Epigenomewide association study (EWAS) and Functional Summary-based Imputation (FUSION) association analyses

INTRODUCTION

Transcriptomewide association statistic z_{TWAS} was originally proposed for gene expression data. For a given Trait of interest **T** for which GWAS summary statistics z_T is available, the corresponding Wald statistic for TWAS is defined such that

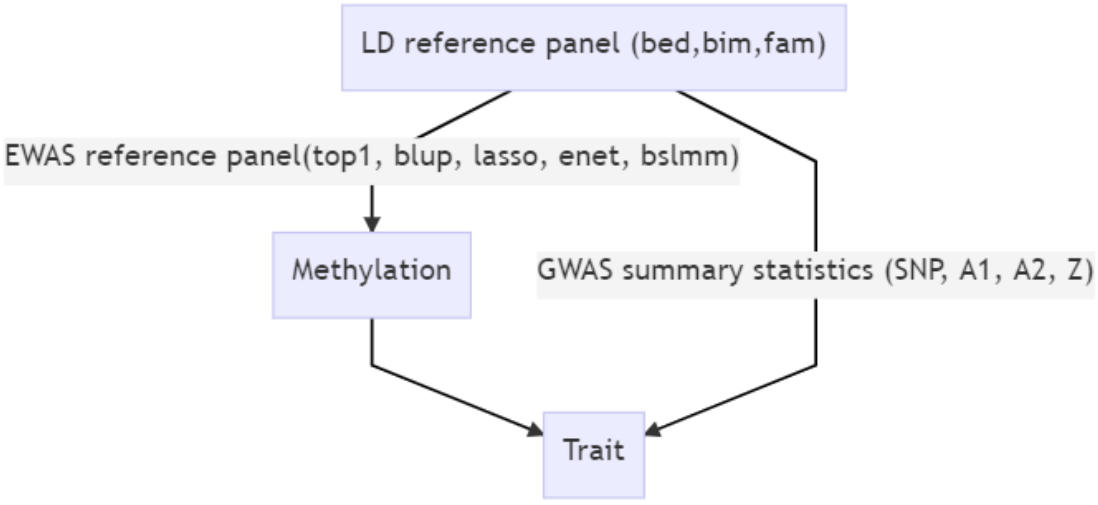
$$z_{TWAS} = \frac{w_{ge}^T z_T}{\sqrt{w_{ge}^T V w_{ge}}}$$

where w_{ge} is a weight associated with gene expression and **V** covariance matrix for z_T, respectively.

By analogy, an epigenomewide association statistic z_{EWAS} is defined through methylation data so that

$$z_{EWAS} = \frac{w_{me}^T z_T}{\sqrt{w_{me}^T V w_{me}}}$$

where w_{me} is the weight associated with methylation. Both approaches allow for imputation using GWAS summary statistics. The derivation of these weights and imputation were done using methods as described in Gusev et al. (2016) called TWAS as well as in Mancuso et al. (2016) called Functional Summary-based Imputation (FUSION). The TWAS statistics from both approaches agreed very well.

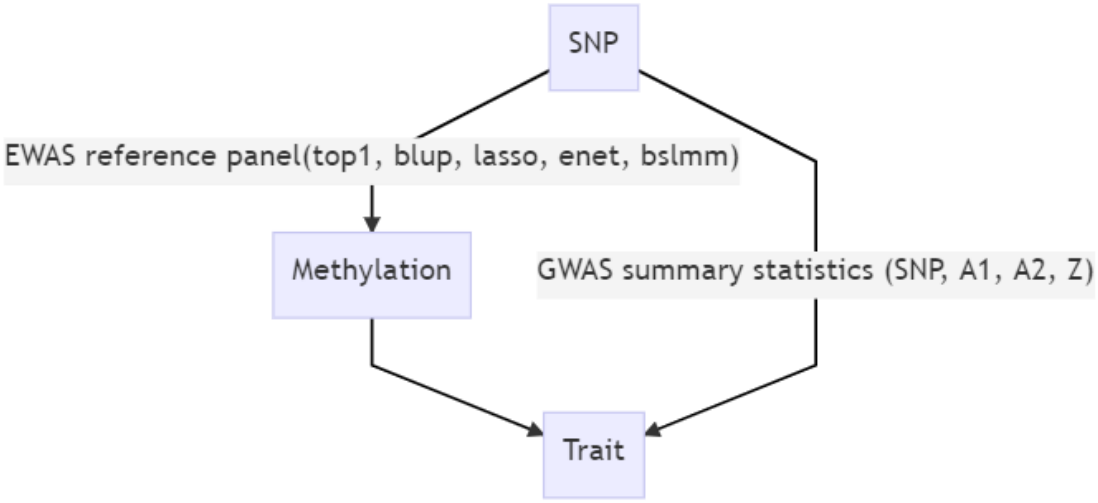


A total of 442,920 CpG sites based on Illumina humanmethylation450 chips on 1,.146 individuals in EPIC-Norfolk study were available. Among these, 1,117 individuals also had genotype data from Affymetrix BioBank Axiom chips. HapMap2 SNPs from genetic data of these individuals were extracted via PLINK2 according to cis-positions of each probe and

subsequently used to build weight analogous to gene expression data as implemented in computer software TWAS. We filtered probes according to their heritabilities estimated from software GCTA at significant level of 0.01. We then performed EWAS for given GWAS summary statistics. The weight generation and methylation imputation was implemented in software called TWAS-pipeline, which allows for whole epigenome computation. After filtering, 78,133 probes reached significant level 0.01.

The FUSION framework has several advantages: First, it integrates heritability estimation and covariate adjustment for whole-chromosomes with additional models such as LASSO, elastic net, BLUP. Second, it offers cross-validation, joint/conditional analyses with the output also informing top hit SNPs and inferred methylation quantitative trait locus (meQTL). Besides, the new software uses modified GCTA software (gcta_nr_robust) leading to higher yield of probes with heritabilities reaching statistical significance, GEMMA giving BSLMM estimates and ability to align strands with reference panels. As both the increased number of models and cross-validation led to excessive computing time, we dropped BSLMM models and conducted five cross-validations. As a result our reference panel for EWAS imputation contains 77,372 probes reaching the heritability p value threshold of 0.01. The association as well as joint/conditional analysis using our weights and LD panel is implemented in software called EWAS-fusion. Like the original TWAS, our implementation will enable a range of GWAS summary statistics to be used coupled with downstream analysis.

EWAS-fusion is reminiscent of Mendelian Randomisation as shown below,



INSTALLATION

- To begin, the software FUSION including dependencies such as `plink2R` and `reshape` is required. The latest version also requires jlimR. Other facilities to be required are Sun grid engine (sge) or GNU parallel for Linux clusters.

- Install the repository on your system, you will need weights based on epigenetic data or to generate them as described in **Weight generation** below.

FILE	Description
EWAS-weights/	directory for EWAS weights
glist-hg19	Probe list
LDREF/	Reference for LD
EWAS-weights.pos	Definition of regions
EWAS-weights.profile*	Probe profiles

* It contains information about the probes but not directly involved in the association analysis. Earlier version of EWAS-fusion used EWAS/, RDat.pos, and RDat.profile.

USAGE

The syntax is as follows,

```
ewas-fusion.sh input-file
```

These will send jobs to the Linux clusters. The sge error and output, if any, should be called EWAS.e and EWAS.o in your HOME directory.

Input

The input file contains GWAS summary statistics similar to .sumstats as in LDSC with the following columns.

Column	Name	Description
1	SNP	RS id of SNPs
2	A1	Effect allele (first allele)
3	A2	Other allele (second allele)
4	Z	Z-scores, taking sign with repect to A1

Output

The results will be in input-file.tmp/ directory.

Annotation

This is furnished with contribution from Dr Alexia Cardona, alexia.cardona@mrc-epid.cam.ac.uk, as follows,

```
Rscript ewas-annotate.R input-file.tmp
```

It is assumed that `HumanMethylation450_15017482_v1-2.csv` is available from the directory containing `ewas-annotate.R`but this can be at different location

```
Rscript ewas-annotate.R input-file.tmp
manifest_location=/at/different/location
```

Q-Q and Manhattan plots using R/gap can be obtained from

```
Rscript ewas-plot.R input-file.tmp
```

Example

The script test.sh uses data reported in Wood, et al. (2015). It downloads and generates an input file called `height` to `ewas-fusion.sh`.

```
ewas-fusion.sh height
```

The results will be in `height.tmp/` once it is done.

The annotation is done with

```
Rscript ewas-annotate.R height.tmp
```

The Q-Q and Manhattan plots are generated with

```
Rscript ewas-plot.R height.tmp
```

Weight generation

This is a revised and much simplified implementation of codes available from TWAS-pipeline. Under our sge it is furnished with

```
qsub get_weight.qsub
```

or

```
qsub get_weight.qsub 22
```

for chromosome 22.

Inputs to these are summarised as follows,

File	Description
FUSION.pheno	PLINK phenotype file containing data for all probes
FUSION.covar	PLINK covariate file containing covariates such as PCs
CpG.txt	CpG ID, chromosome and position

In addition, PLINK binary pedigree file for each CpG also requires to be prepared, as in files. Although it was not done, it is possible to use code as in 1KG.sh to get around gerneration of these individual files by using a combined one. Note the setup takes advantage of the compact storage of non-genetic data.

The results will be available from the EWAS-fusion directory to be profiled and used for association analysis above. As the number of files is fairly large, cp_weight.qsub is written to put weights from their temporary directories in place while ewas-profile.sh profiles these weights as well as prepares for LDREF. For the version with FUSION, it can be done as follows,

```
wget -qO- https://data.broadinstitute.org/alkesgroup/FUSION/LDREF.tar.bz2 |
tar xfj - --strip-components=1
seq 22|awk -vp=1000G.EUR. '{print p $1}' > merge-list
plink-1.9 --merge-list merge-list --make-bed --out FUSION
rm 1000G.EUR.* merge-list
sort -k2,2 FUSION.bim > EUR.bim
```

To mirror FUSION, which uses glist-hg19, an equivalent for EWAS needs to be built.

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APPENDIX

Additional information for Illumina infinium humanmethylation450 beadchip as in Illumina website

Column Name	**Description**
Index	Probe Index
TargetID	Identifies the probe name. Also used as a key column for data import.
ProbeID_A	Illumina identifier for probe sequence A

ProbeID_B	Illumina identifier for probe sequence B
IlmnID	Unique CpG locus identifier from the Illumina CG database
Name	Unique CpG locus identifier from the Illumina CG database
AddressA_ID	Address of probe A
AlleleA_ProbeSeq	Sequence for probe A
AddressB_ID	Address of probe B
AlleleB_ProbeSeq	Sequence for probe B
Infinium*Design*Type	Defines Assay type - Infinium I or Infinium II
Next_Base	Base added at SBE step - Infinium I assays only
Color_Channel	Color of the incorporated baseá (Red or Green) - Infinium I assays only
Forward_Sequence	Sequence (in 5'-3' orientation) flanking query site
Genome_Build	Genome build on which forward sequence is based
CHR	Chromosome - genome build 37
MAPINFO	Coordinates - genome build 37
SourceSeq	Unconverted design sequence
Chromosome_36	Chromosome - genome build 36
Coordinate_36	Coordinates - genome build 36
Strand	Design strand
Probe_SNPs	Assays with SNPs present within probe >10bp from query site
Probe*SNPs*10	Assays with SNPs present within probe ?10bp from query site (HM27 carryover or recently discovered)
Random_Loci	Loci which were chosen randomly in the design proccess
Methyl27_Loci	Present or absent on HumanMethylation27 array
UCSC*RefGene*Name	Gene name (UCSC)
UCSC*RefGene*Accession	Accession number (UCSC)
UCSC*RefGene*Group	Gene region feature category (UCSC)
UCSC*CpG*Islands_Name	CpG island name (UCSC)
Relation*to*UCSC*CpG*Island	Relationship to Canonical CpG Island: Shores - 0-2 kb from CpG island; Shelves - 2-4 kb from CpG island.
Phantom	FANTOM-derived promoter
DMR	Differentially methylated region (experimentally determined)
Enhancer	Enhancer element (informatically-determined)
HMM_Island	Hidden Markov Model Island
Regulatory*Feature*Name	Regulatory feature (informatically determined)
Regulatory*Feature*Group	Regulatory feature category
DHS	DNAse hypersensitive site (experimentally determined)

Bioconductor packages

These are **IlluminaHumanMethylation450kanno.ilmn12.hg19** and **IlluminaHumanMethylation450kmanifest** as shown in **minfiDataEPIC**.

```
library(IlluminaHumanMethylation450kanno.ilmn12.hg19)
data(IlluminaHumanMethylation450kanno.ilmn12.hg19)
data(Locations)
data(Other)
data(Manifest)
data(SNPs.Illumina)
data(Islands.UCSC)
```

and for instance we have

```
> data(IlluminaHumanMethylation450kanno.ilmn12.hg19)
> IlluminaHumanMethylation450kanno.ilmn12.hg19
IlluminaMethylationAnnotation object
Annotation
  array: IlluminaHumanMethylation450k
  annotation: ilmn12
  genomeBuild: hg19
Available annotation
  Islands.UCSC
  Locations
  Manifest
  Other
  SNPs.132CommonSingle
  SNPs.135CommonSingle
  SNPs.137CommonSingle
  SNPs.138CommonSingle
  SNPs.141CommonSingle
  SNPs.142CommonSingle
  SNPs.144CommonSingle
  SNPs.146CommonSingle
  SNPs.147CommonSingle
  SNPs.Illumina
Defaults
  Locations
  Manifest
  SNPs.137CommonSingle
  Islands.UCSC
  Other
```