

EWAS meta-analysis and the PACE consortium

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By the end of this session, you will be able to...

- Explain the rationale behind meta-analysis in EWAS
- List different meta-EWAS approaches
- List different meta-EWAS tools
- Interpret meta-EWAS results
- List different consortia to facilitate meta-EWAS
- Design a meta-EWAS project pipeline
- Describe a real-life example of a meta-EWAS

Why meta-analyse?



- Meta-analysis is the statistical synthesis of information from multiple independent studies
- Improve power
- Reduce false positive findings

Principles of meta-analysis

“Absolute”

- Pooled individual-level data

“Relative”

- Compare summary statistics (e.g. coefficients and standard error) from multiple studies
- Can be as efficient in terms of statistical power as pooling individual-level data (*Lin & Zeng, 2009, Genetic Epidemiology*)

Sources of summary statistics

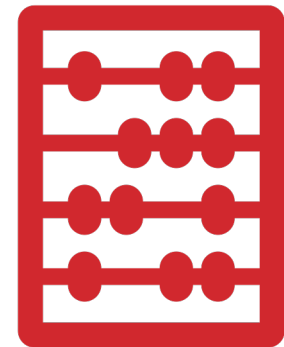
- Directly from other studies
- Online repositories of summary statistics (e.g. EWAS catalog, OSF, etc)
- Online repositories of publicly-available individual level data
- Papers



Meta-analysis models for EWAS

Calculating the average effect size estimate (ES).

- **Inverse variance weighting (IVW)**: more weight given to some studies than others. Weighting by the inverse of the variance of the study specific ES means more precise studies “count for more” and minimises the SE of the combined ES.
- **Fixed-effect IVW MA** assumes there is one true ES shared by all included studies and any observed difference arises from sampling error alone.
- **Random-effects IVW MA** allows true effect to vary from study to study



Meta-analysis models

- **Choosing between FE and RE**
- Assumptions of FE are less likely to be met than RE
- RE is less powerful than FE

"fixed-effects meta-analysis is a simple method that provides useful well-calibrated inference about an average effect, under mild conditions. **Fixed-effects meta-analysis can and often should be used in situations where effects differ between studies.** This recommendation in no way rules out statistical **analyses of heterogeneity**, and fixed-effects meta-analysis has natural complements (Cochran's Q, meta-regression) that provide them."
Rice, Higgins & Lumley, 2018

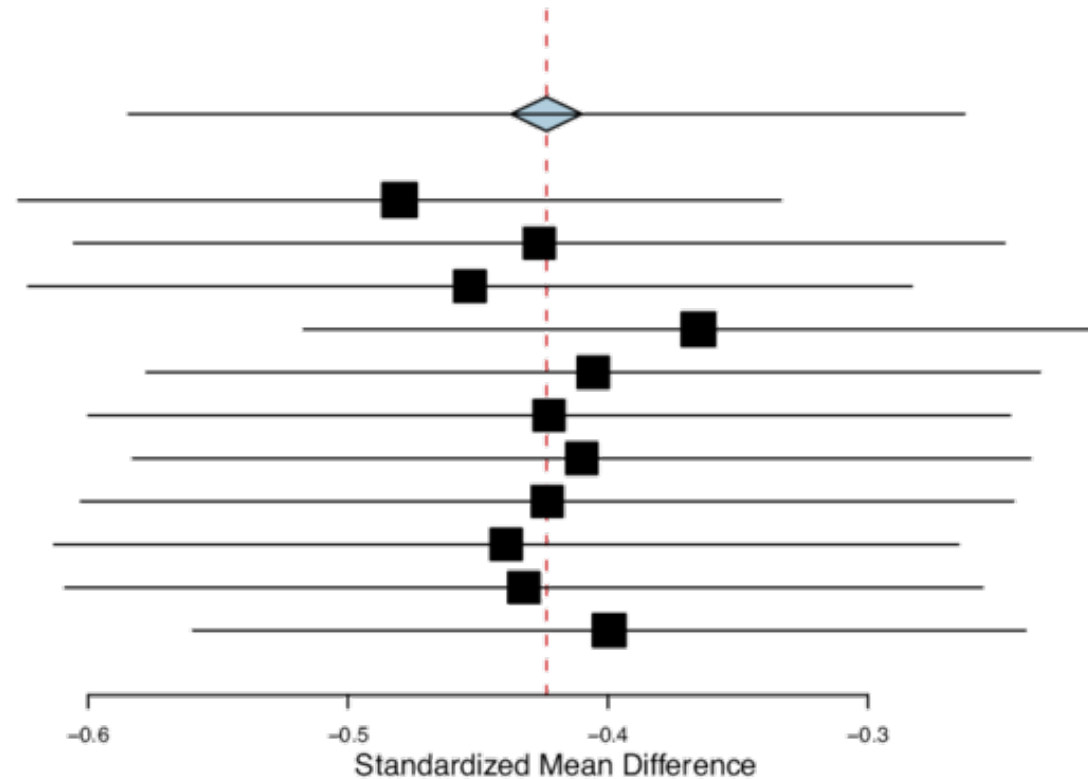
Heterogeneity

- Cochran's Q test
- Q = weighted sum of squared differences between individual study ESs and the combined ES.
- I^2 = the percentage of variation across studies that is due to heterogeneity rather than chance.
- Tau^2 = an estimate of the variance of the distribution of true effect sizes (i.e. the magnitude of heterogeneity)

Sensitivity analyses

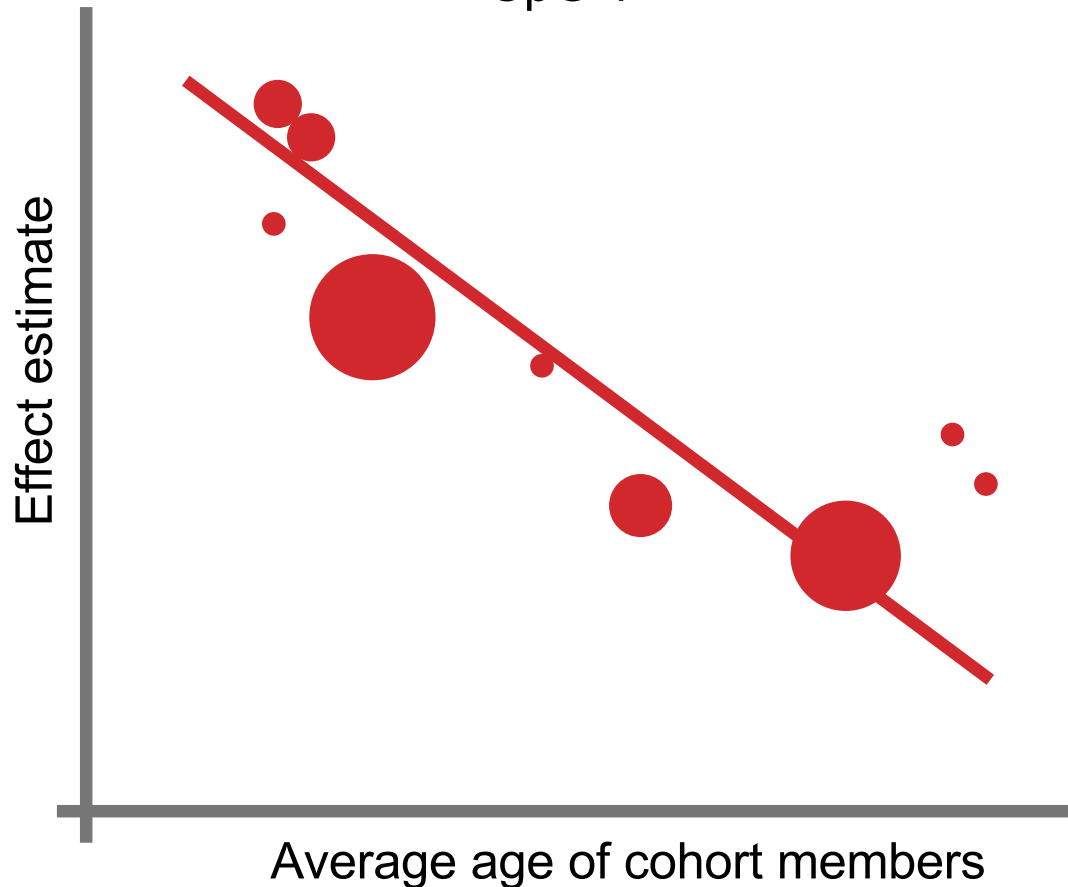
- Split studies by characteristics
- Leave-one-out analysis

Studies	Estimate (95% C.I.)
Overall	-0.424 (-0.584, -0.263)
- Labad 2015	-0.480 (-0.627, -0.333)
- Day 2014	-0.426 (-0.606, -0.247)
- Cullen 2014	-0.453 (-0.623, -0.283)
- Mondelli 2015	-0.365 (-0.517, -0.213)
- Pruessner 2015	-0.406 (-0.578, -0.234)
- Pruessner 2013	-0.423 (-0.600, -0.245)
- Aas 2011	-0.410 (-0.583, -0.237)
- Mondelli 2010	-0.423 (-0.603, -0.244)
- Pruessner 2008	-0.439 (-0.613, -0.265)
- Hempel 2010	-0.432 (-0.609, -0.256)
- Monteleone 2014	-0.399 (-0.560, -0.239)



Meta-regression

CpG 1



- Examines the impact of moderator variables on combined ES using regression-based techniques
- Study weights are also incorporated
- Potential problems: too few studies, too many sources of diversity, low power

Meta-EWAS tools: METAL

- Developed for GWAS meta-analysis.
- http://genome.sph.umich.edu/wiki/METAL_Program
- There are two possible approaches:

1) SAMPLE SIZE BASED

- Combines P-values across studies (taking sample size and direction of effect into account)

2) INVERSE VARIANCE BASED

- Combines coefficients and standard errors
- NOTE: in the official release of METAL, you can ONLY run FE MA

Meta-EWAS tools: metafor



- Very versatile
- Much slower than METAL
- Leave-one-out and meta-regression

EWAS consortia

- GoDMC - The genetic basis of DNA methylation variation (<http://www.godmc.org.uk/>)
- PACE – Pregnancy and Childhood Epigenetics (PACE) (<https://academic.oup.com/ije/article/47/1/22/4157905>)
- CHARGE - The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (<http://www.chargeconsortium.com/>)

Fetal exposures in pregnancy

- Air pollution
- Alcohol use
- Body mass index
- Diet
- Diseases
- Gestational weight gain
- Smoking
- Stress



Methodologic topics

- Causality
- Cell composition adjustment
- Differentially methylated regions
- Integrative omics analyses

PACE

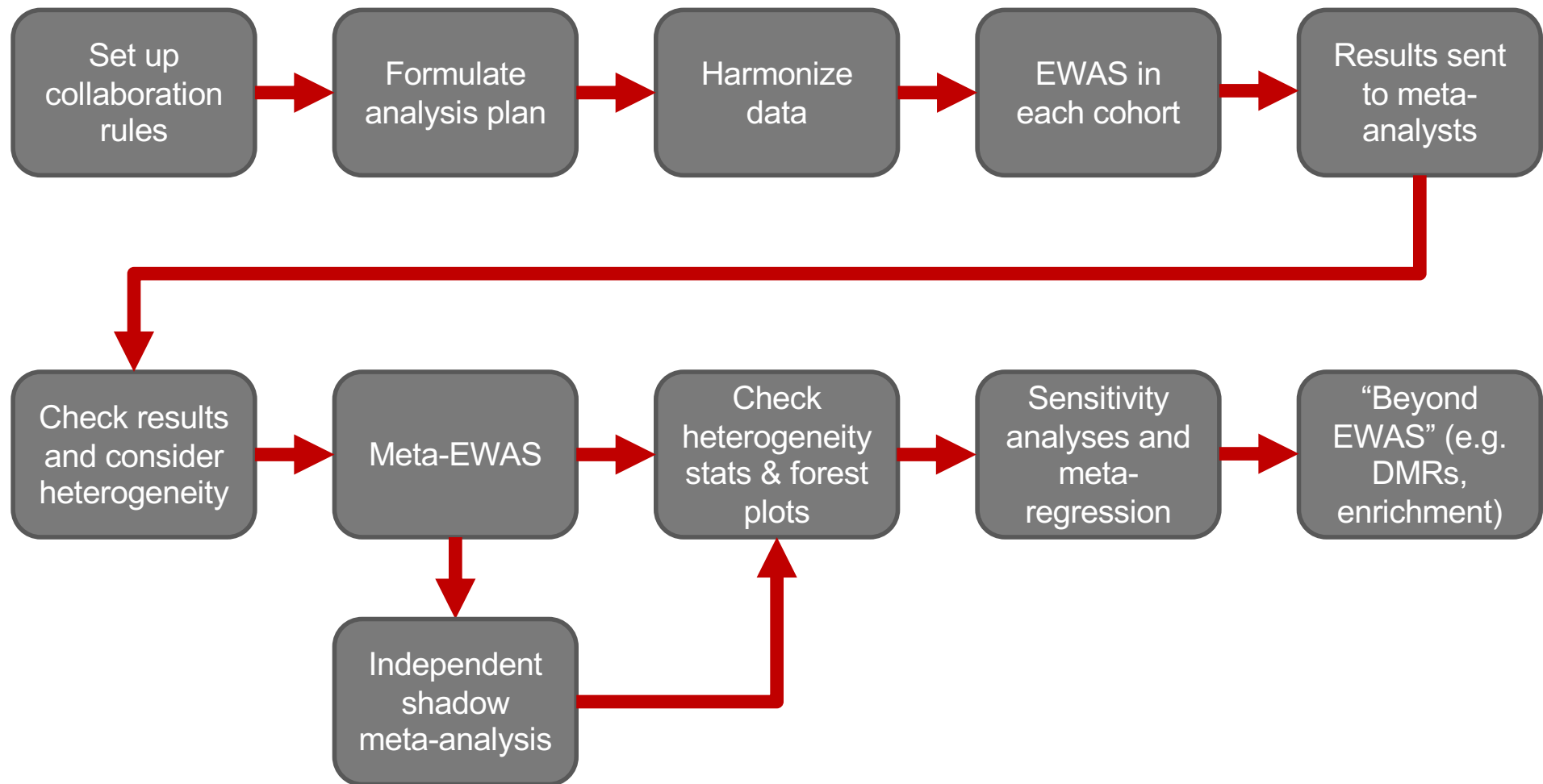
Pregnancy And Childhood Epigenetics

Birth, infancy and childhood exposures/outcomes

- Birth weight, gestational age
- Cardio-metabolic: body mass index, blood pressure
- Eczema, atopy
- Neuro-developmental
- Otitis media
- Respiratory: wheezing, asthma, lung function

Felix et al., **Cohort Profile: Pregnancy And Childhood Epigenetics (PACE) Consortium**, *International Journal of Epidemiology*, Volume 47, Issue 1, February 2018, Pages 22–23u, <https://doi.org/10.1093/ije/dyx190>

Example meta-EWAS pipeline



Example of meta-EWAS: Paternal BMI

Analysis plan:

https://github.com/ammegandchips/PACE_Paternal_BMI/blob/master/analysis_plan_bmi.md



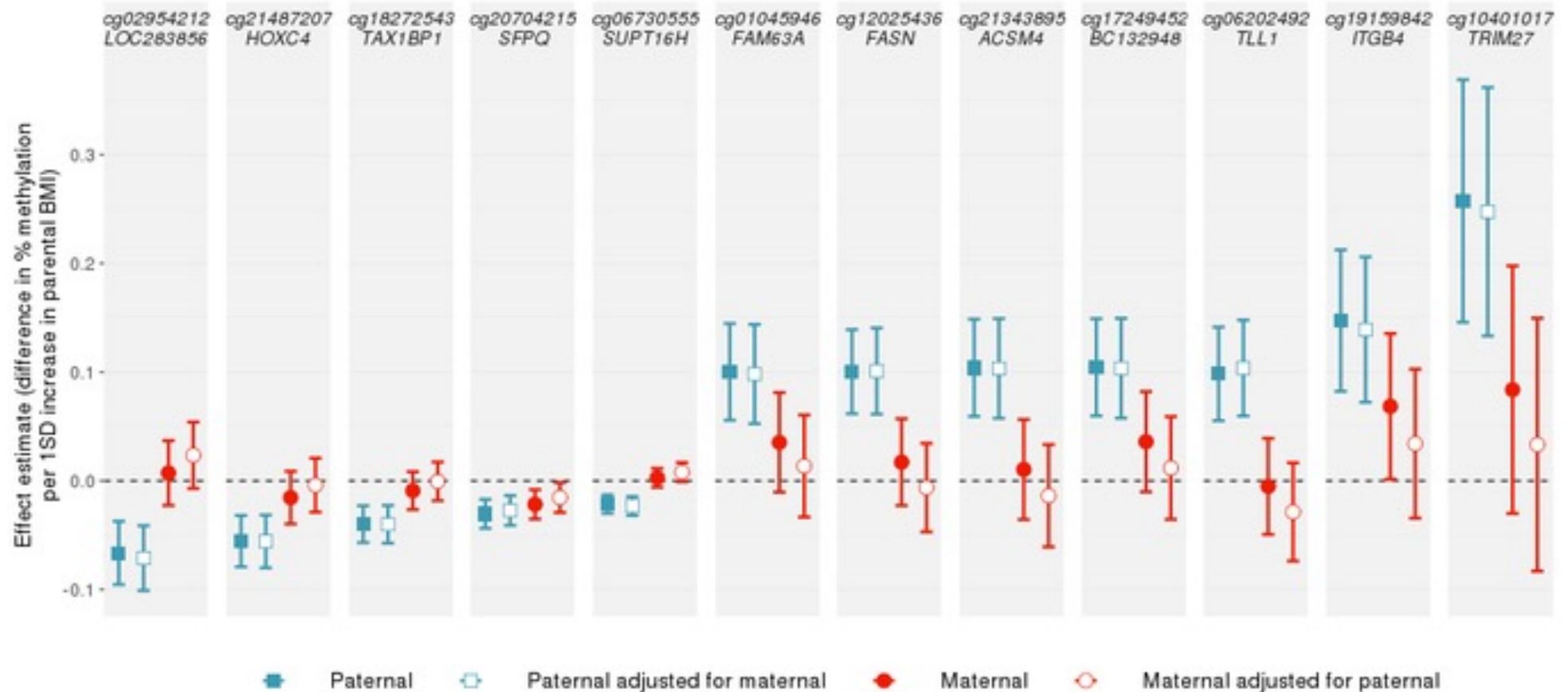
Cord blood at birth
13 datasets
4894 father-baby pairs

Peripheral blood in childhood
6 datasets
1982 father-child pairs

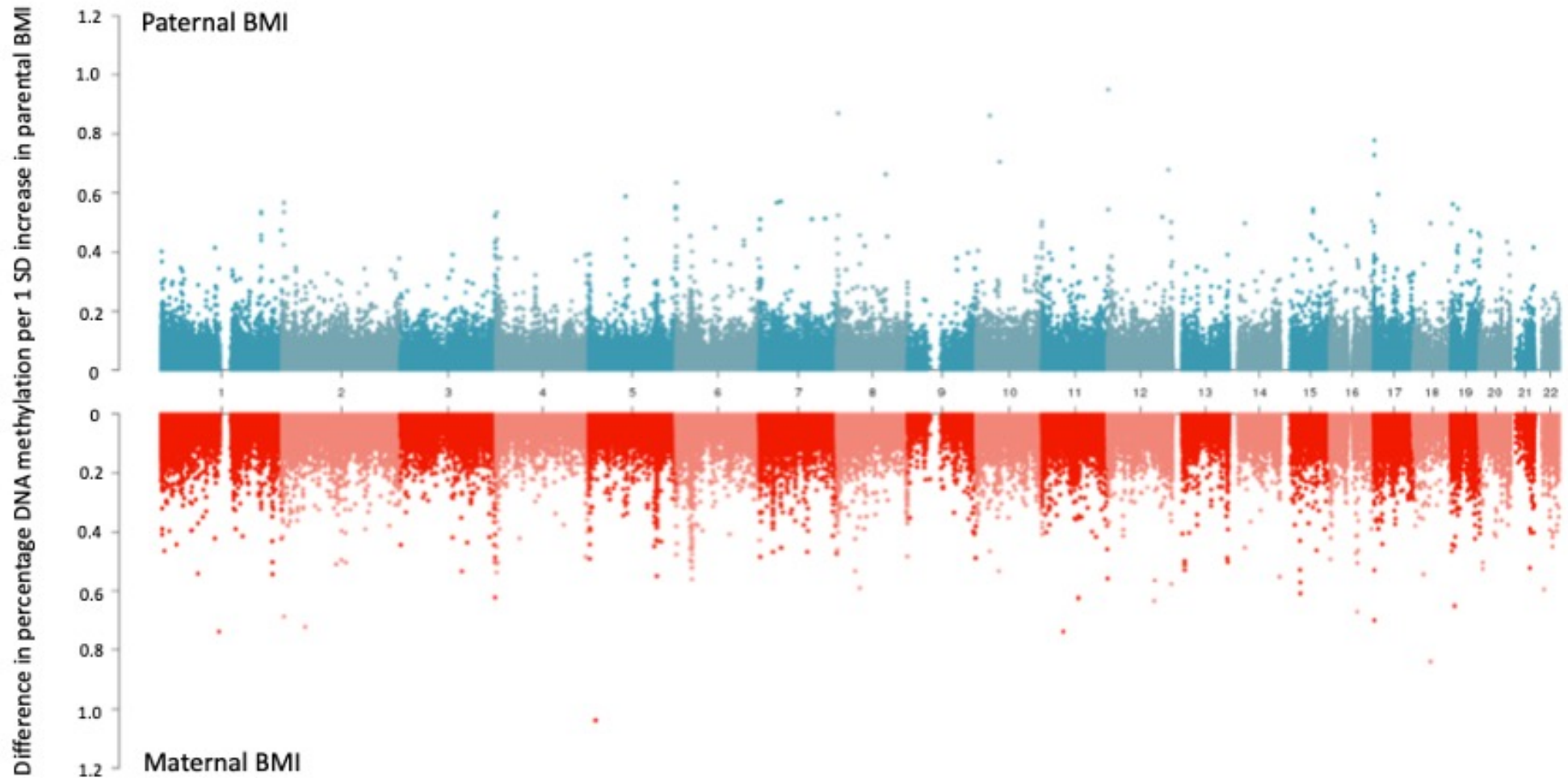
Sharp et al. (2021) Paternal body mass index and offspring DNA methylation: findings from the PACE consortium, *International Journal of Epidemiology*

Example of meta-EWAS: Paternal BMI

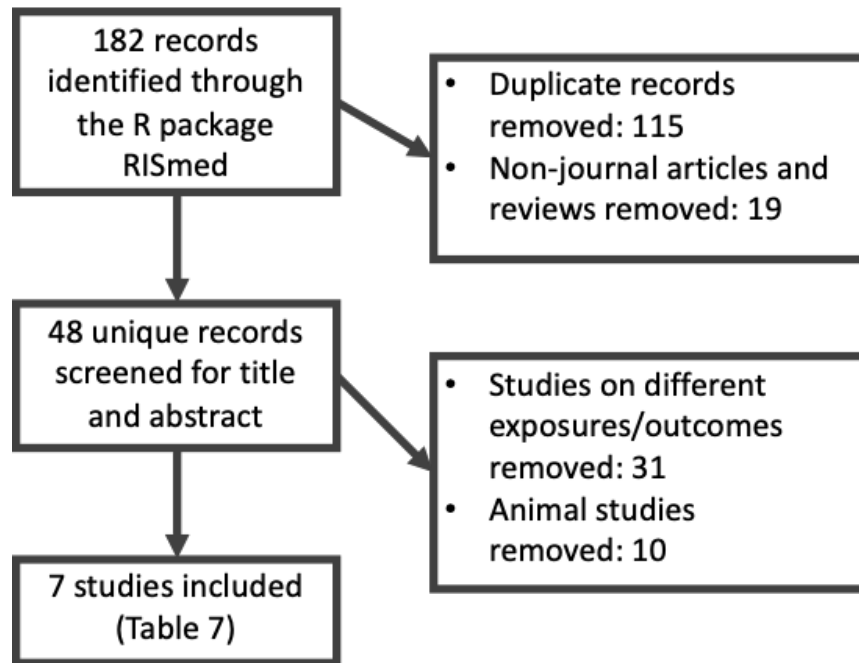
CpGs showing strongest statistical evidence of association with paternal BMI



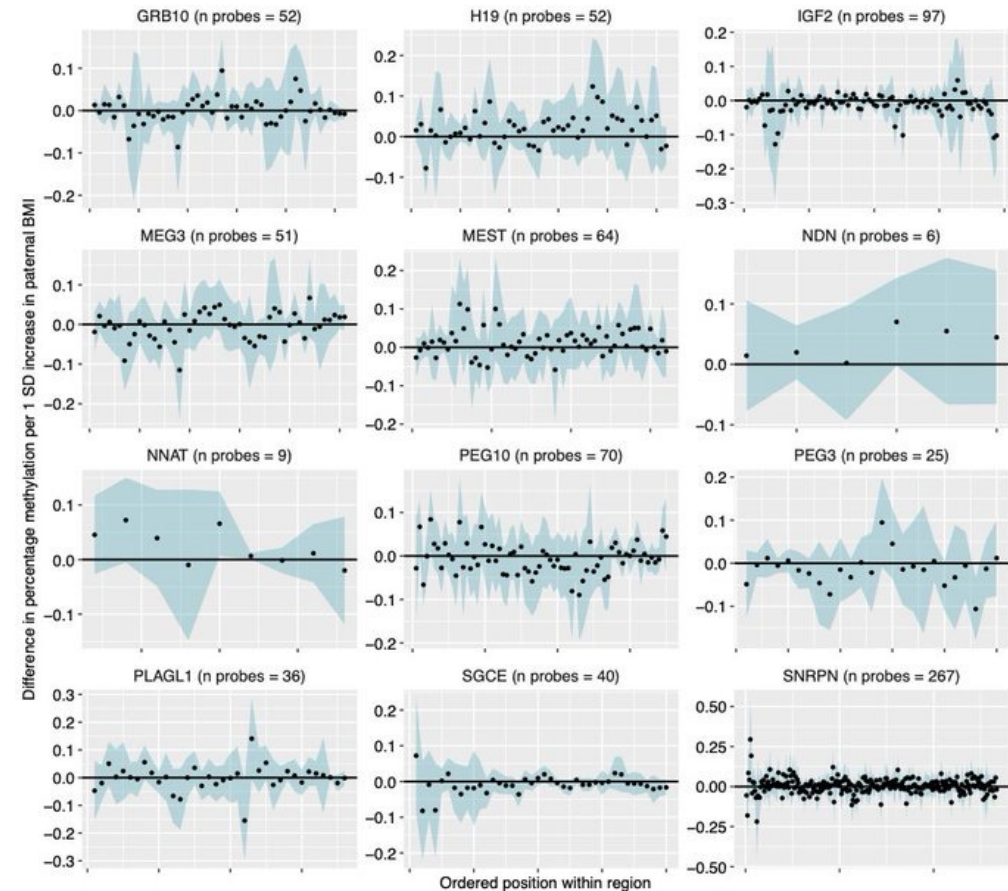
Example of meta-EWAS: Paternal BMI



Example of meta-EWAS: Paternal BMI



Associations between paternal BMI (adjusted for maternal BMI) and offspring methylation at birth in imprinted regions



Example of meta-EWAS: Paternal BMI

Sharp et al. (2021) Paternal body mass index and offspring DNA methylation: findings from the PACE consortium, *International Journal of Epidemiology*

Summary

- Meta-EWAS can improve power and reduce false positives
- Most meta-EWAS combine summary statistics from multiple cohorts
- METAL and metafor are commonly used tools
- Heterogeneity should be considered and sensitivity analyses and meta-regression can aid interpretation
- There are multiple consortia to facilitate meta-EWAS, including the PACE consortium
- Pre-specified, well-designed analysis plans are essential to meta-EWAS projects
- Meta-EWAS won't necessarily guarantee you positive findings!