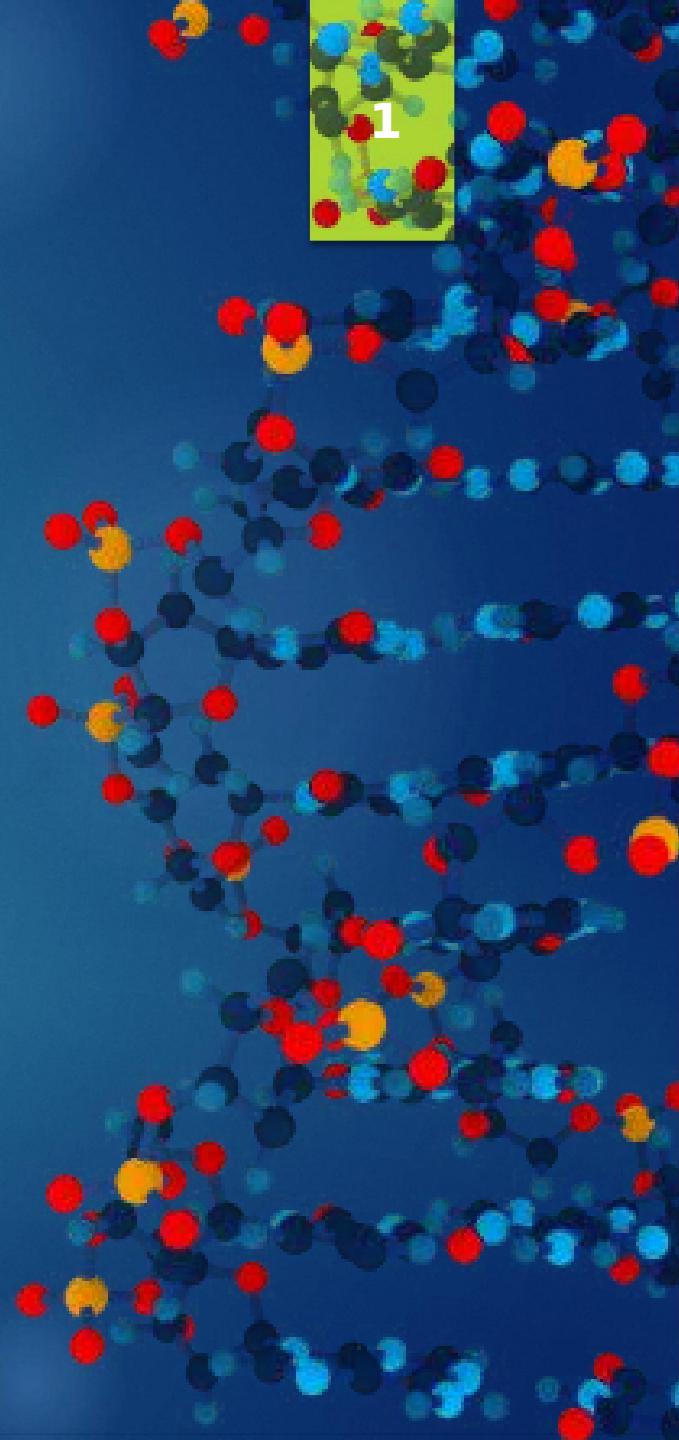


Celiac Disease Triggers

MATTHIJS KNIGGE



Overview

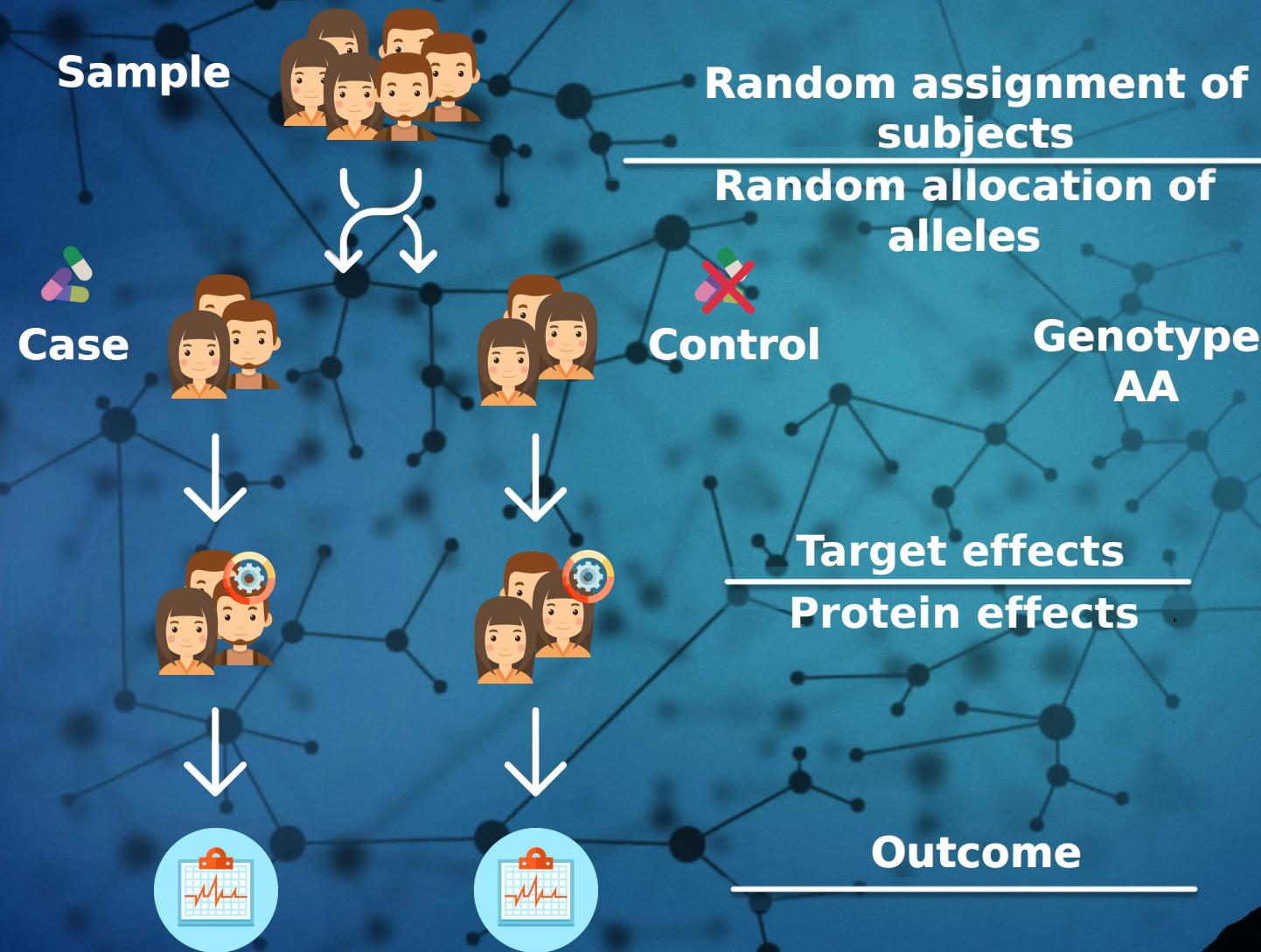
- ▶ Project goal
- ▶ Mendelian Randomization
- ▶ Methods
- ▶ Data
- ▶ Pipeline
- ▶ Preliminary results
- ▶ Future steps



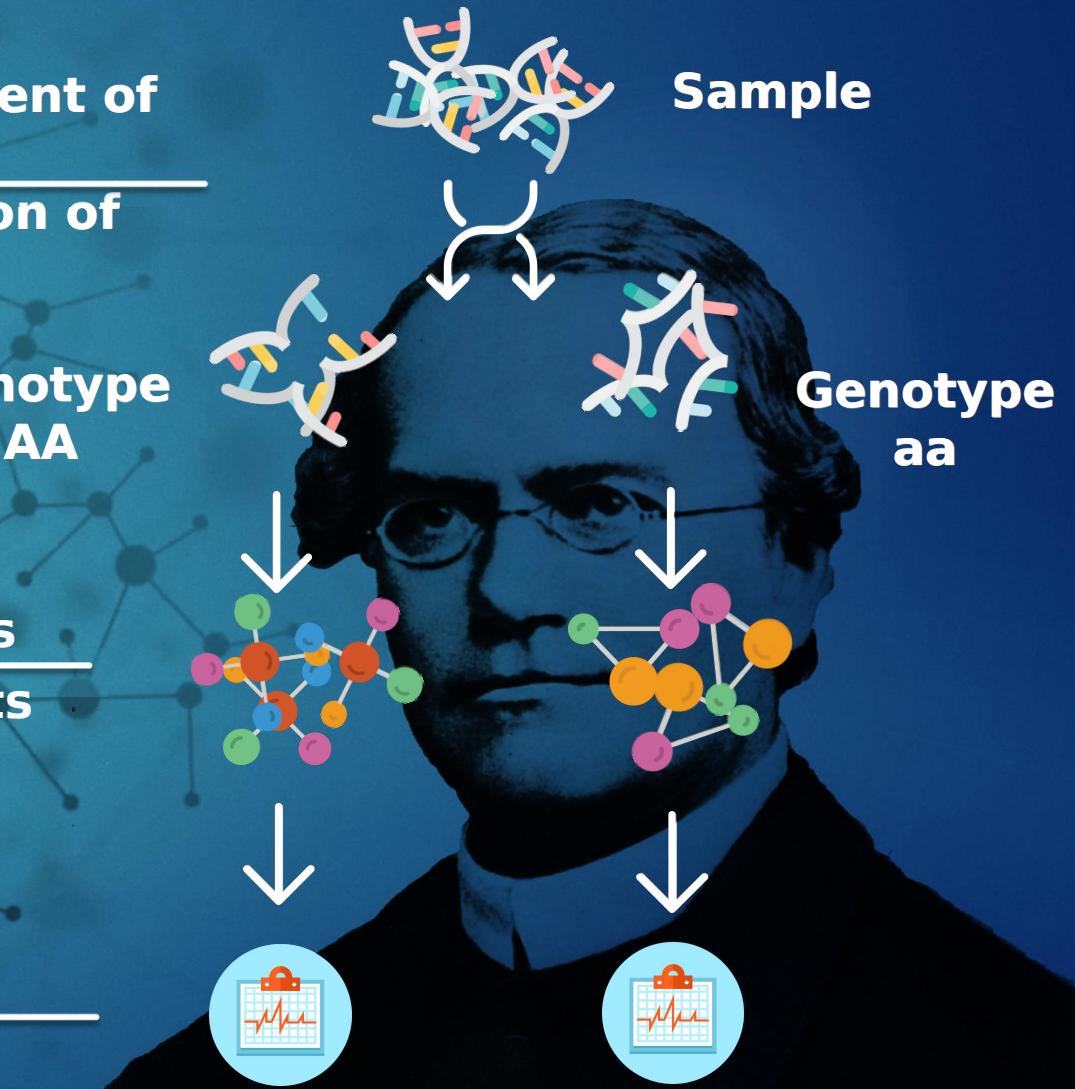
Project Goal

- ▶ Inspect > 70.000 clinical parameters and molecular mechanisms to;
 - ▶ Find identification of factors that cause or protect against Celiac Disease
 - ▶ Quantify the impact of causal or protective factors

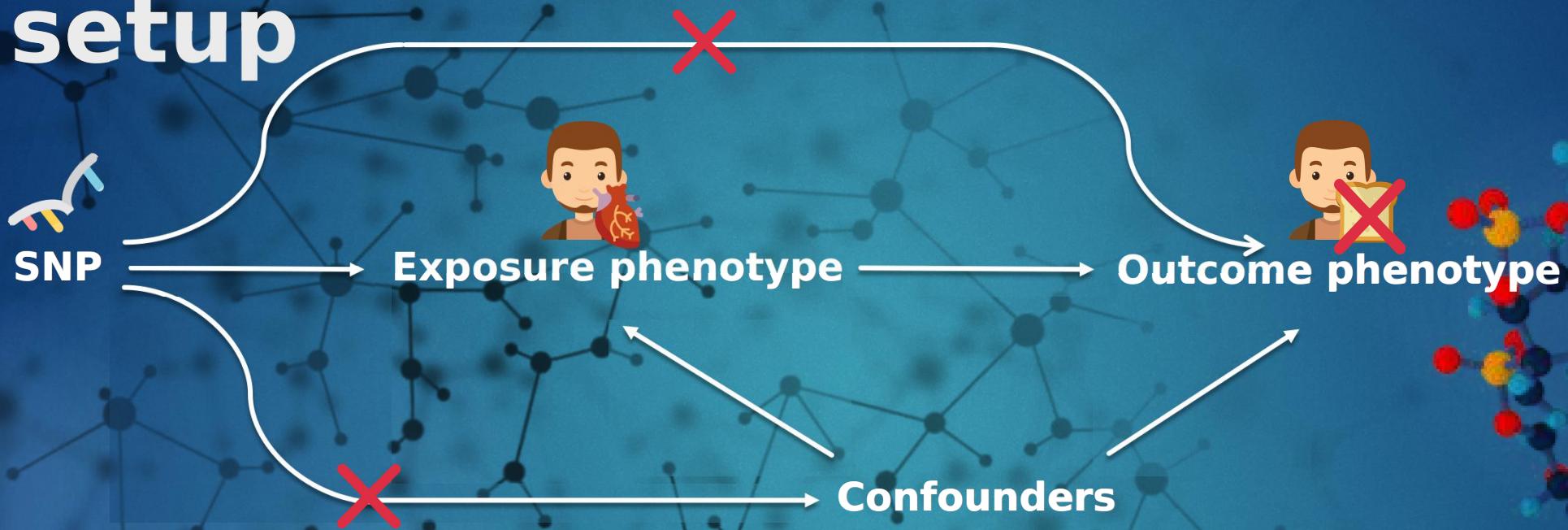
Randomized Controlled Trials (RCT)



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	32432	Exposure
MRbase Methylation levels	GWAS on methylation levels in whole blood across 5 time points	33256	Exposure
additional downloaded GWAS	GWAS	1308	Exposure
Total		70059	

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.



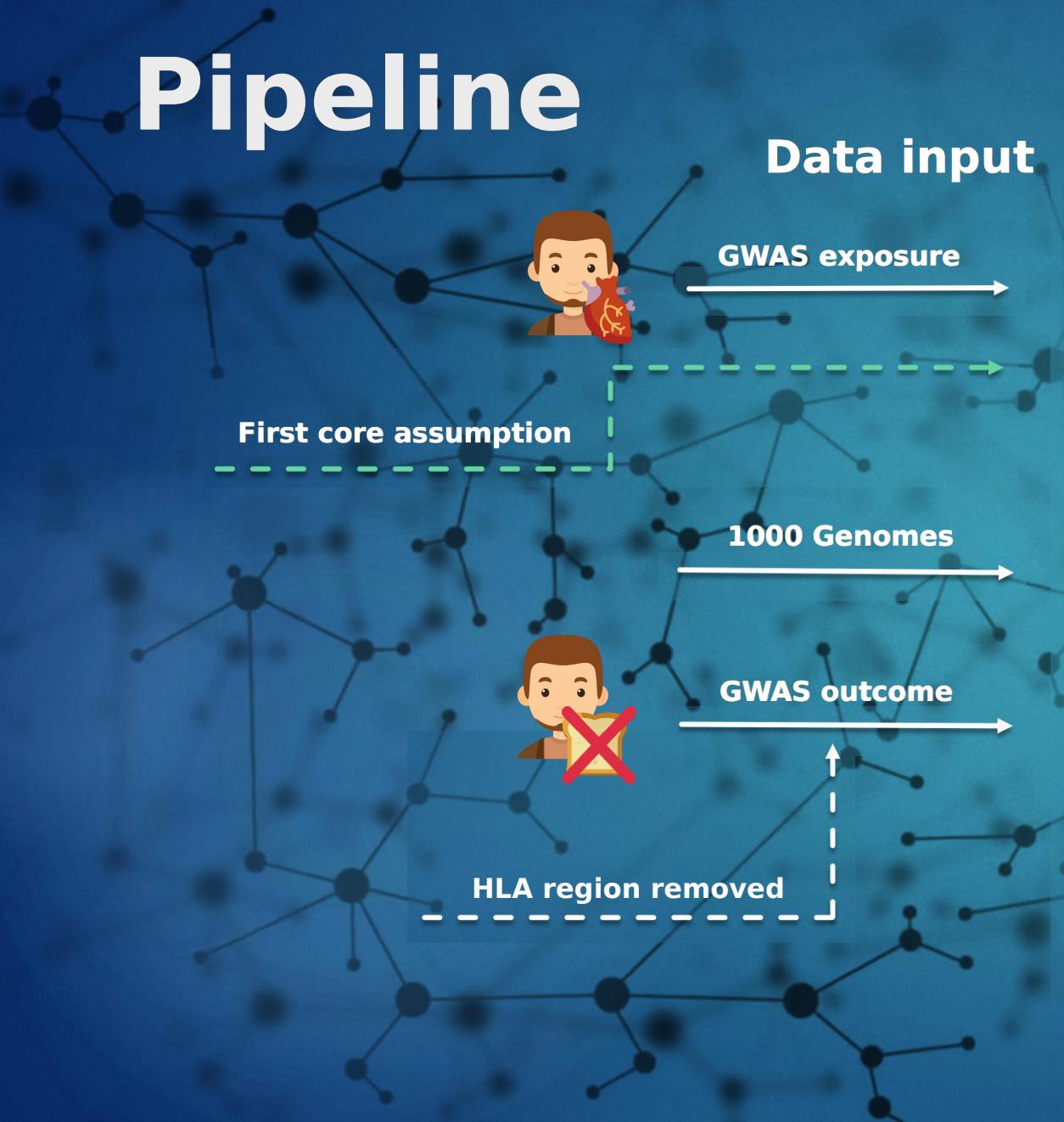
GWAS →

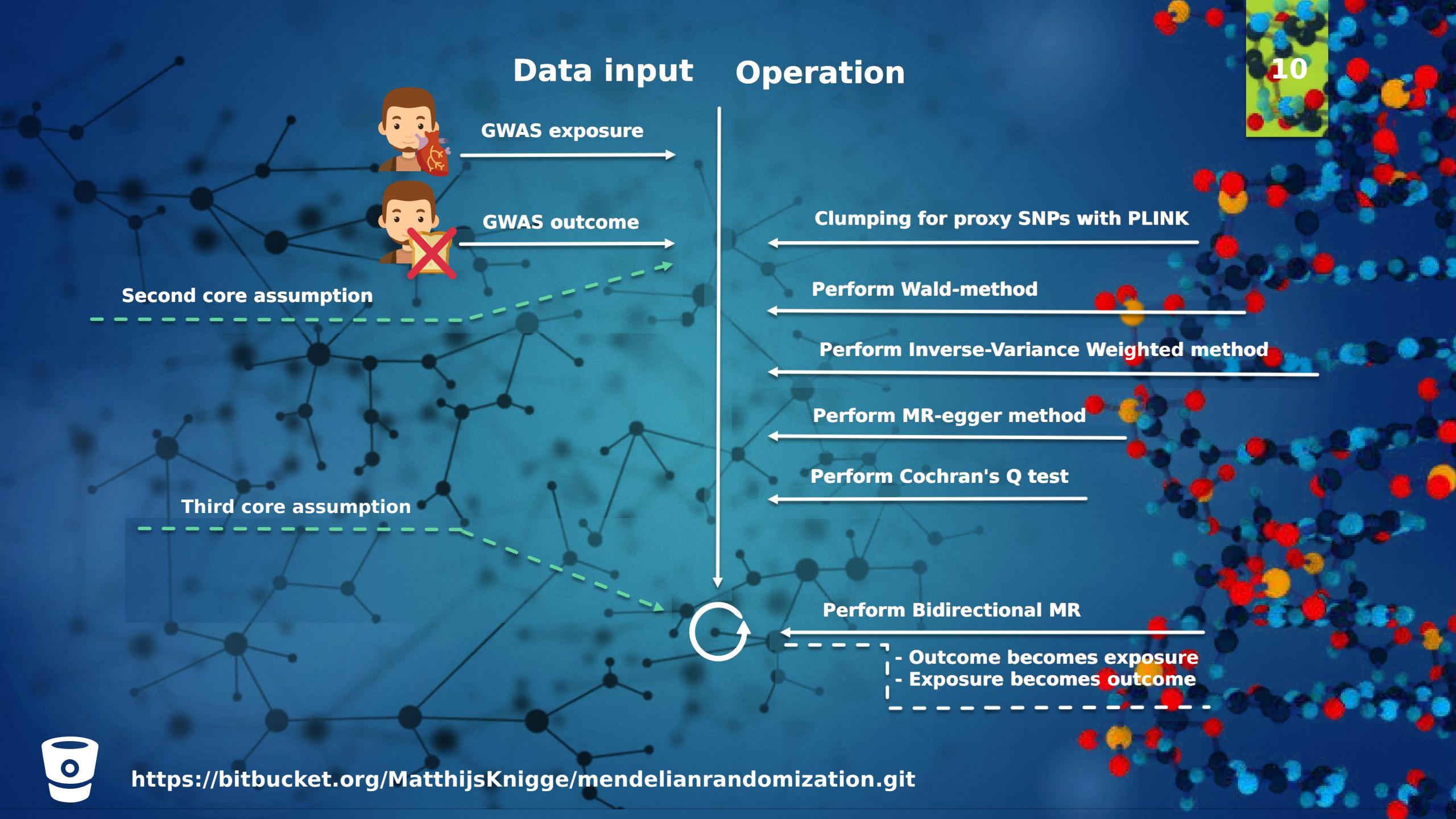
SNP	effect_allele	beta	se	p
rs314253	C	-0.0201900	0.002646	2.471e-14
rs7775397	G	-0.0369352	0.004845	2.721e-14
....

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





Preliminary results

► Total

Celiac 2011

Celiac 2010

483

434

567

► Considerd FDR < .05 for MR-egger | IVW

Celiac 2011

Celiac 2010

63

21

36

Preliminary results

- ▶ Celiac 2011, Gosia Trynka et al.
 - ▶ 63 significant potential exposures
- ▶ Top 10

Exposure	IVW	Egger	nSNP
Rheumatoid Arthritis	3.171316e-06	4.627945e-15	53
eosinophils + basophils count	1.361791e-05	8.413819e-08	28
eosinophils count	1.482109e-07	2.594648e-06	32
lymphocytes count	7.274596e-01	1.892860e-05	27
plateletcrit	5.517519e-08	2.612460e-05	19
platelet count	1.679860e-07	3.664932e-05	18
eosynophils percentage	2.691746e-04	1.070842e-03	27
platelets distribution width	8.302935e-03	1.199338e-03	14
hematocrit	1.125320e-02	3.785512e-03	9
hemoglobin	7.628868e-03	4.910752e-03	9

Preliminary results

- ▶ Celiac 2010, Patrick Dubois et al.
- ▶ 36 significant potential exposures

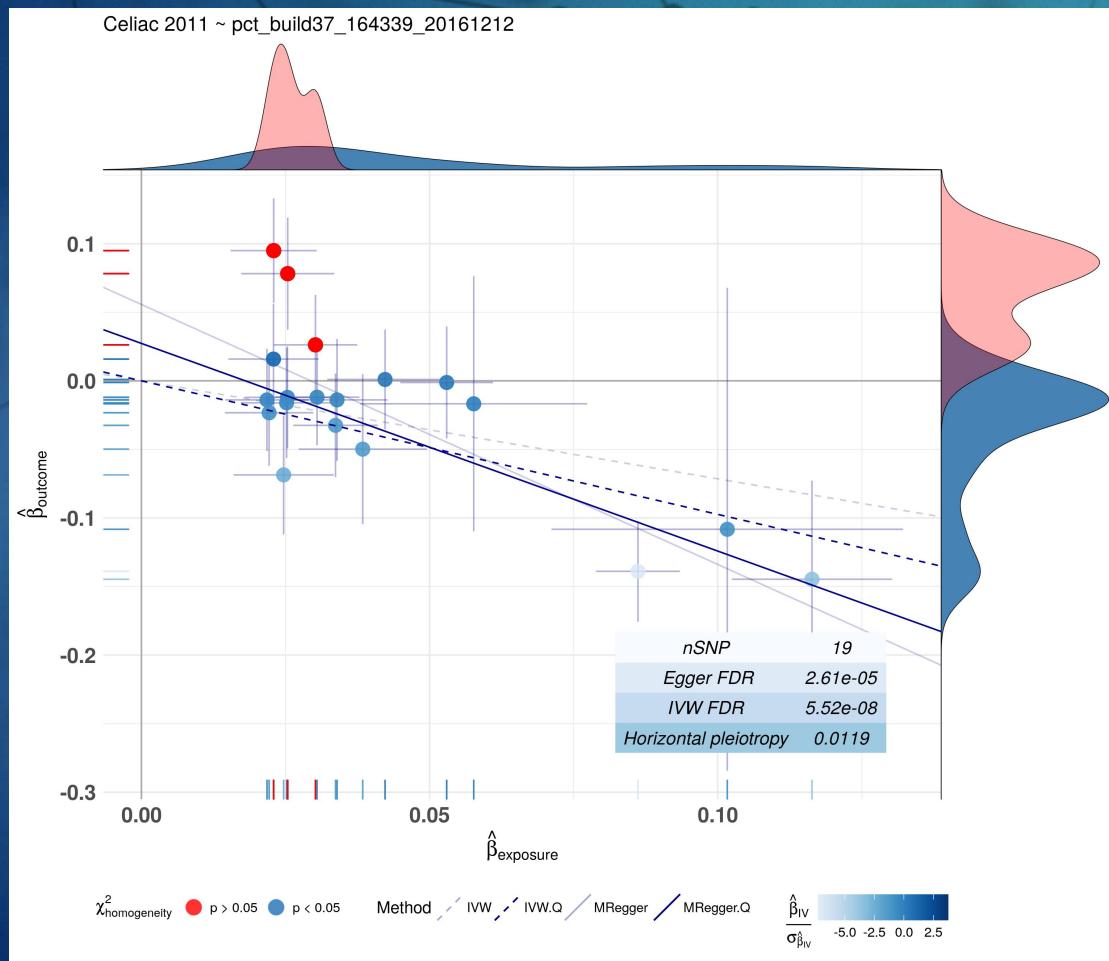
- ▶ Top 10

Exposure	IVW	Egger	nSNP
eosinophils count	2.457824e-05	0.0344422831	25
hematocrit	3.635149e-01	0.0344422831	22
neutrophils percentage	2.694008e-02	0.0344422831	25
eosinophils % granulocytes	6.095143e-03	0.0500008203	21
eosinophils + basophils count	6.892643e-06	0.0545461874	24
eosynophils percentage	3.581443e-03	0.0881148661	22
Type 1 Diabetes	1.036351e-04	0.5464139213	24
Multiple Sclerosis	2.899315e-02	0.5587373911	17
Packed cell volum	4.829159e-02	0.5587373911	8
CD39+ Activated CD4 + CD8 - Treg % Parent	4.356029e-02	0.5924281553	19

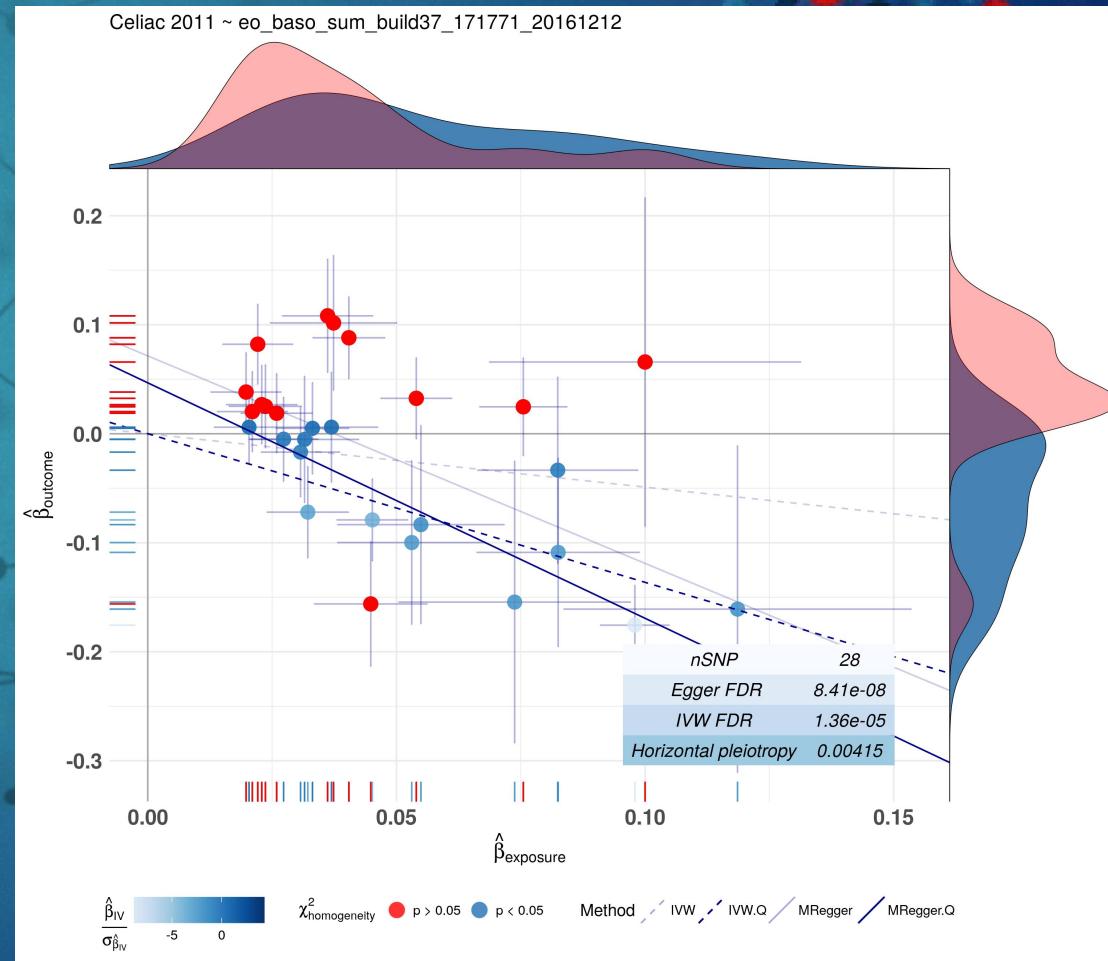
Preliminary results

Celiac 2011, Gosia Trynka et al.

An increase in 1 SD of platelecrit is responsible for an Odds ratio of 0.3356451 in celiac disease.



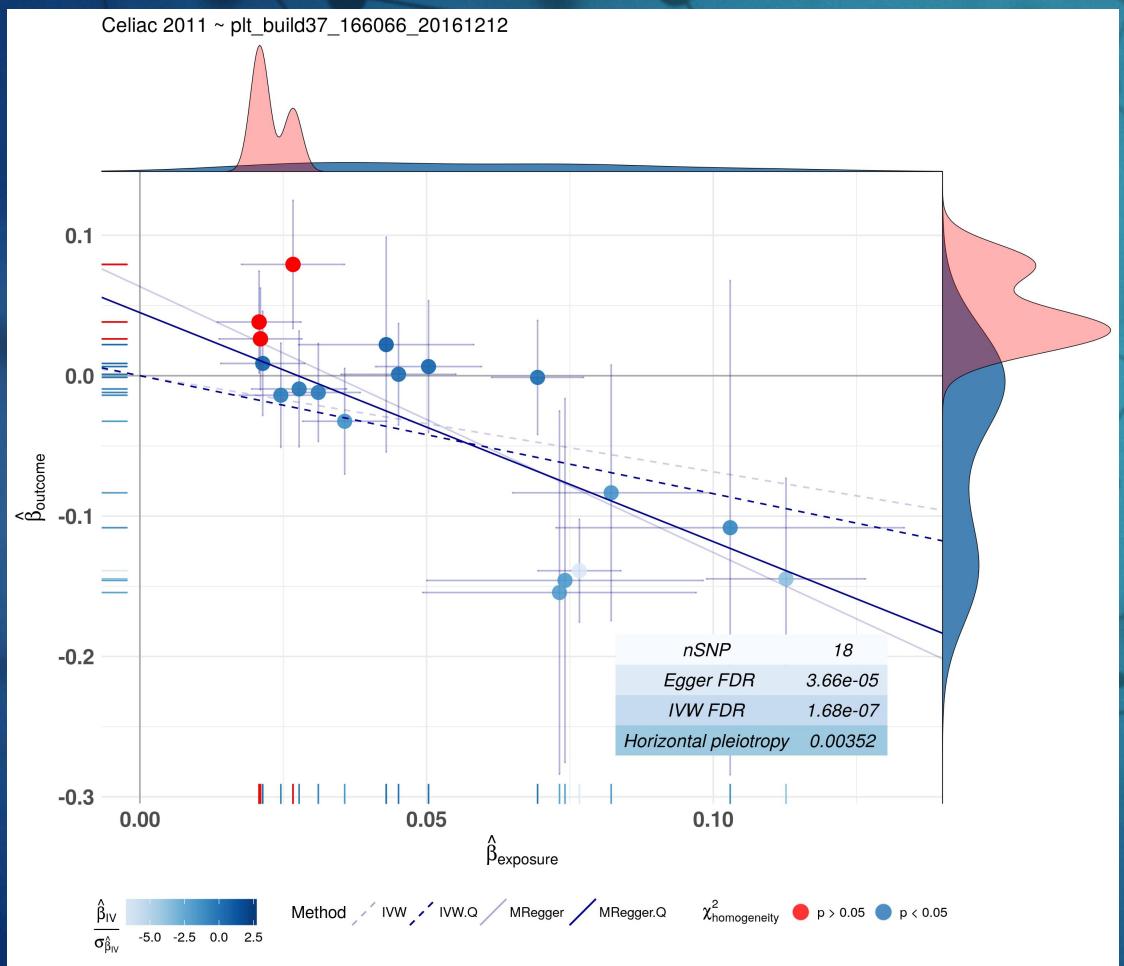
An increase in 1 SD of eosinophils + basophils count is responsible for an Odds ratio of 0.7140582 in celiac disease.



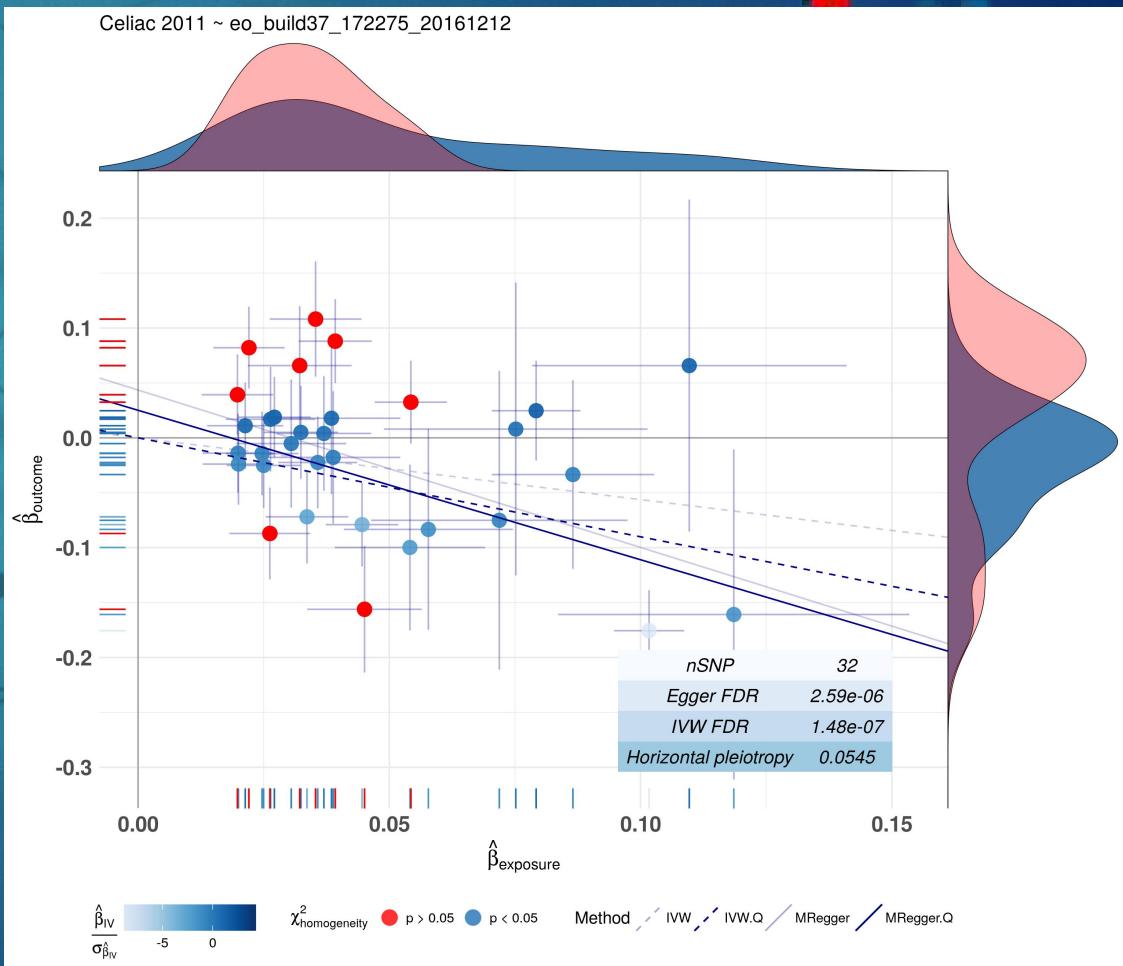
Preliminary results

► Celiac 2011, Gosia Trynka, et al.

An increase in 1 SD of platelet count is responsible for an Odds ratio of 2.928006 in celiac disease.



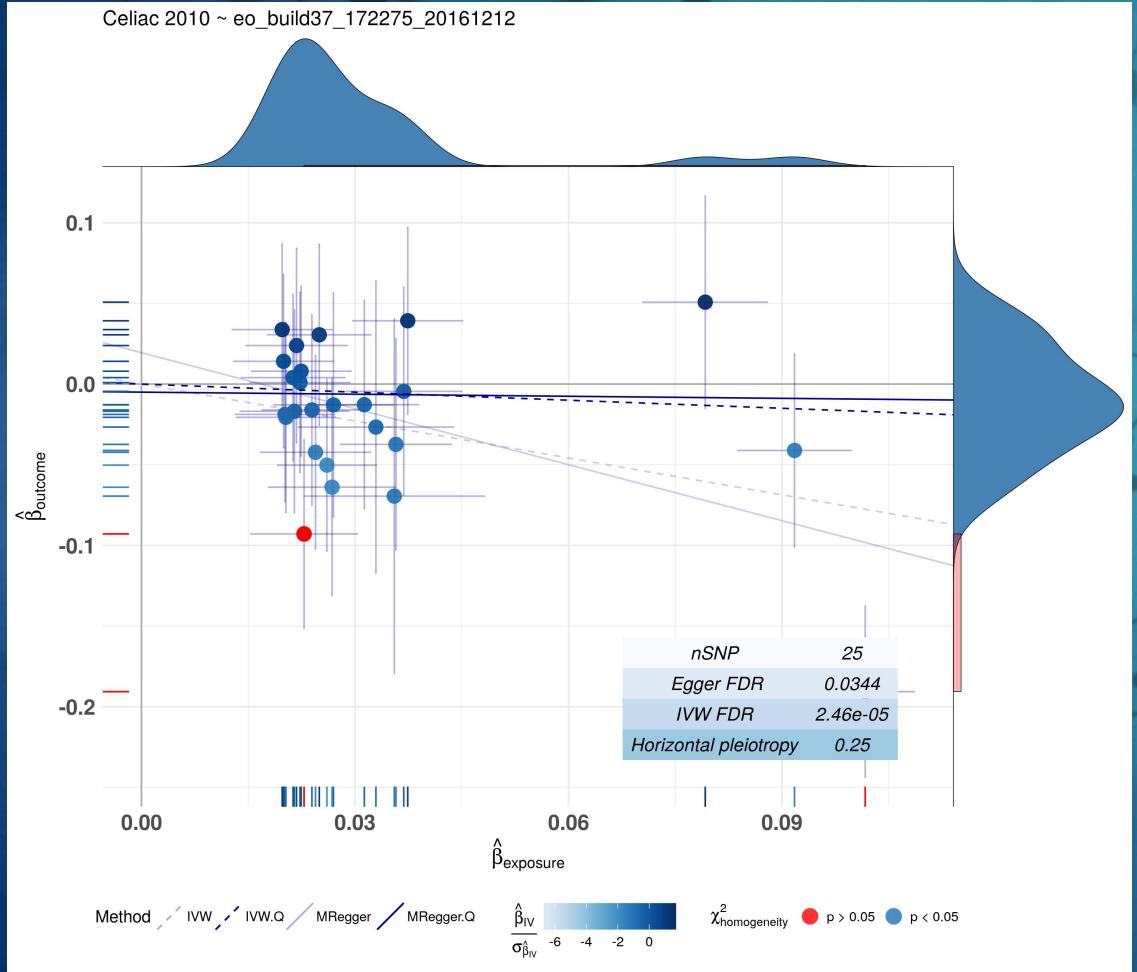
An increase in 1 SD of eosinophils count is responsible for an Odds ratio of 0.5757044 in celiac disease.



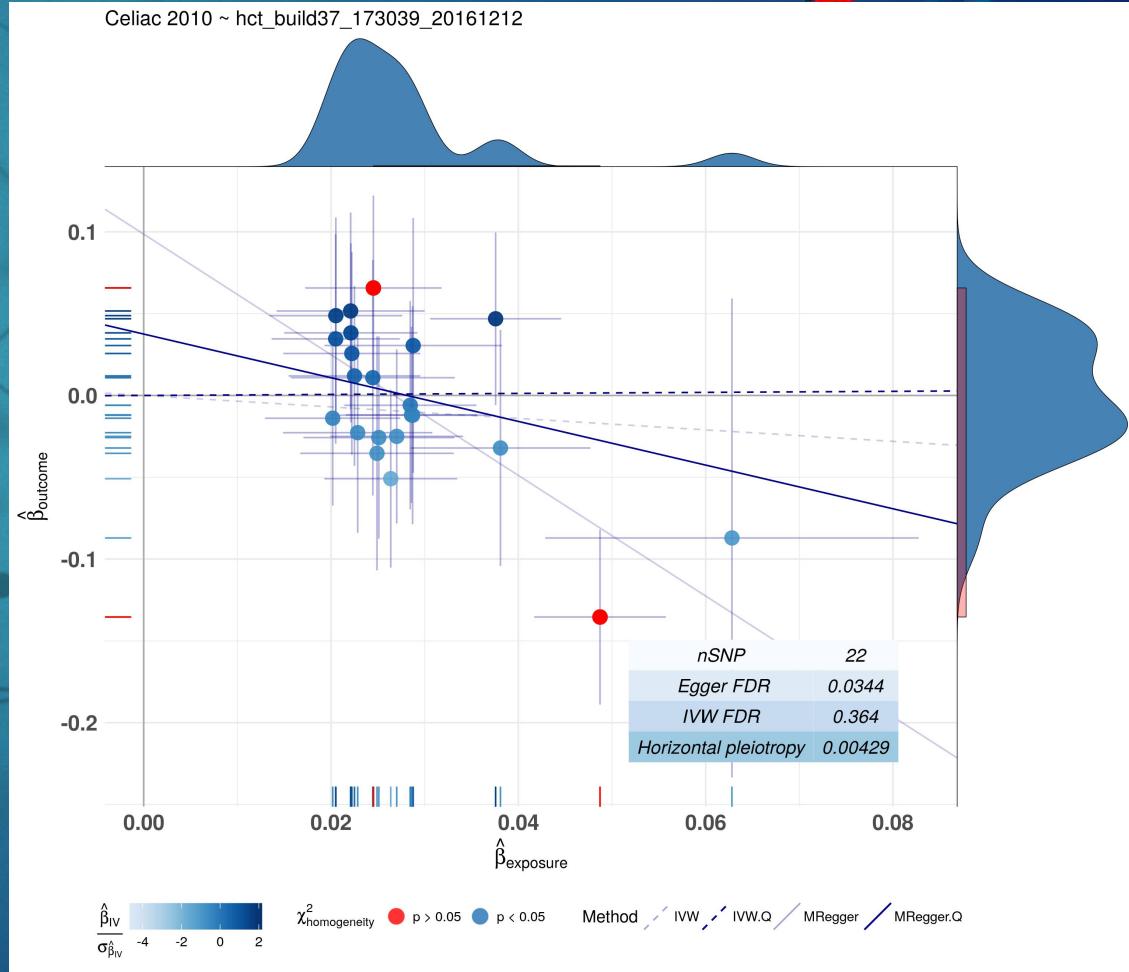
Preliminary results

► Celiac 2010, Patrick Dubois et al.

An increase in 1 SD of eosinophils count is responsible for an Odds ratio of 0.2703218 in celiac disease.



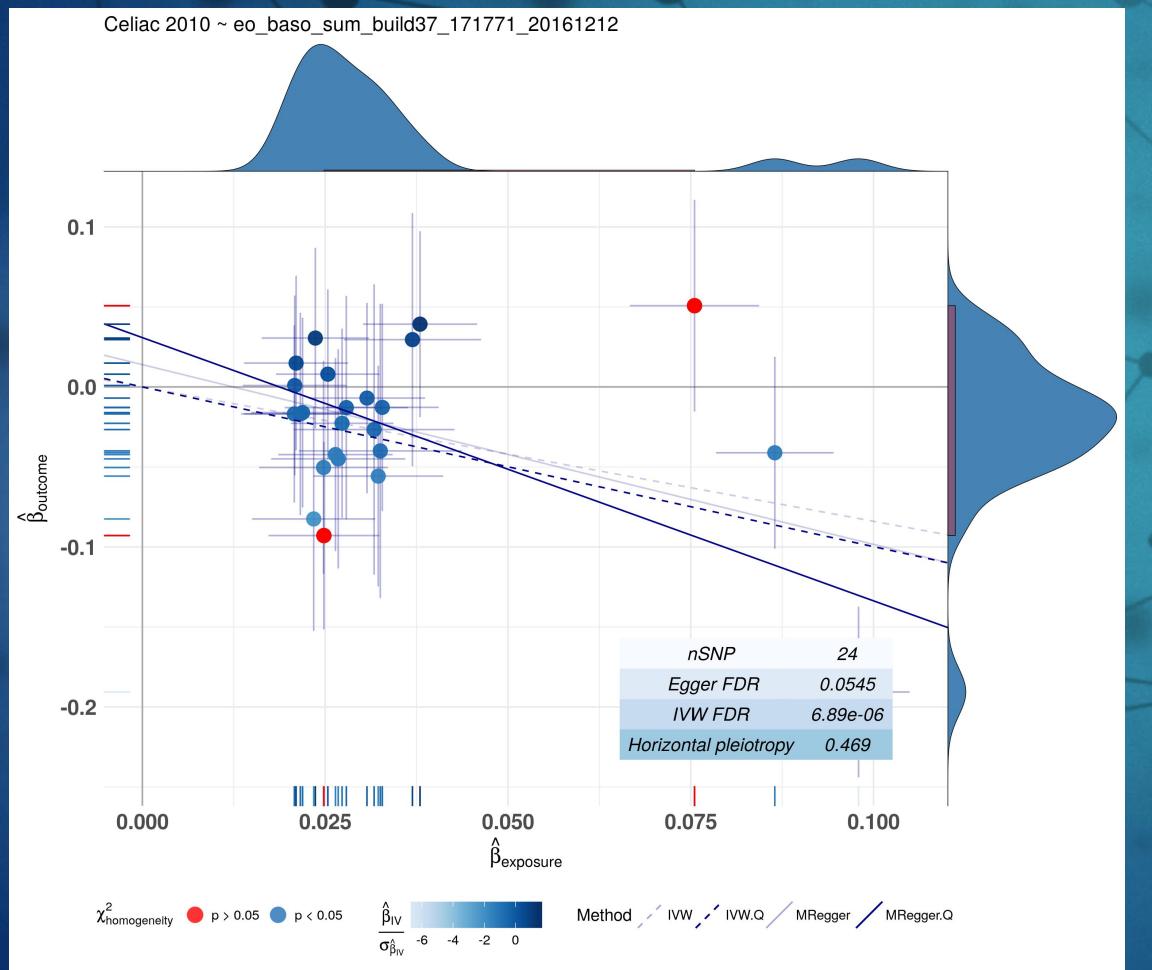
An increase in 1 SD of hematocrit is responsible for an Odds ratio of 1.048042 in celiac disease.



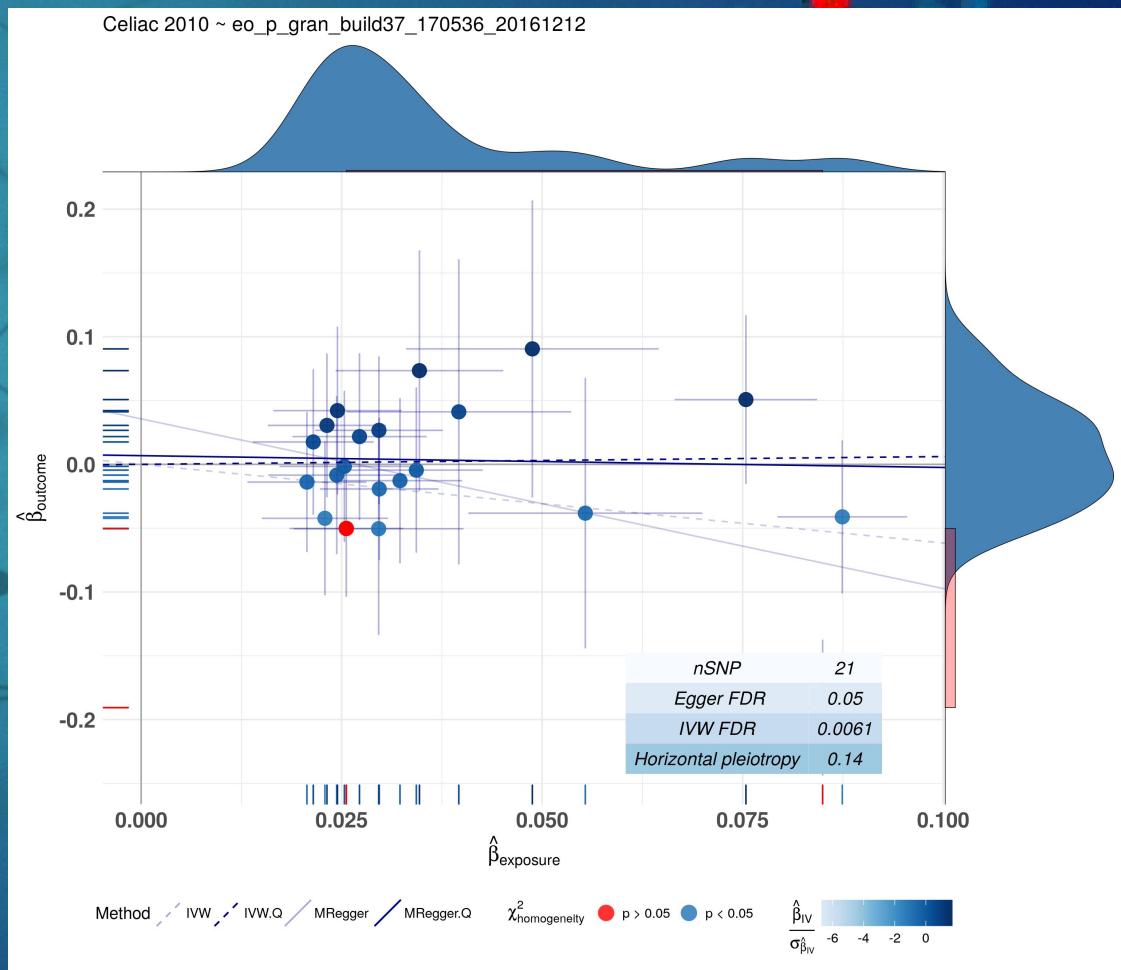
Preliminary results

Celiac 2010, Patrick Dubois et al.

An increase in 1 SD of eosinophils + basophils count is responsible for an Odds ratio of 0.1749221 in celiac disease.



An increase in 1 SD of eosinophils percentage is responsible for an Odds ratio of 0.4843092 in celiac disease.

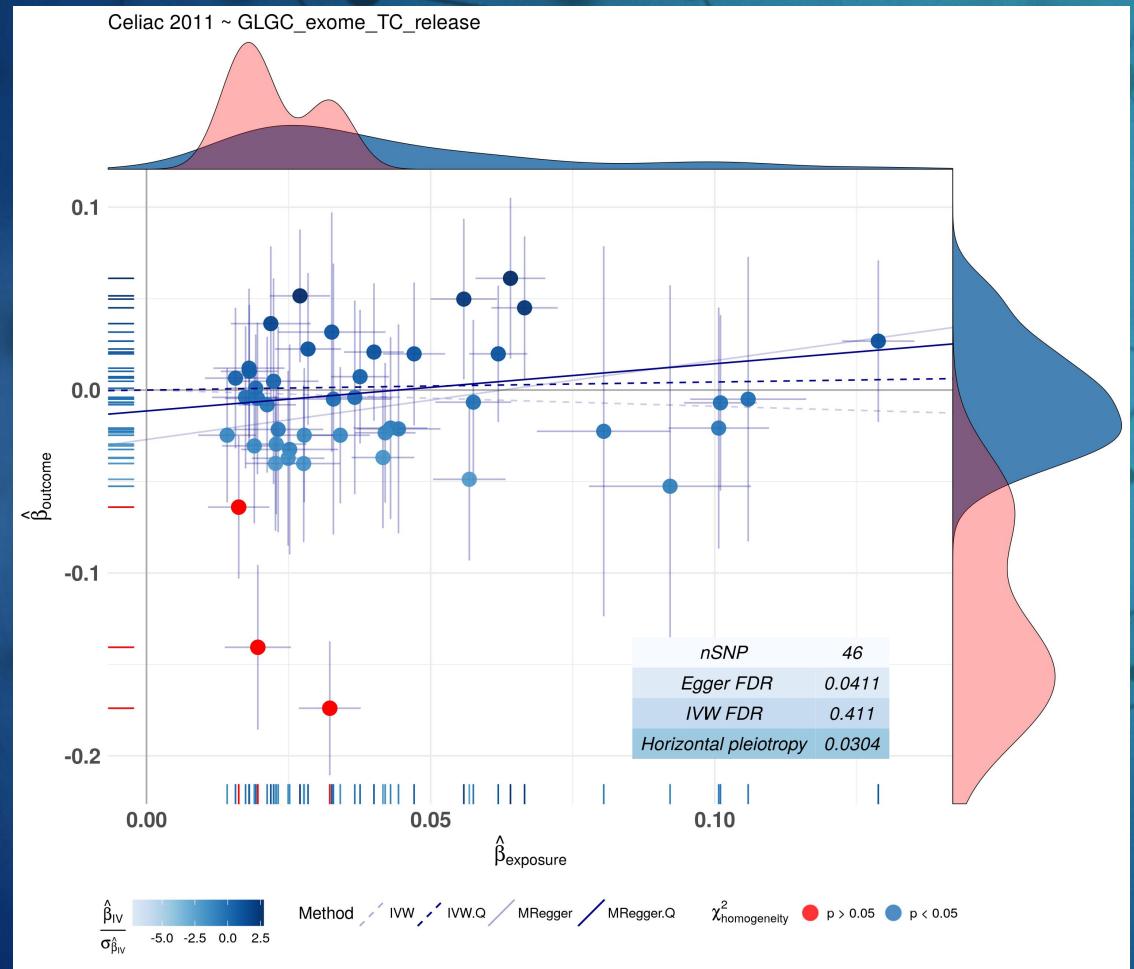


Preliminary results Bidirectional MR

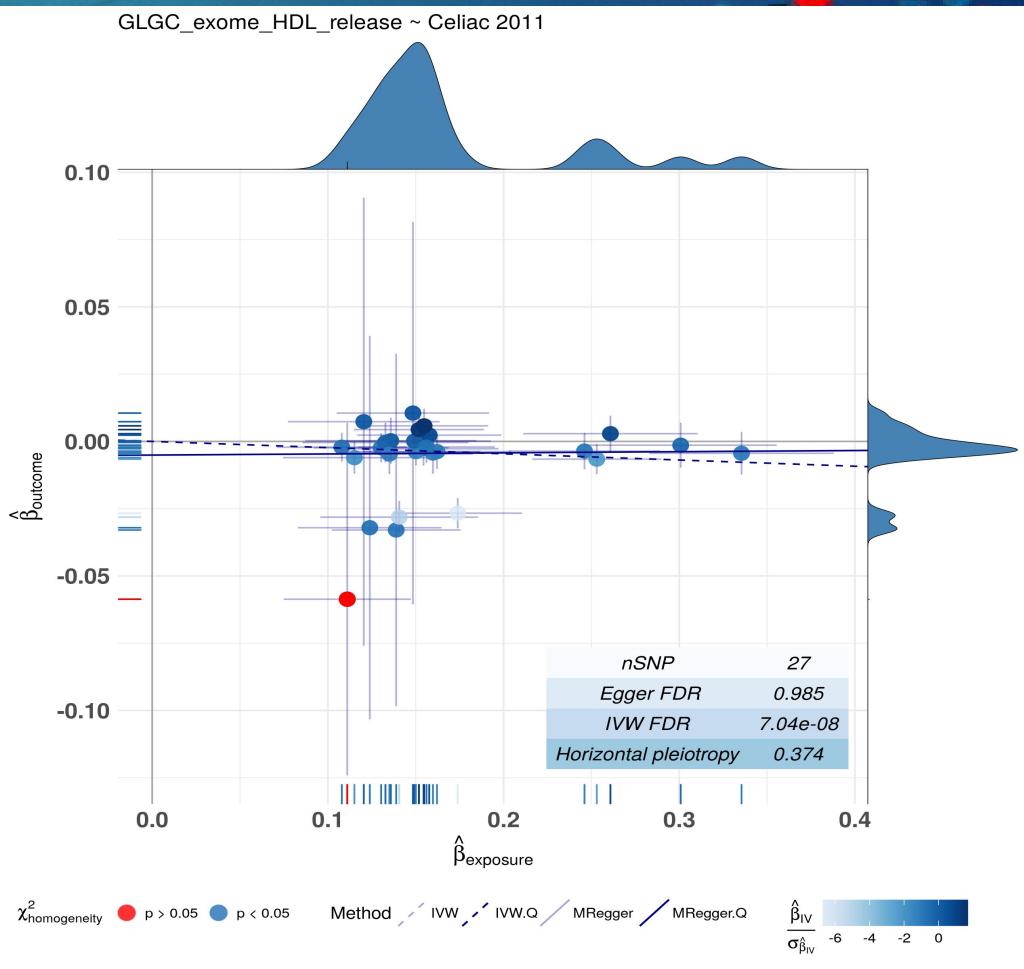
18

Celiac 2011, Gosia Trynka et al.

An increase in 1 SD of LDL + HDL + Cholesterol is responsible for an Odds ratio of 2.423787 in celiac disease.



An increase in 1 SD of celiac is responsible for an Odds ratio of 3.545413 in LDL + HDL + Cholesterol

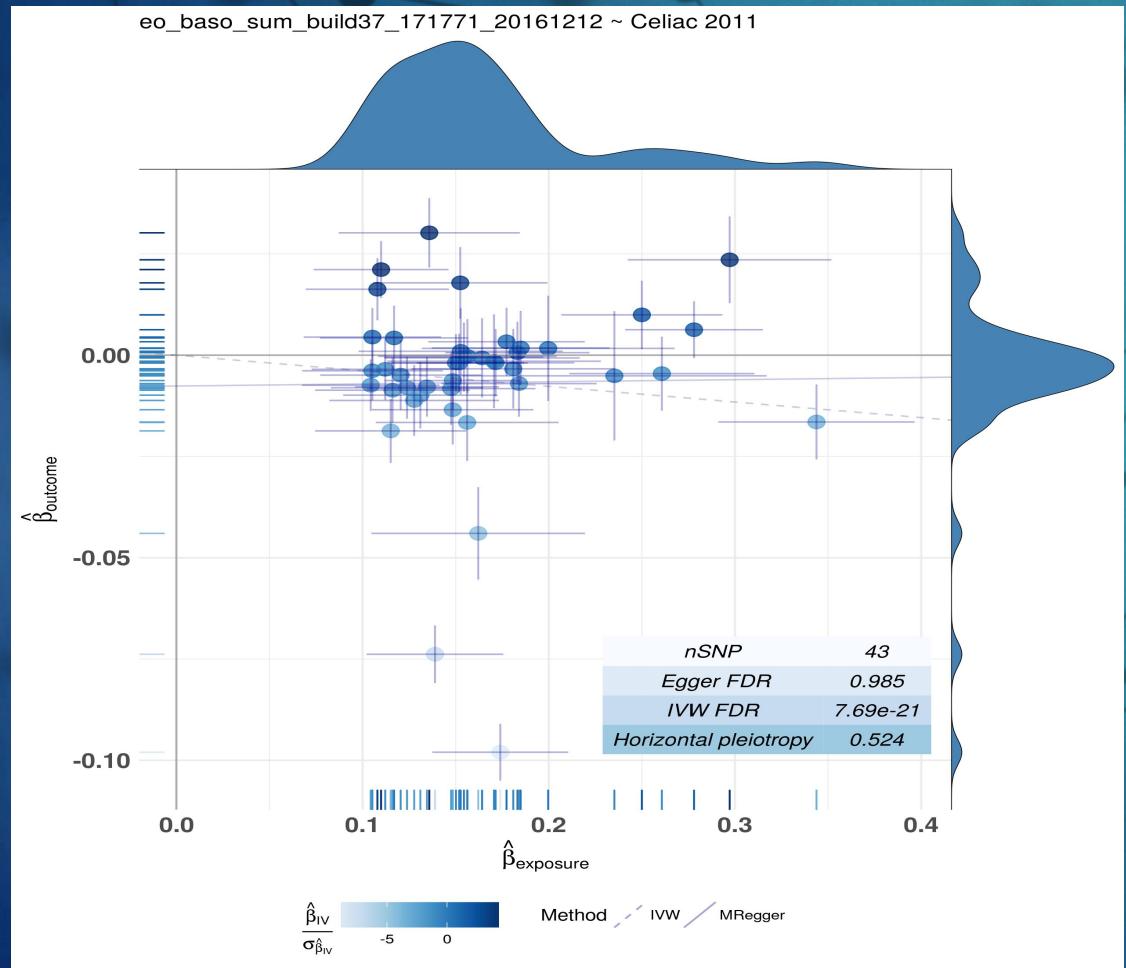


Preliminary results Bidirectional MR

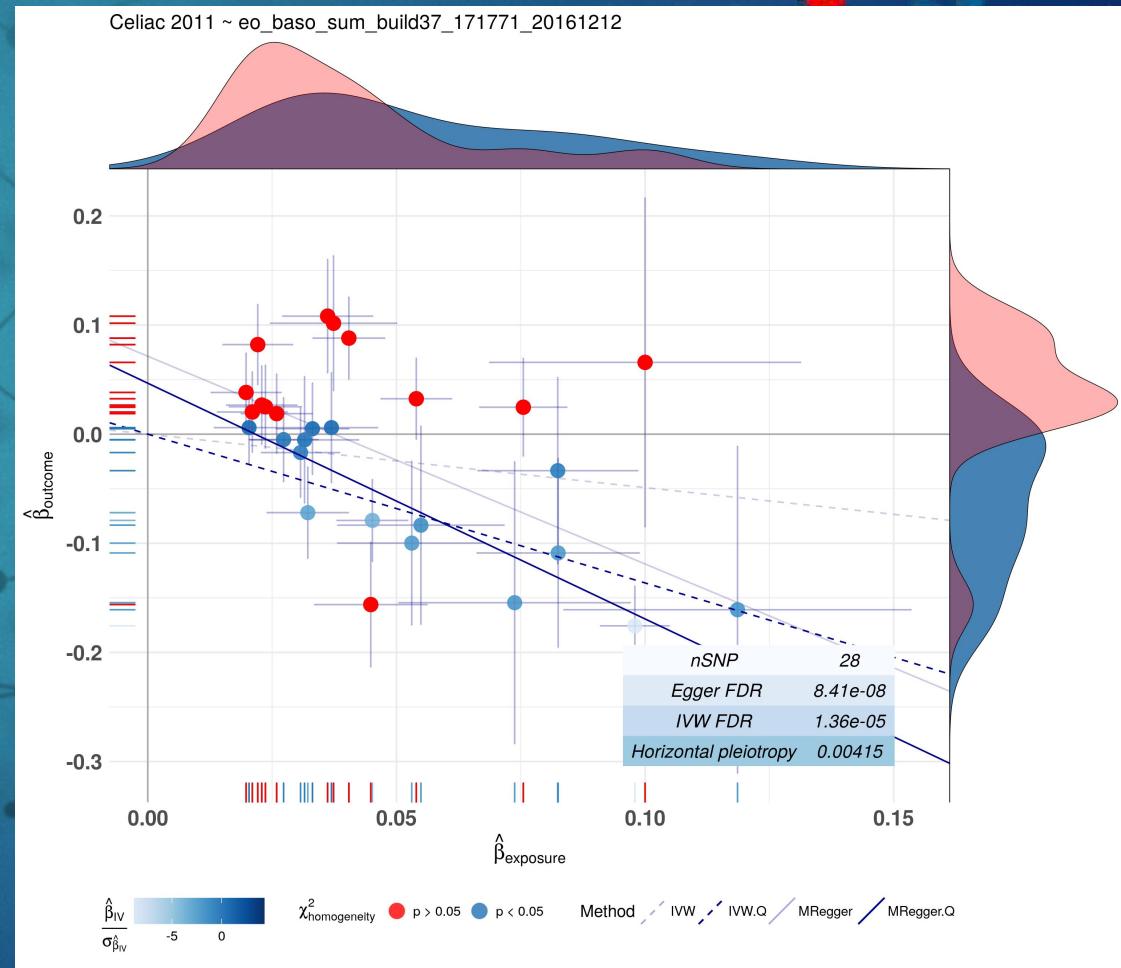
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Celiac 2011, Gosia Trynka et al.

An increase in 1 SD of celiac is responsible for an Odds ratio of 3.256354 in eosinophils + basophils count.



An increase in 1 SD of eosinophils + basophils counts responsible for an Odds ratio of 0.7140582 in Celiac



Conclusions

► Celiac 2011

Phenotype	Conclusion
Rheumatoid Arthritis	Low Rheumatoid Arthritis is protective for Celiac Disease
eosinophils + basophils count	High eosinophils + basophils count is protective for Celiac Disease
eosinophils count	High eosinophils count is protective for Celiac Disease
lymphocytes count	Low lymphocytes count is protective for Celiac Disease
plateletcrit	High plateletcrit is protective for Celiac Disease
platelet count	High platelet count is protective for Celiac Disease
eosynophils percentage	No causal relationship
platelets distribution width	Low platelets distribution width protects for Celiac Disease
hematocrit	Low hematocrit is protective for Celiac Disease
hemoglobin	Low hemoglobin is protective for Celiac Disease

Conclusions

► Celiac 2010

Phenotype	Conclusion
eosinophils count	High eosinophils count protects for Celiac Disease
hematocrit	High hematocrit protects for Celiac Disease
neutrophils percentage	No causal relationship
eosinophils % granuloctyes	No causal relationship
eosinophils + basophils count	High eosinophils + basophils count protects for Celiac Disease
eosynophils percentage	No causal relationship
Type 1 Diabetes	Low Type 1 Diabetis protects for Celiac Disease
Multiple Sclerosis	Low Multiple Sclerosis protects for Celiac Disease
Packed cell volume	High packed cell volume protects for Celiac Disease
CD39+ Activated CD4 + CD8 - Treg % Parent	Low CD39+ Activated CD4 + CD8 - Treg % Parent protects for Celiac 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