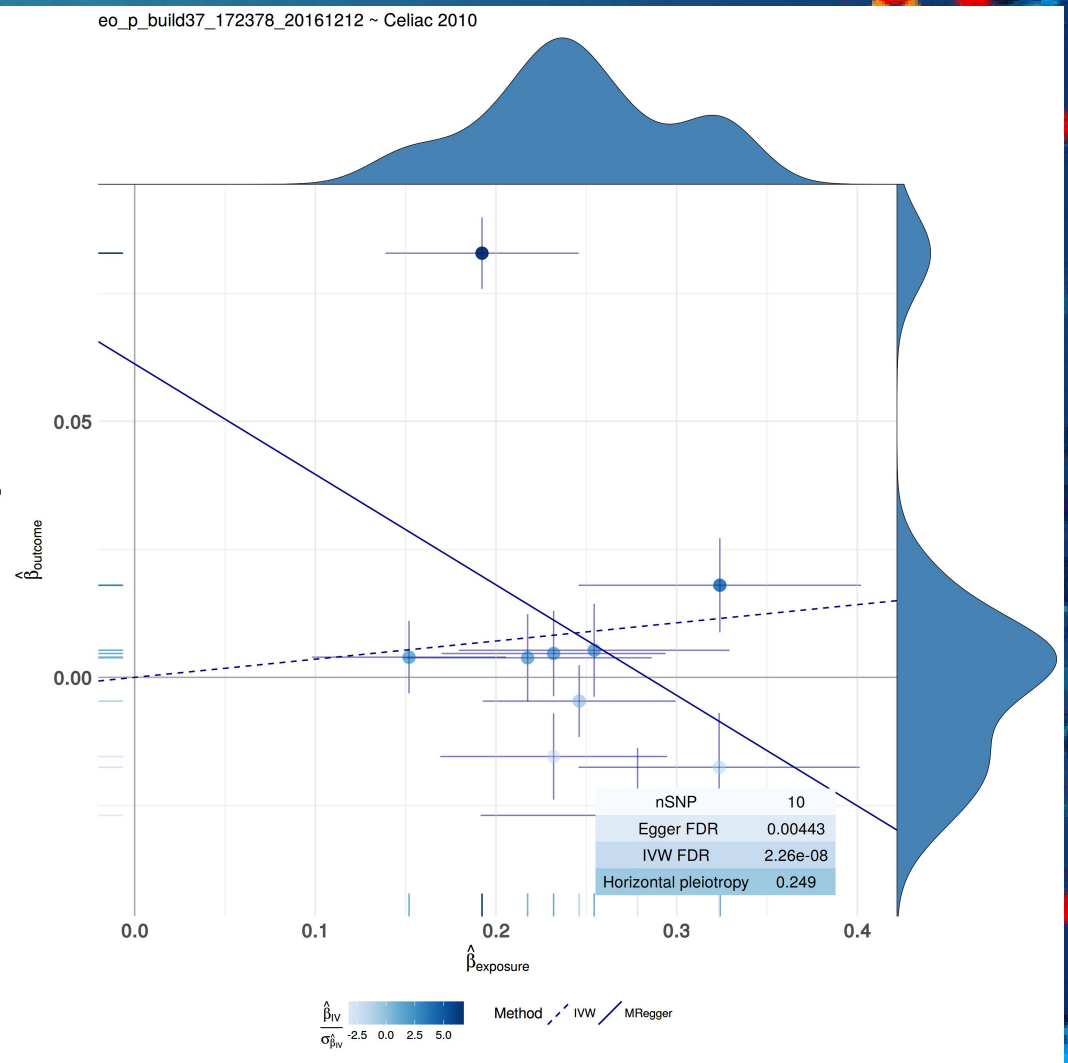
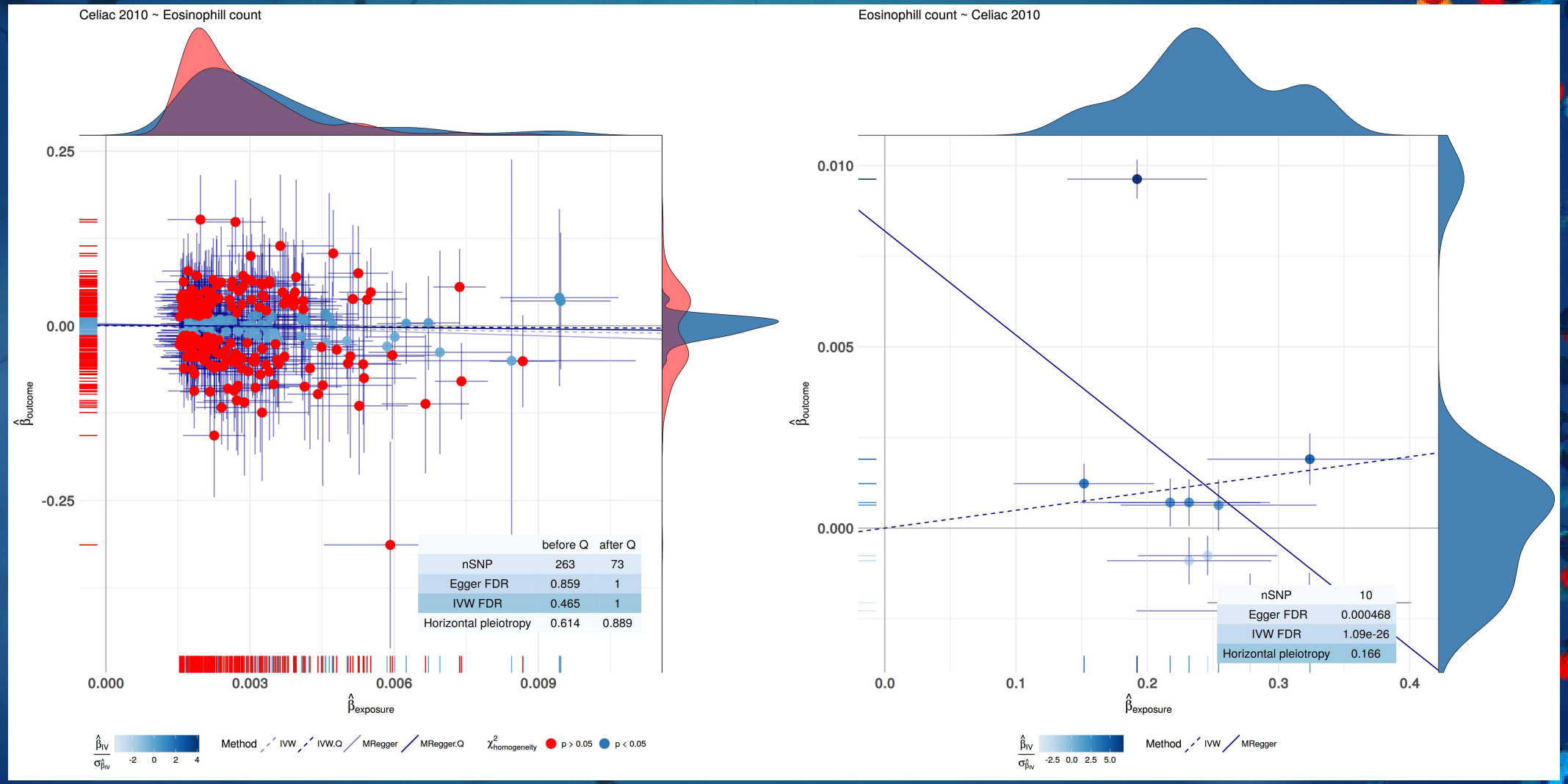




Preliminary results Bidirectional MR

- **Celiac 2010, Patrick Dubois et al.**



Conclusions

- **Celiac 2011, Gosia Trynka et al. ~ Exposure**

Phenotype	Conslusion
T1D_meta_2015_25751624_hg19	lower risk for Type 1 Diabetes protects for Celiac Disease
Type 1 Diabetes	lower risk for Type 1 Diabetes protects for Celiac Disease
Inflammatory Bowel Disease	lower risk for Inflammatory Bowel Disease protects for Celiac Disease
Ulcerative colitis	lower risk for Ulcerative colitis protects for Celiac Disease
Crohns disease	lower risk for Crohns Disease protects for Celiac Disease
plateletcrit	higher level of plateletcrit protects for Celiac Disease
IL18RAP	higher level of IL18RAP protects for Celiac Disease
lymphocytes count	higher level of lymphocytes count protects for Celiac Disease
platelet count	lower level of platelet count protects for Celiac Disease
Packed cell volume	lower lever of packed cell volume protects for Celiac Disease

Conclusions

- **Celiac 2010, Patrick Dubois et al. ~ Exposure**

Phenotype	Conslusion
T1D_meta_2015_25751624_hg19	lower risk for Type 1 Diabetes protects for Celiac Disease
Type1 Diabetes	lower risk for Type 1 Diabetes protects for Celiac Disease
Inflammatory Bowel Disease	lower risk for Inflammatory Bowel Disease protects for Celiac Disease
Ulcerative colitis	lower risk for Ulcerative colitis protects for Celiac Disease
Primary biliary cirrhosis	lower risk for Primary biliary cirrhosis protects for Celiac Disease
Coronary artery disease mi additive	lower risk for Coronary artery disease protects for Celiac Disease
Crohns disease	lower risk for Crohns Disease protects for Celiac Disease
eosinophils+basophils count	Seemingly a lower level of eosinophils+basophils count protects for Celiac Disease but this is driven by outliers
eosinophils count	Seemingly a lower level of eosinophils count protects for Celiac Disease but this is driven by outliers
platelet count	Seemingly a lower level of platelet count protects for Celiac Disease but this is driven by outliers

Future steps

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