EWAS for Incident CVD Events

WGCNA detects incident CVD-associated modules

WGCNA was performed on the full set of CpG sites passing quality control steps in WHI. Associations with incident CVD were assessed using Cox models with adjustment for only DNA isolation batch and Houseman estimated cell counts.

module	modSize	varExpl	p	enrichment	islandLocEnrich	geneLocEnrich
blue	29441	0.45	0.000274	organism development	islands	1stExon
brown4	953	0.53	0.004550	immune activation	Open sea	
lavenderblush3	568	0.45	0.005000	T cell activation	Open sea	Body
firebrick4	371	0.43	0.018800	cell development	Islands	TSS1500
darkolivegreen4	366	0.37	0.019900	development	Shores	TSS1500
brown	21296	0.52	0.035800	immune+development	Open sea;Shores	Body

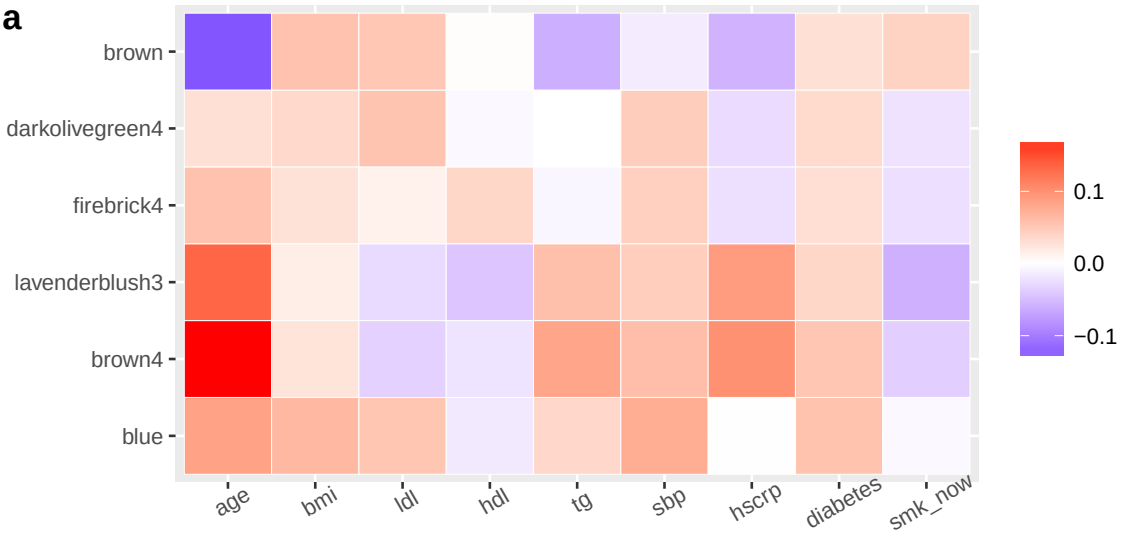
Six modules were at least nominally associated (before adjustment for any cardiovascular risk factors). The first principal components of these modules explained notable fractions of the variability (40-50%), so are reasonable proxies for module association analyses. The modules were enriched primarily for either immune function or developmental processes.

Moderate enrichment for constituent CpGs of Horvath's DNAm age predictor was observed for the blue (p=1.69e-05) and brown (p=0.000267) modules.

Modules associate moderately with known risk factors

Correlations (heatmap) and partial correlations (network; qgraph package) between module eigenCpGs and cardiovascular risk factors (log-transformed) are shown. EigenCpGs were oriented as necessary such that their cell count-adjusted association with incident CVD was positive.

a


brown
darkolivegreen4
firebrick4
lavenderblush3
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age
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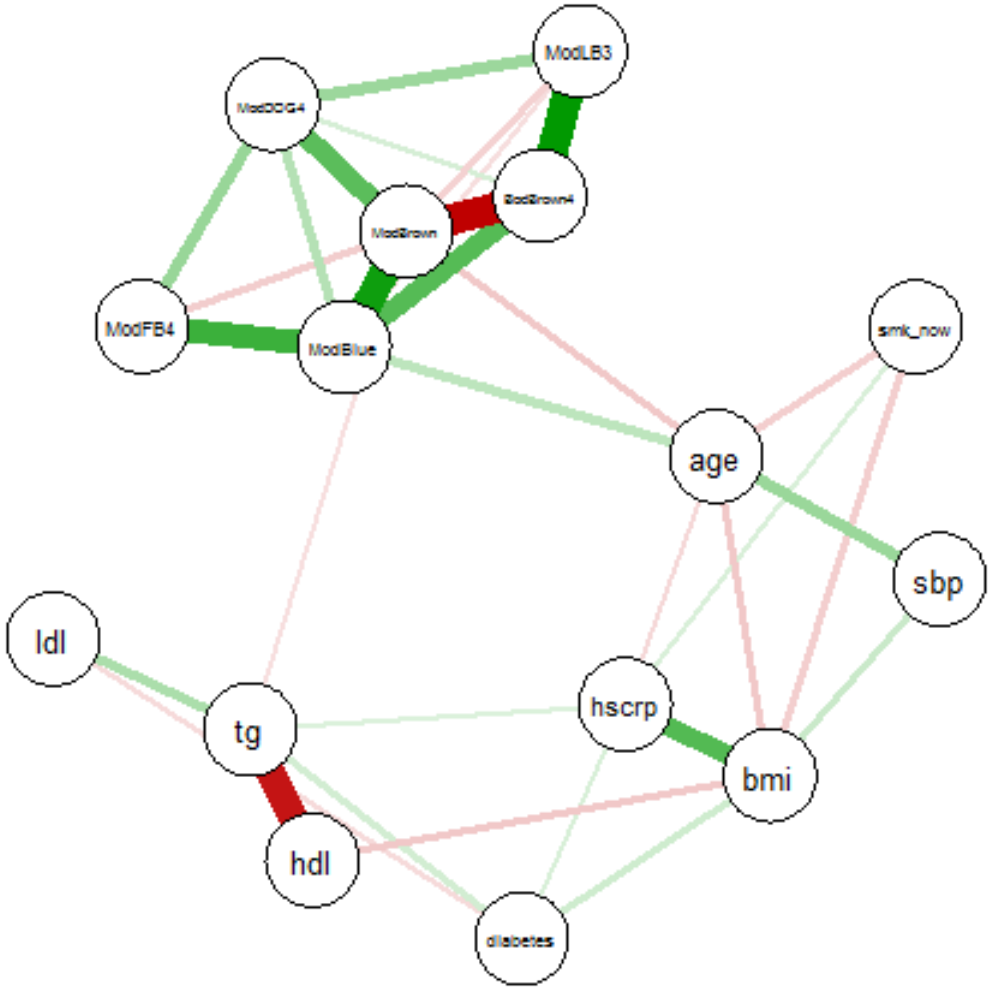
b

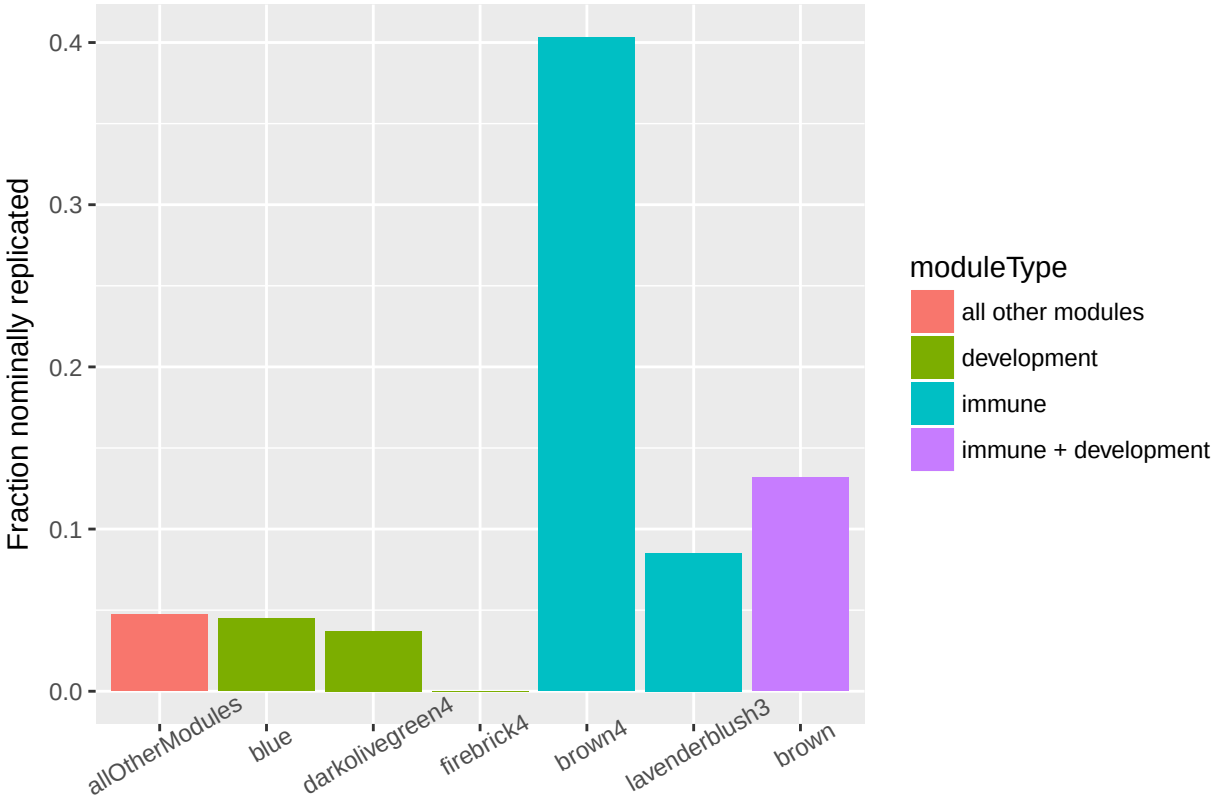

ModLB3
ModDOG4
ModBrown4
ModBrown
ModFB4
ModBlue
smk_now
age
sbp
ldl
tg
hscrp
bmi
hdl
diabetes

Some modules are related to age, but none (other than possibly brown4) seem to be strongly tracking age, and the other major risk factors even less so. The PC network reveals a general clustering of all modules with each other and away from the risk factors, suggesting that these modules may represent novel mechanisms of cardiovascular risk.

Investigation of strong signals at the single-CpG level

All CpGs in these 6 modules were analyzed individually to find specific signals that may be driving the associations. The models were the same as in my previous described EWAS (Cox models with extreme outlier filtering and correction for age/race/bmi/smoking/cell counts/DNA batch) The same relationships were tested in FHS as a validation dataset, additionally correcting for sex and FHS-specific technical covariates.

CpG	coef.whi	p.whi	fdr	coef.fhs	p.fhs	module	annot_gene
cg09155044	4.10	6.63e-09	0.000351	2.16	0.0856	brown	VKORC1
cg11691298	4.95	3.41e-08	0.000904	0.55	0.732	blue	FAM59B
cg18335681	3.95	4.82e-07	0.00775	-2.30	0.182	blue	MUM1
cg17611074	4.12	5.85e-07	0.00775	2.37	0.182	brown	CCDC85A
cg02379107	2.15	8.02e-07	0.0085	0.38	0.605	blue	KIAA1755
cg25246158	11.40	1.95e-06	0.0172	-0.60	0.916	blue	CSNK1G2
cg21172322	2.75	3.63e-06	0.0253	0.01	0.989	blue	BCAT1
cg07196571	5.26	3.83e-06	0.0253	0.44	0.845	brown	SNX22
cg18772376	10.37	6.41e-06	0.0376	5.35	0.251	blue	ST8SIA1
cg03725309	-4.50	7.09e-06	0.0376	-6.66	0.00231	brown	SARS
cg11403708	3.56	8.75e-06	0.0411	-0.70	0.622	brown	
cg01422797	3.83	1.04e-05	0.0411	1.07	0.43	blue	PRDM13
cg24968336	5.39	1.06e-05	0.0411	0.04	0.988	blue	KCNK6
cg08866794	2.85	1.08e-05	0.0411	1.20	0.539	brown	C6orf132



One single site was found to reach FDR<0.05 in WHI (this FDR calculation is biased because the modules were pre-selected for association – can discuss more) and replicated at the Bonferroni level in FHS. There was a notable lack of replication for the majority of sites.

However, it seems that in general, the use of module-level associations as an initial filter was helpful in finding biologically relevant associations: of all sites w/ nominal p<0.05 in the discovery set, 8.5% of those found in relevant modules replicated at p<0.05 in the validation set, whereas only 4.7% of those found in other modules replicated at p<0.05 (p=1.56e-25, Chi-sq. test). Exploring this idea further...

- This enrichment of successful validation occurs in only immune-related module CpGs, not development-related (particularly strong in the small brown4 immune-related module – see plot above).
- This may be explained by sex differences! Examining associations between eigenCpGs for these same modules in FHS:
  - Development-related modules all show negative interactions between male sex and module activation
  - Immune-related modules all show (non-significant) positive interactions between male sex and module activation
  - Suggestion: immune-related modules are replicating because of a shared mechanistic association across datasets, while development-related modules track potentially female-specific mechanisms.

Regional approach

Comb-p was used to uncover regional associations, using the same set of ~50k CpGs constituting the 6 modules of interest (These regions are somewhat smaller in general due to the decreased "tiling density" resulting from the module filter).

Table 3: Comb-p regions with multiple test-corrected p<0.05 in WHI and uncorrected p<0.05 in FHS

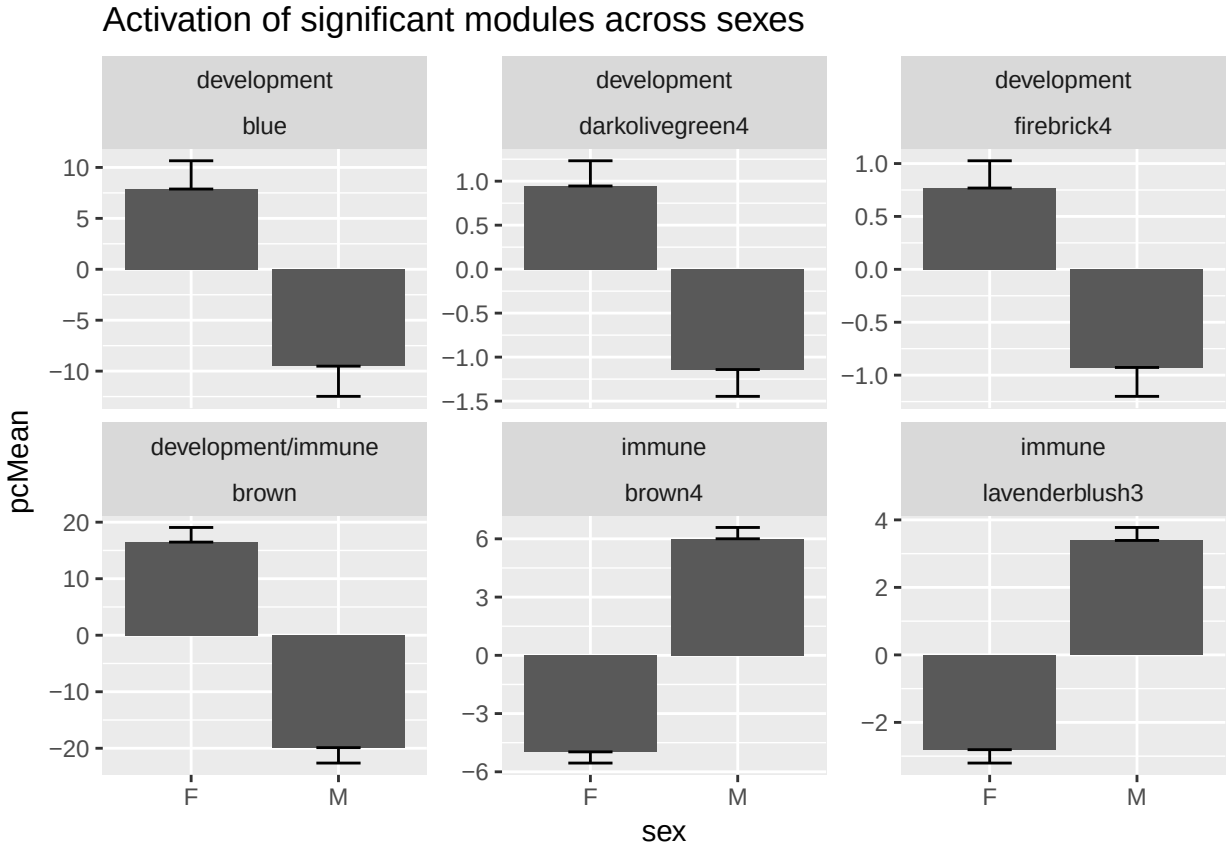
Chrom	# CpGs	Annot. gene	Genomic region	Rel. to island	P.whi	Padj.whi	P.fhs
chr18	4	BCL2	Body	OpenSea	1.03e-05	0.00093	0.00884
chr16	2	VKORC1	TSS1500	S_Shore	7.93e-08	0.00105	0.0266
chr16	2	DPEP2	TSS1500	OpenSea	7.37e-06	0.00173	0.00677
chr7	4	MAD1L1	Body	OpenSea	5.46e-05	0.00506	0.0435
chr16	2	NDRG4	Body	Island	1.7e-05	0.00635	0.0272
chr3	5	NBEAL2	Body;TSS1500;3'UTR	Island	9.48e-05	0.00673	0.00187
chr14	3	REM2	TSS1500	N_Shelf;OpenSea	5.39e-05	0.0073	0.00133
chr1	6			Island;S_Shore	1e-04	0.00914	0.0469
chr22	3	SEC14L4	1stExon;5'UTR;Body	Island	3.2e-05	0.0145	0.0199
chr5	2			Island;N_Shore	4.21e-05	0.016	0.0305
chr10	6			Island	0.000368	0.0167	0.00833
chr10	6	TMEM26	1stExon;5'UTR;TSS200;TSS1500	S_Shore	0.000139	0.018	0.0307
chr21	2	ABCG1	Body	Island	2.91e-05	0.025	0.0282
chr16	4	SLC9A3R2	Body	N_Shelf	0.00039	0.0289	0.00785
chr11	2	MADD	5'UTR	S_Shelf;S_Shore	0.000292	0.0328	0.0236
chr1	5	CD164L2	TSS200;1stExon;5'UTR	S_Shore;Island	7.12e-05	0.0334	0.038
chr1	6	FLJ37453	Body	Island;N_Shore	0.000455	0.0368	0.00306

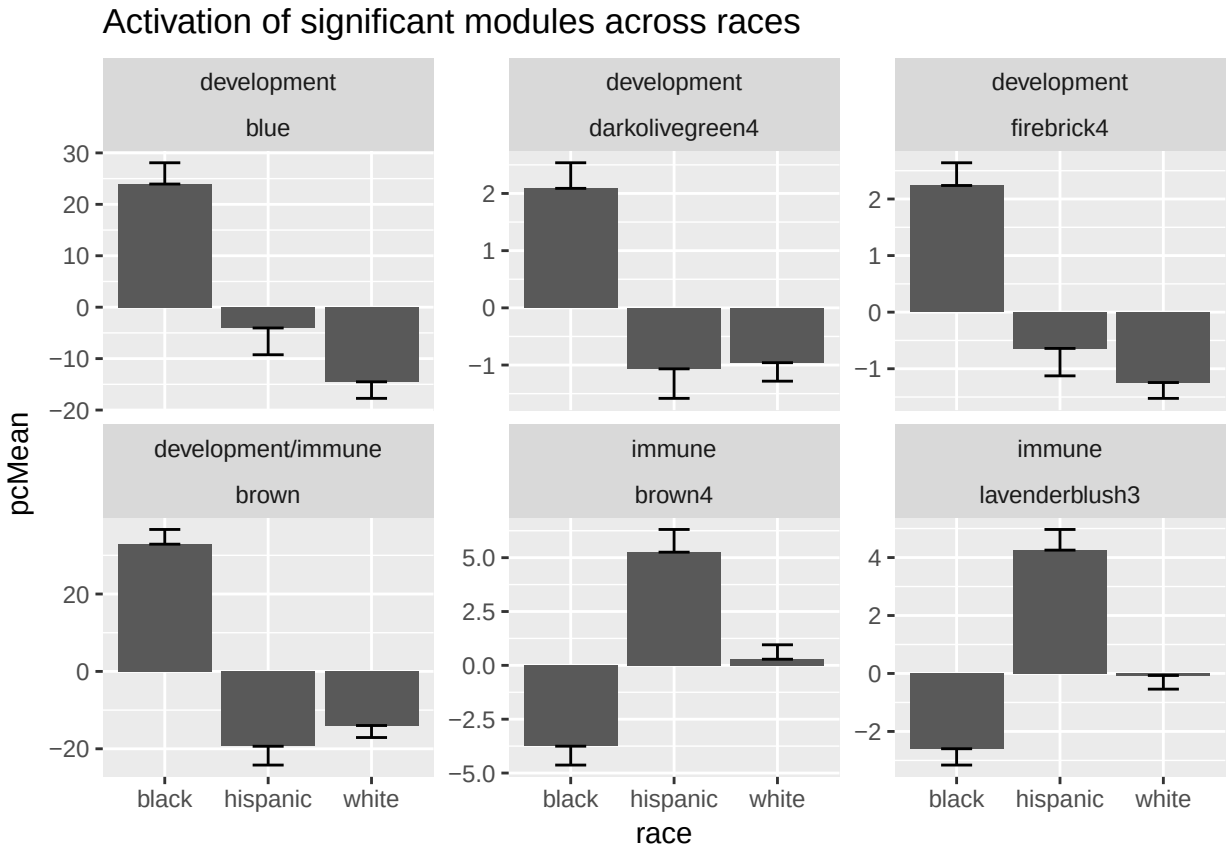
PhenoAge

The recent PhenoAge metric proposed by Horvath's group shows strong predictive power alone in both cohorts (p~10e-12 in WHI and p<10e-16 in FHS), but these associations become only moderate (p~5e-3) after adjustment for age and BMI.

Further demographic comparison – possibly of interest

Cross-sectional differences in principal component "activations" were examined across sex (in FHS) and race (in WHI).


Activation of significant modules across sexes
development
blue
development
darkolivegreen4
development
firebrick4
development/immune
brown
immune
brown4
immune
lavenderblush3
pcMean
sex
F
M


Activation of significant modules across races
development
blue
development
darkolivegreen4
development
firebrick4
development/immune
brown
immune
brown4
immune
lavenderblush3
pcMean
race
black
hispanic
white