Identifying methylome-metabolome interactions

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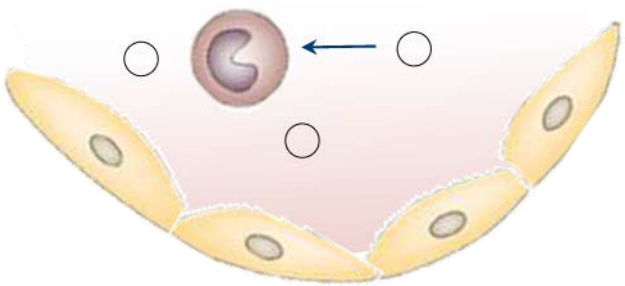
Part 1: Blood lipids influence DNA methylation in circulating immune cells

- Atherosclerosis is a lipid-driven immune disease

- Lipids epigenetically prime circulating immune cells, possibly affecting the development of atherosclerosis



Dekkers et al. *Curr Opin Lipidol.* (2016)

Biobank-based Integrative Omics Study

BBMRI.nl
Biobanking and
BioMolecular resources
Research Infrastructure
The Netherlands

- Six cohorts
  - CODAM, LL, LLS, NTR, PAN, RS
  - 3296 subjects
  - Whole blood

- Measurements
  - Methylome - 450k array
  - Genome - imputed SNP arrays
  - Transcriptome - RNA-Seq (n = 2044)
  - Lipids
  - Cell counts

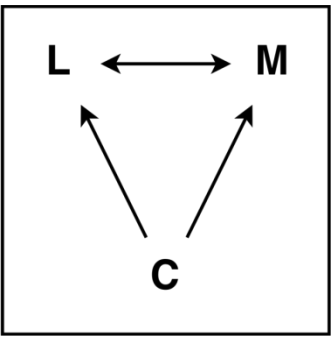
Dekkers et al. *Genome Biol.* 2016

EWAS on lipids

- Linear model per cohort per CpG
  - DNA methylation ~ lipid + gender + age + cell counts + batches
  - Correction for inflation

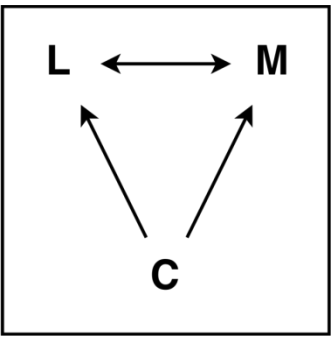
- Meta-analysis per CpG
  - Fixed-effect
  - Correction for inflation
  - Correction for multiple testing (FDR < 0.05)

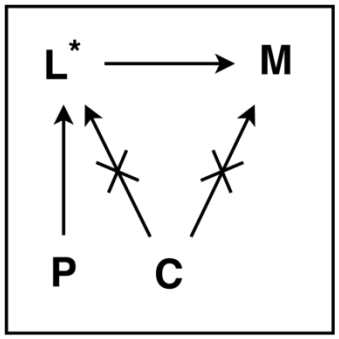
EWAS on lipids



TG	21
LDL-C	3
HDL-C	4

Mendelian randomization





	Variants	F-stat	R^2
Triglycerides	30	157	5.6%
LDL cholesterol	28	99	3.6%
HDL cholesterol	60	164	5.1%

TG	21
LDL-C	3
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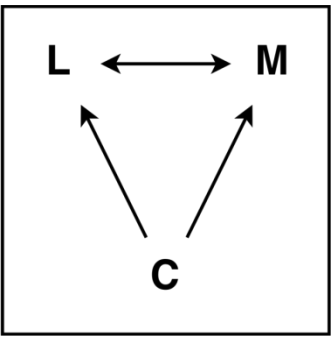
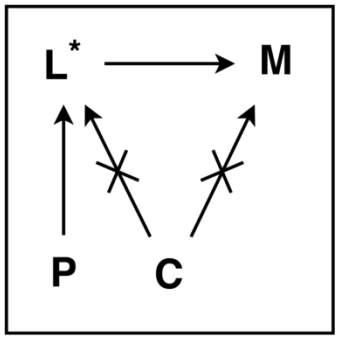
1. lipid ~ PS + gender + age + cell counts + batches
2. DNA methylation ~ predicted lipid + gender + age + cell counts + batches

Willer et al. *Nat Genet.* (2013)

Mendelian randomization





TG	21	9
LDL-C	3	2
HDL-C	4	2

	Variants	F-stat	R^2
Triglycerides	30	157	5.6%
LDL cholesterol	28	99	3.6%
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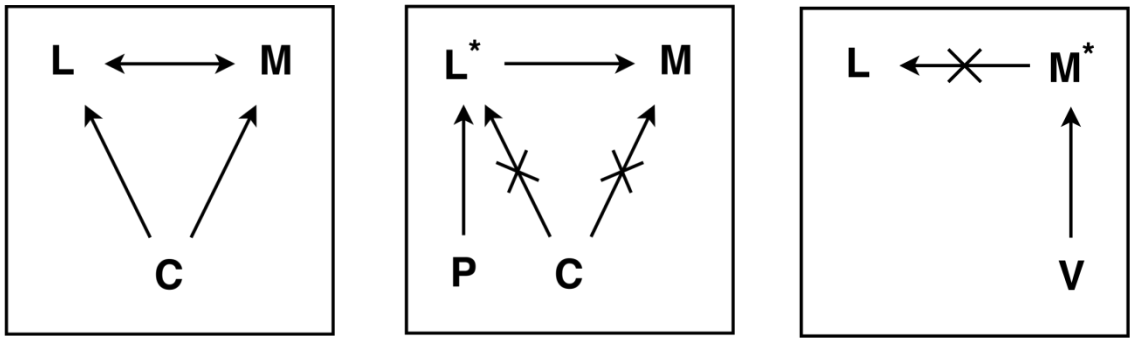
Negative control



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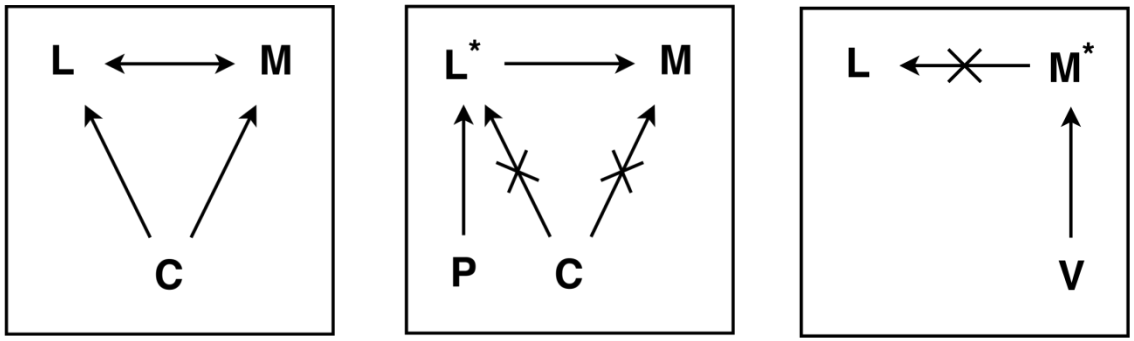

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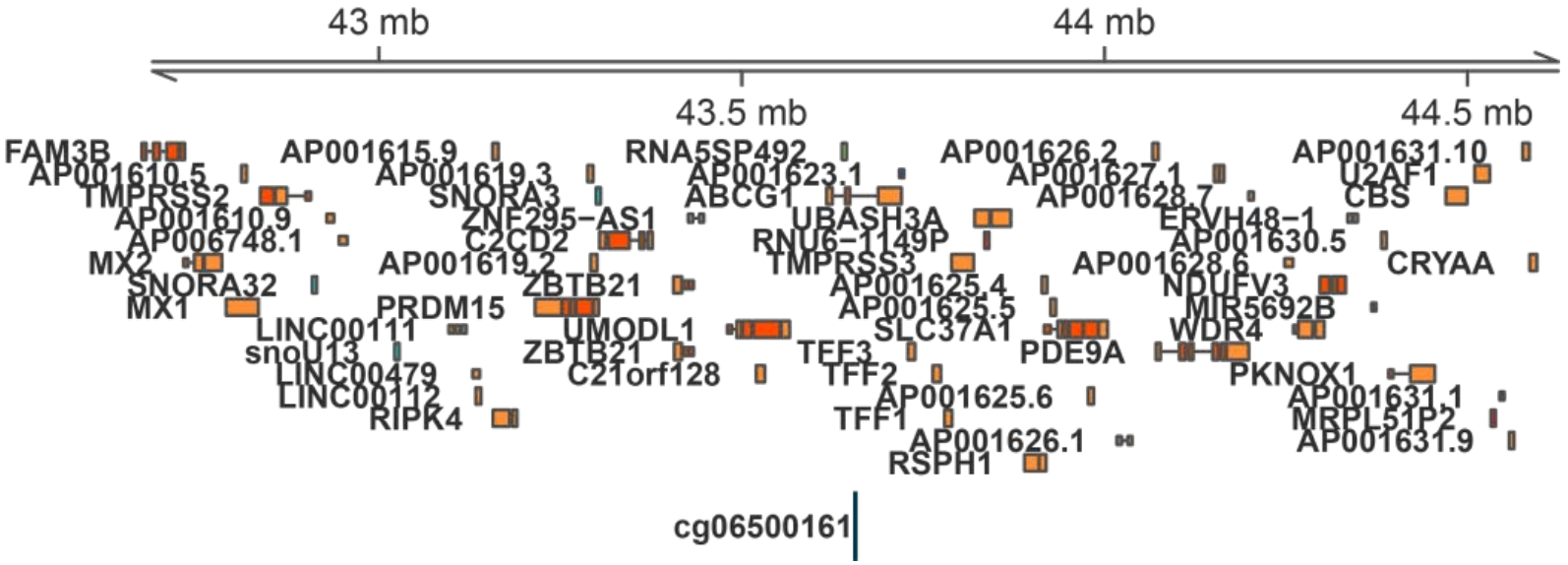
LDL-C	3	2	2
HDL-C	4	2	2

Blood lipids influence DNA methylation

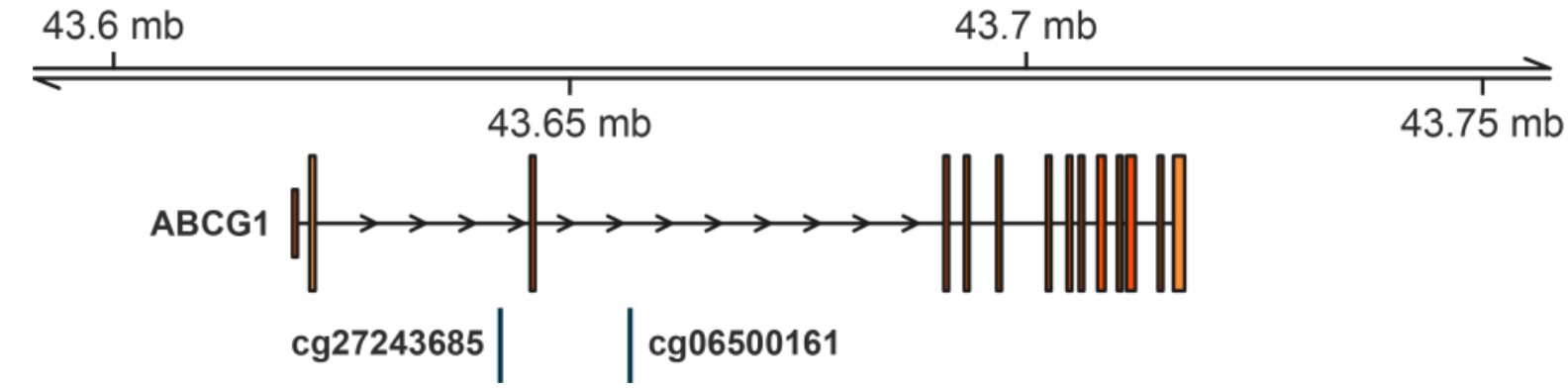
- Additional analyses to correct for pleiotropic effects

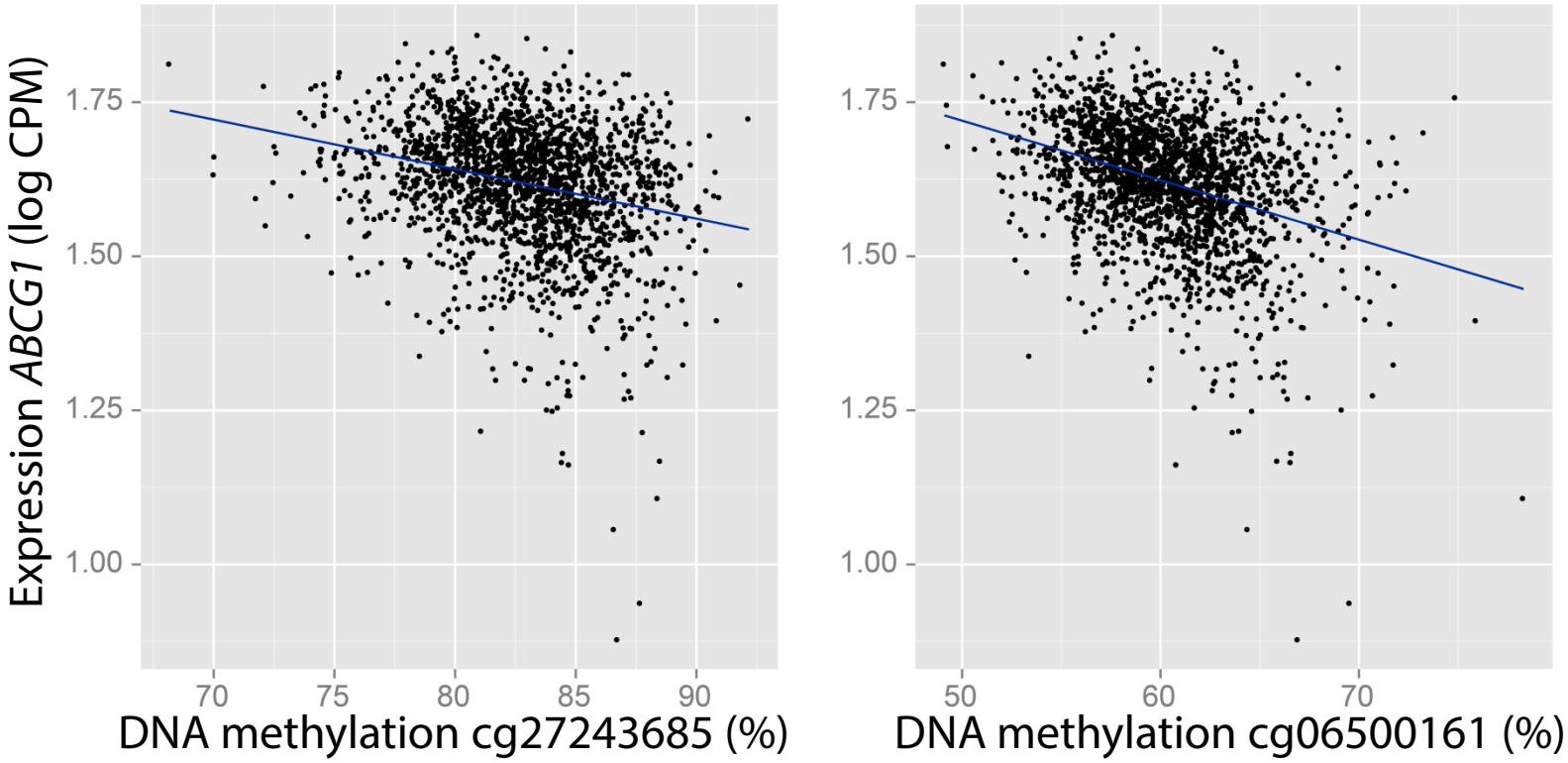
	EWAS P-value	MR P-value
Triglycerides		
cg00574958	1.0×10^{-28}	1.5×10^{-3}
cg17058475	1.4×10^{-11}	2.5×10^{-3}
cg11024682	4.2×10^{-16}	5.4×10^{-3}
LDL cholesterol		
cg27168858	2.3×10^{-8}	3.1×10^{-3}
HDL cholesterol		
cg27243685	5.4×10^{-15}	2.2×10^{-3}
cg06500161	2.9×10^{-19}	3.5×10^{-4}

Linking DNA methylation to gene expression



Linking methylation to expression





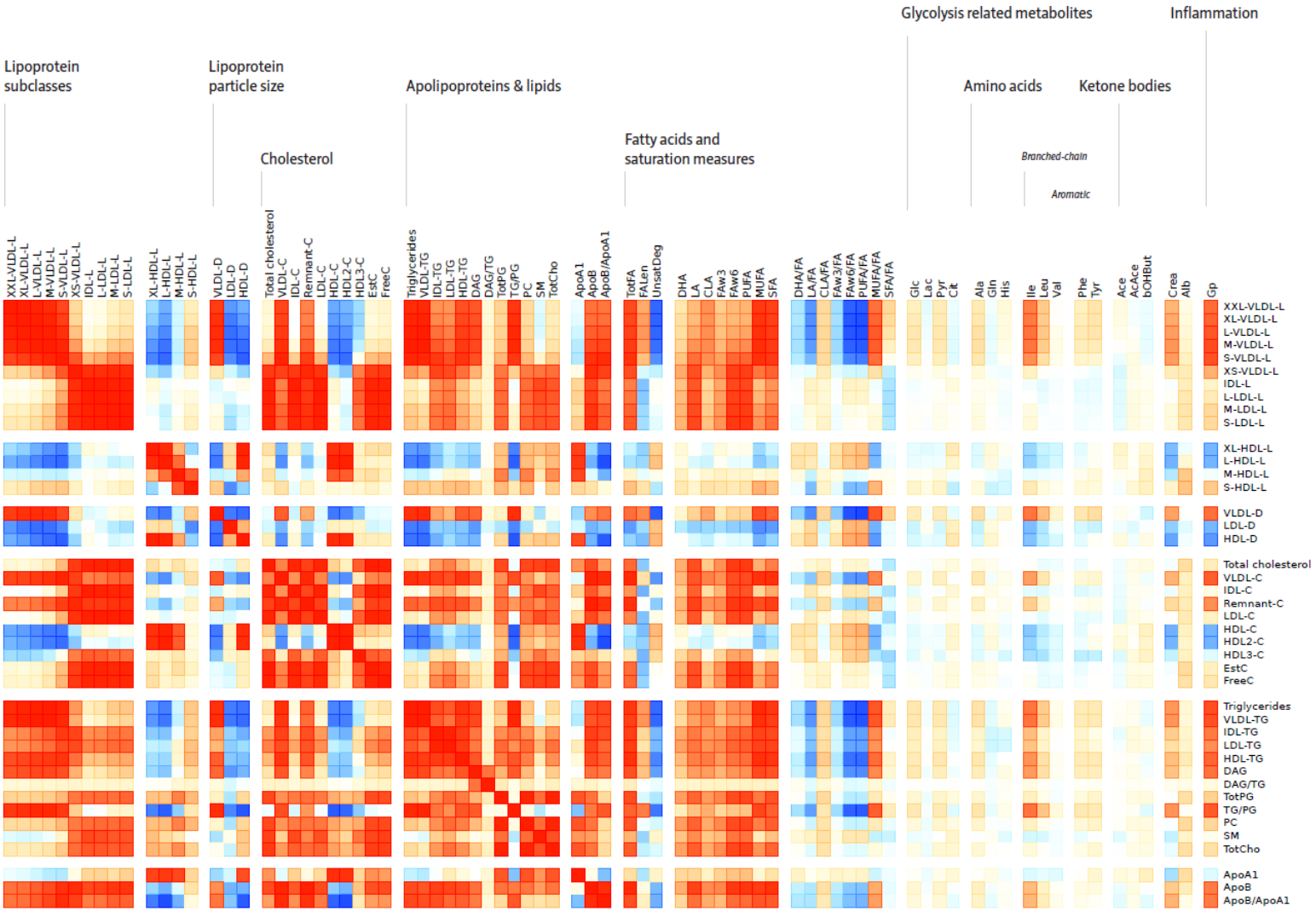
Linking DNA methylation to gene expression

	Gene	Function
Triglycerides		
cg00574958	*CPT1A*	lipid catabolism
cg17058475	*CPT1A*	lipid catabolism
cg11024682	*SREBF1*	regulation of lipid synthesis
LDL cholesterol		
cg27168858	*DHCR24*	cholesterol synthesis
HDL cholesterol		
cg27243685	*ABCG1*	mediates cholesterol efflux to HDL
cg06500161	*ABCG1*	mediates cholesterol efflux to HDL

→ **end-product feedback control**

Part 2: Brainshake metabolomics platform

- NMR-based detection of 250 metabolites



Metabolomics EWAS

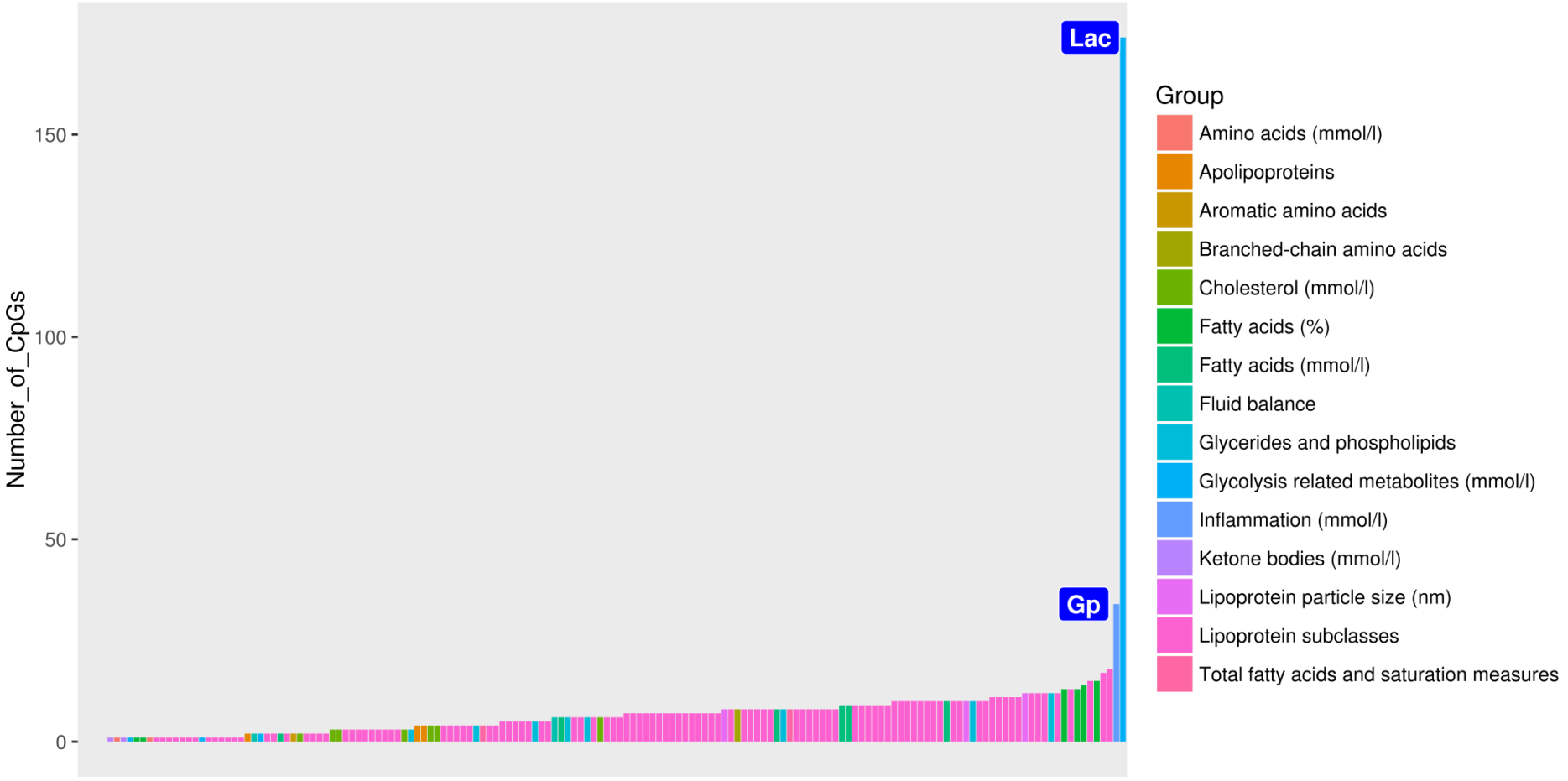
- Combines two BBMRI rainbow projects
  - RP3 (DNA methylation) with RP4 (Metabolomics)

- Preliminary results: metabolomics data not yet complete

- LL, LLS, NTR, RS

Number of associating CpGs per metabolite

- 156 metabolites associate with at least 1 CpG



Number of associating metabolites per CpG

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Conclusions

- Metabolites associate with DNA methylation near genes involved in metabolism in circulating cells
- For blood lipids we show DNA methylation changes are the consequence of inter-individual variation in lipid levels
- Next: Mendelian randomization to infer causality for other metabolites
- Europe-wide meta-analysis: 8 cohorts, n > 6000
  - University of Oulu (Johannes Kettunen)
  - *LL*, *LLS*, *NTR*, *RS*, FINRISK, KORA, ALSPAC and NFBC

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Leiden Longevity Study
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Netherlands Twin Register
- Dorret Boomsma

Prospective ALS study
- Jan Veldink

Rotterdam Study
- Cornelia van Duijn

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Cardio Vasculair Onderzoek Nederland