



# Longitudinal genome-wide association study identifies novel loci and functional follow-up implicates putative effector genes for pediatric bone accrual

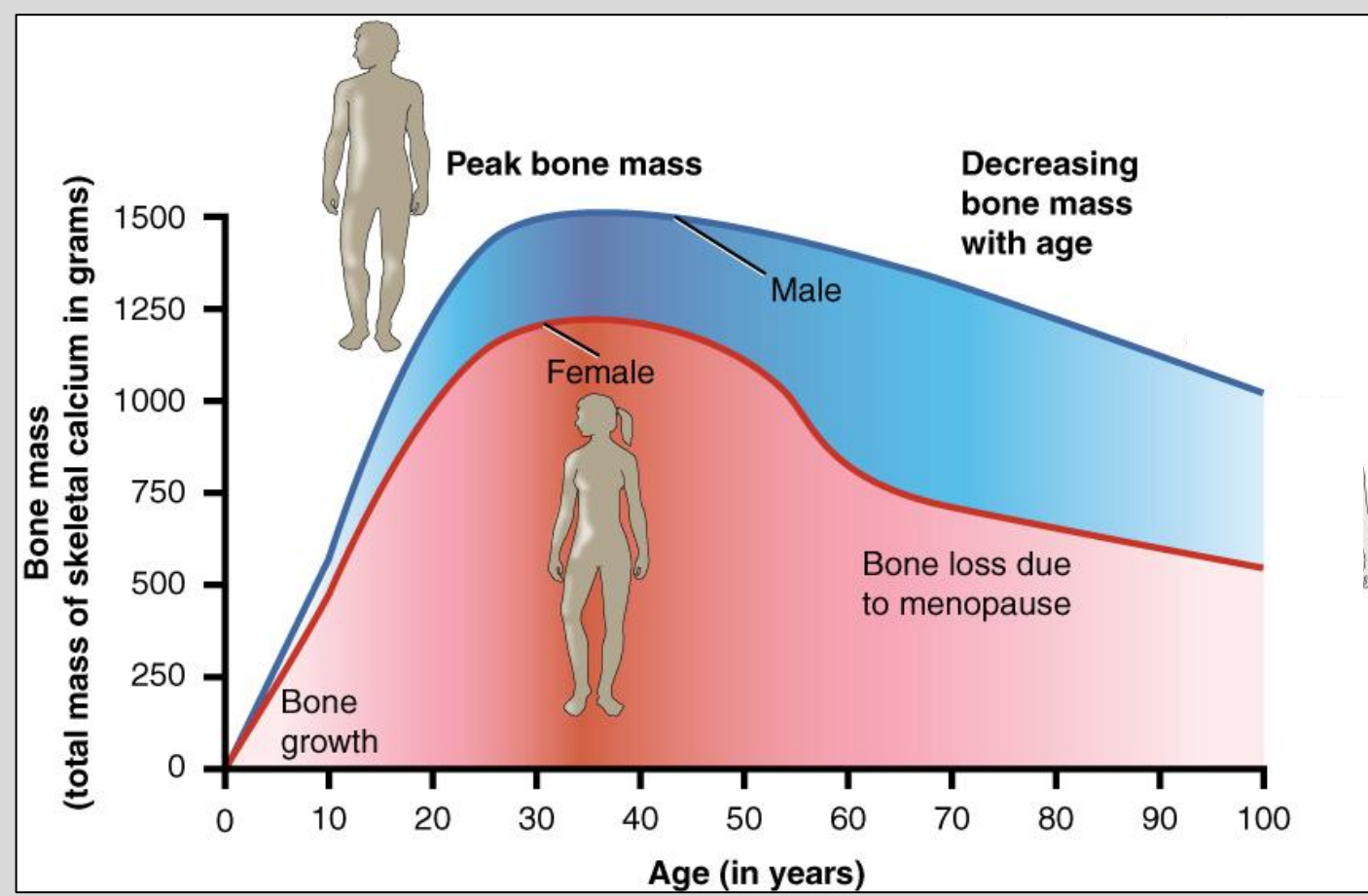


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\*Equal contribution

## BACKGROUND



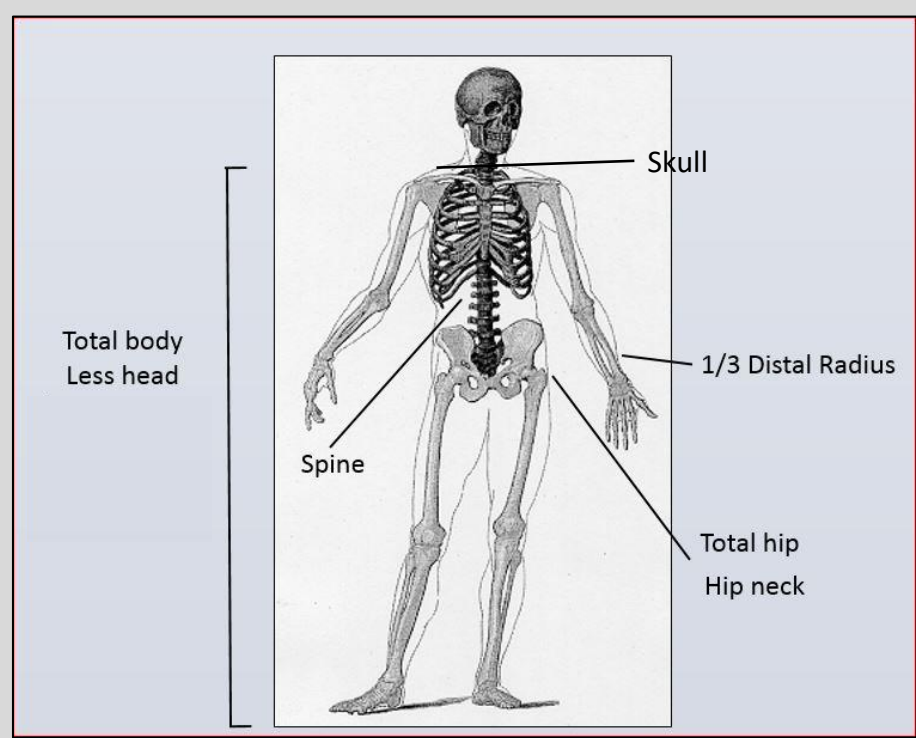
- Vast majority of genetic variants associated with bone density discovered in cross-sectional adult studies
- However, bone accrual is key for maximizing bone health across the life course
- Target effector genes at most associated loci are unknown

**AIM:** Identify genetic loci and target genes impacting bone accrual

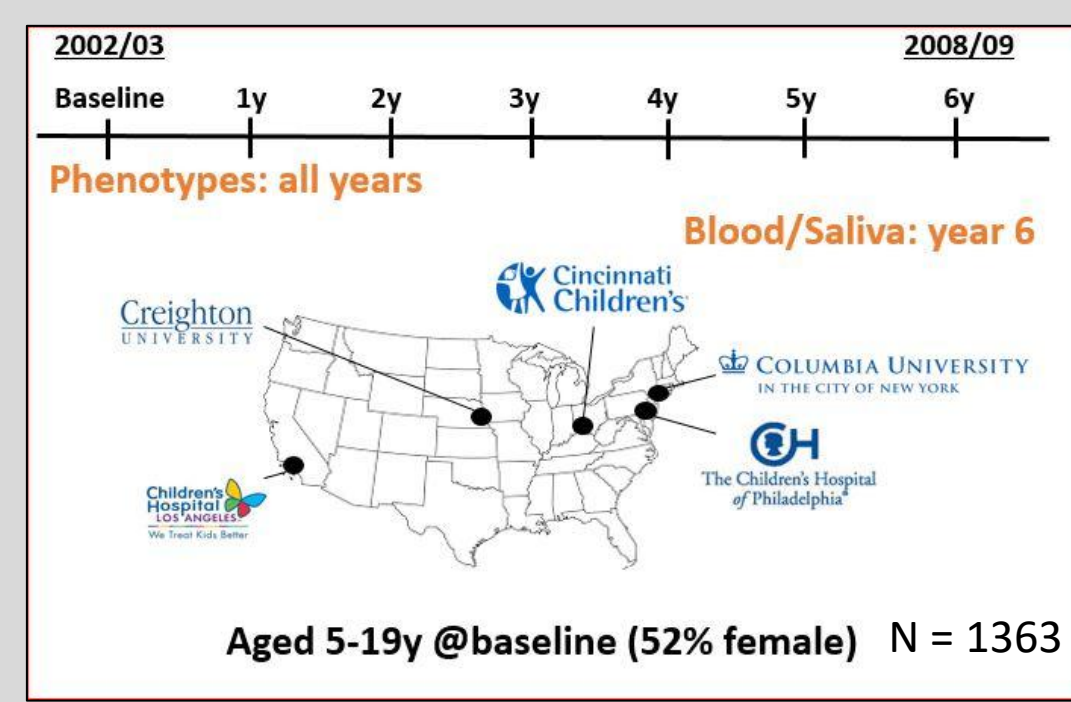
## MATERIALS AND METHODS

### Novel angle 1: Phenotyping

DXA-derived bone mineral density at six skeletal sites



**Bone Mineral Density in Childhood Study**  
Longitudinal, multi-ethnic cohort from 5 clinical centers with up to 7 DXA measurements per subject

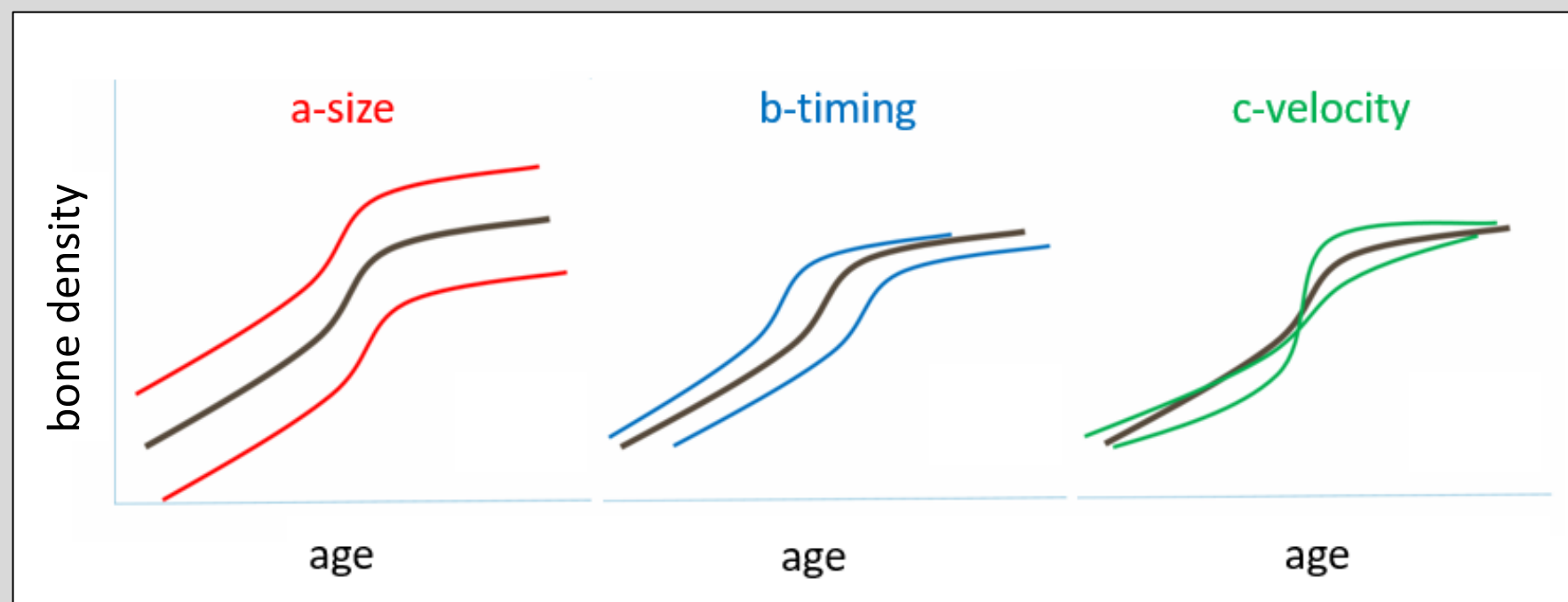


Zemel et al. Revised reference curves for bone mineral content.... (2013) JCEM.

### Novel angle 2: Analytical approach

Longitudinal modeling using Superimposition by Translation and Rotation (SITAR)

Leverages ~11,000 bone scans per skeletal site



Cole et al. SITAR—a useful instrument for growth curve analysis. (2010) IJE.

### Novel angle 3: GWAS of longitudinal trait

36 models (6 skeletal sites x BMD (g/cm<sup>2</sup>) and BMC (g/cm) x 3 SITAR parameters)

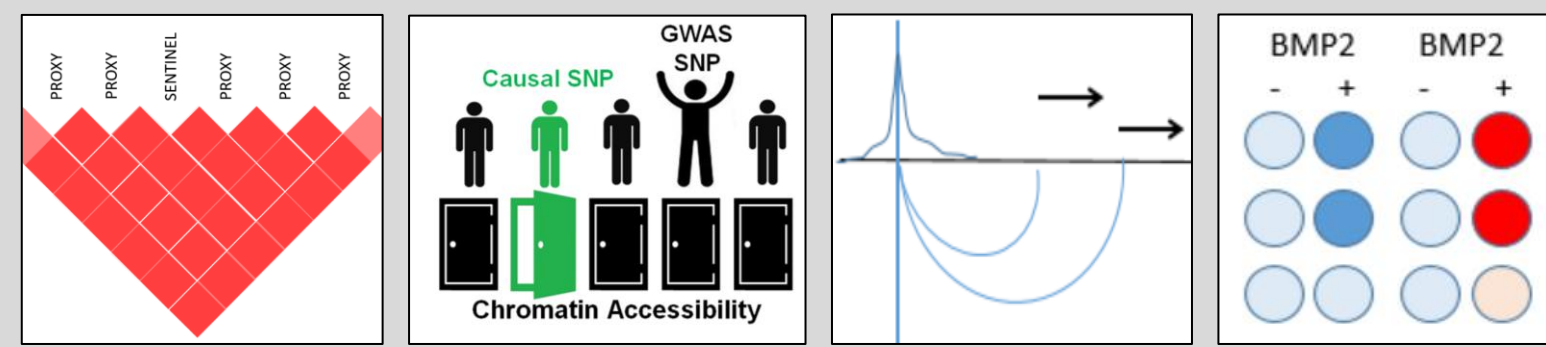
PhenoSpd determined 16 independent tests

Linear mixed models in GEMMA

### Novel angle 4: TAD-based informatics

Annotated genes in TADs surrounding signals for functional evidence

### Novel angle 5: Functional variant-to-gene mapping



Chesi et al. Genome-scale Capture C promoter interactions implicate effector genes at GWAS loci for bone mineral density. (2019) Nat Commun.

## CONCLUSIONS

Harnessing the depth of information in **longitudinal** and **multi-ethnic cohort + functional follow-up** in appropriate cellular context **greatly improves discovery and identification of target genes** for further functional studies, some of which may be **disease-relevant** (right)

### Lookup in adult fracture

Signal	Phenotype	GWAS Novel/known	Fracture P	Source/skeletal site
8	Radius BMD c	Novel	0.049	Morris, et al (2019)
10	TBLH & Tot hip BMD c	Novel	0.00089	PheWEB/ tibia & fibula
11	Spine BMC c	Novel	0.03	Morris, et al (2019)
			0.0052	PheWEB/ lower limb
23	Radius BMD a	Novel	8.9 x 10 <sup>-5</sup>	PheWEB/ radius
S13	Tot hip BMC a	Known	0.0024	Morris, et al (2019)

PheWeb (<http://pheweb.sph.umich.edu/>)  
Morris et al. An atlas of genetic influences on osteoporosis in humans and mice. (2019) Nat Genet.

### Future directions:

Replication of GWAS signals in independent cohort

CRISPR editing to validate SNP effects on gene expression

## RESULTS

### 1: 40 prioritized loci

GWAS implicated 27 genome-wide significant + 13 suggestive loci shared by multiple traits (3x the # currently known)

35/40 (85%) novel; only one previously seen in pediatric BMD GWAS

### 2: Pathway analysis on TAD genes

Implicates known and new pathways

GO or KEGG term	Obs	Exp	P
Calcium signaling pathway	7	2.84	0.02
FoxO signaling pathway	5	2.05	0.05
Hippo signaling pathway	2	0.45	0.07
Arachidonic acid metabolism	3	0.98	0.07
long-chain fatty acid metabolic process	8	1.30	4.91E-05
negative regulation of toll-like receptor signaling pathway	5	0.46	9.24E-05

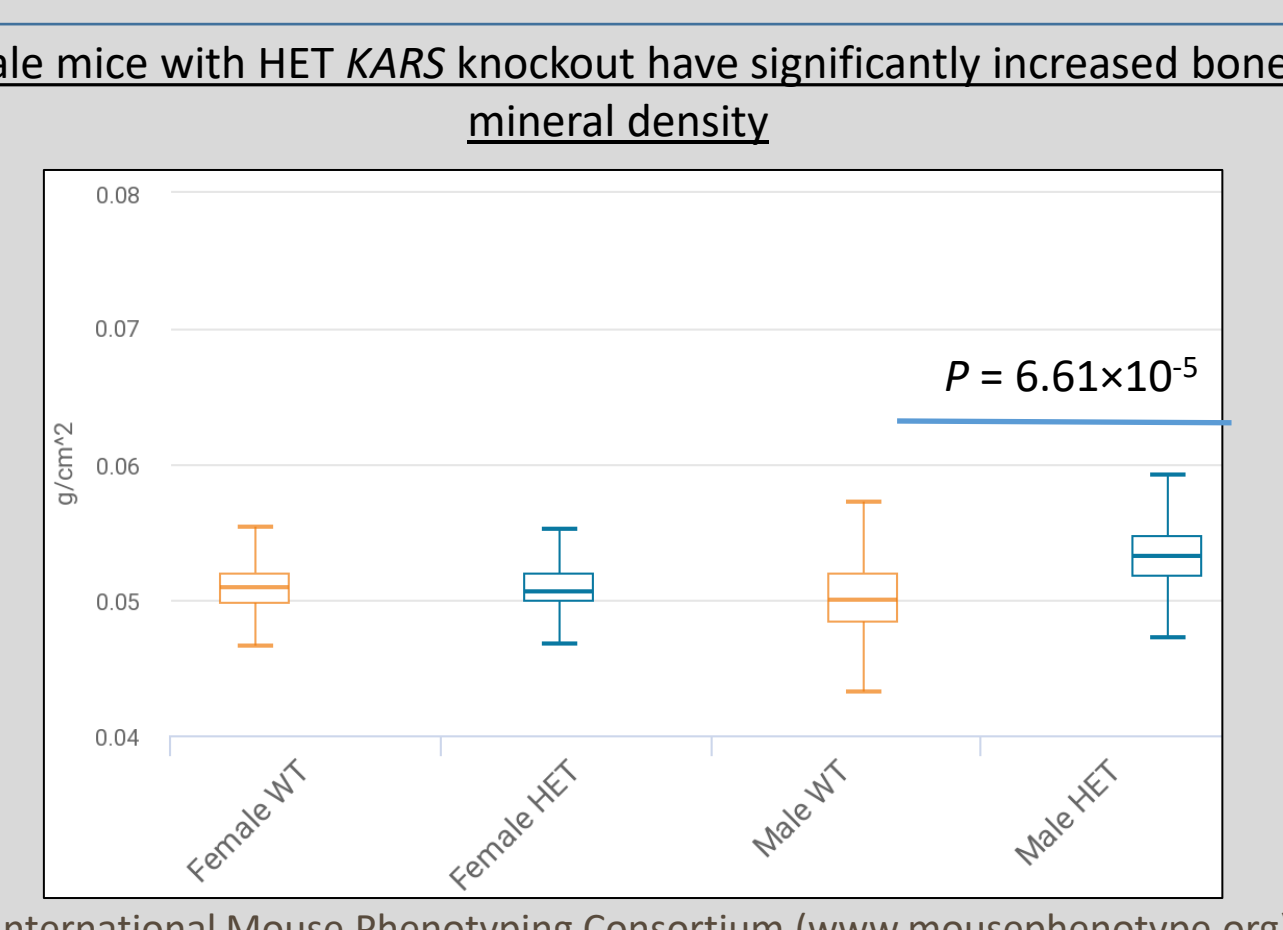
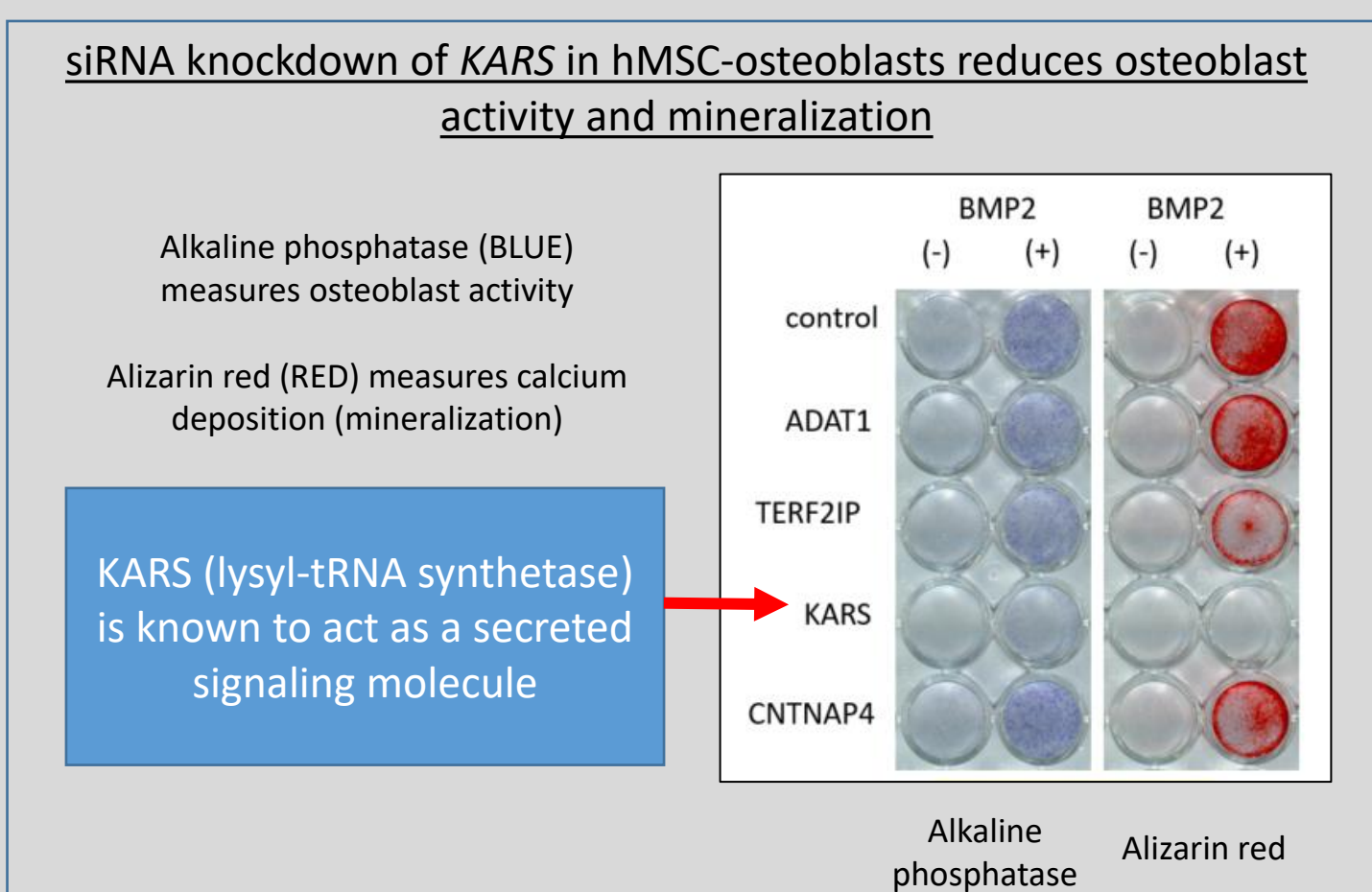
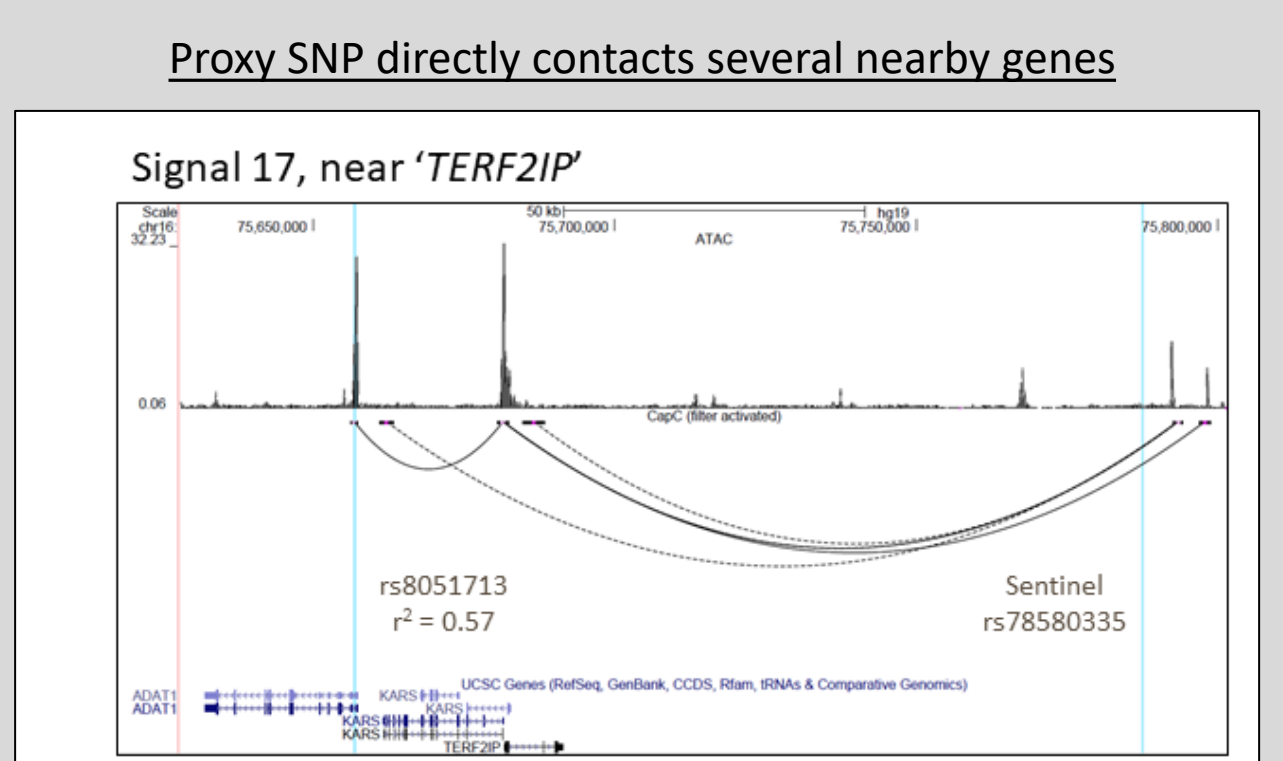
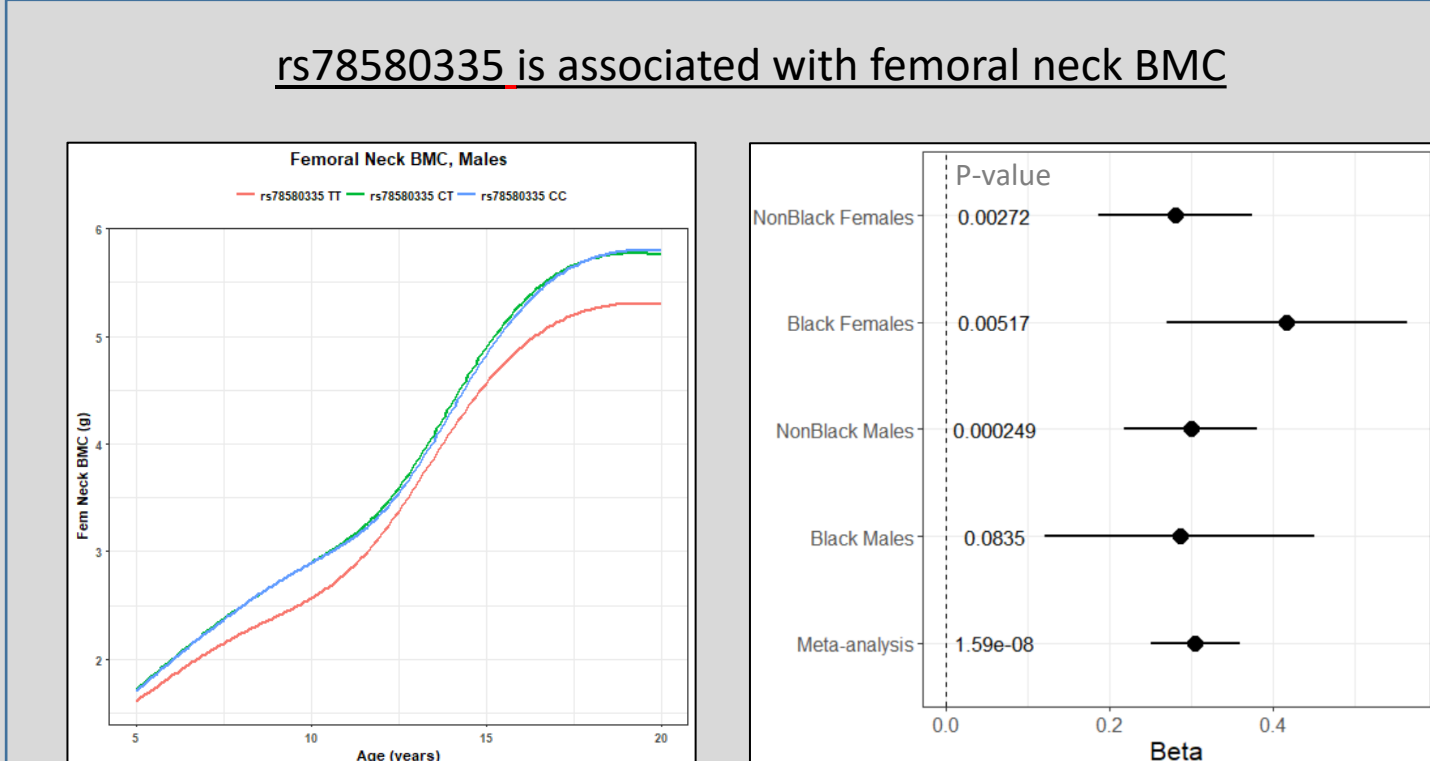
Way et al. Implicating candidate genes at GWAS signals by leveraging topologically associating domains. (2017) Eur J Hum Genet.

### 3: Candidate genes

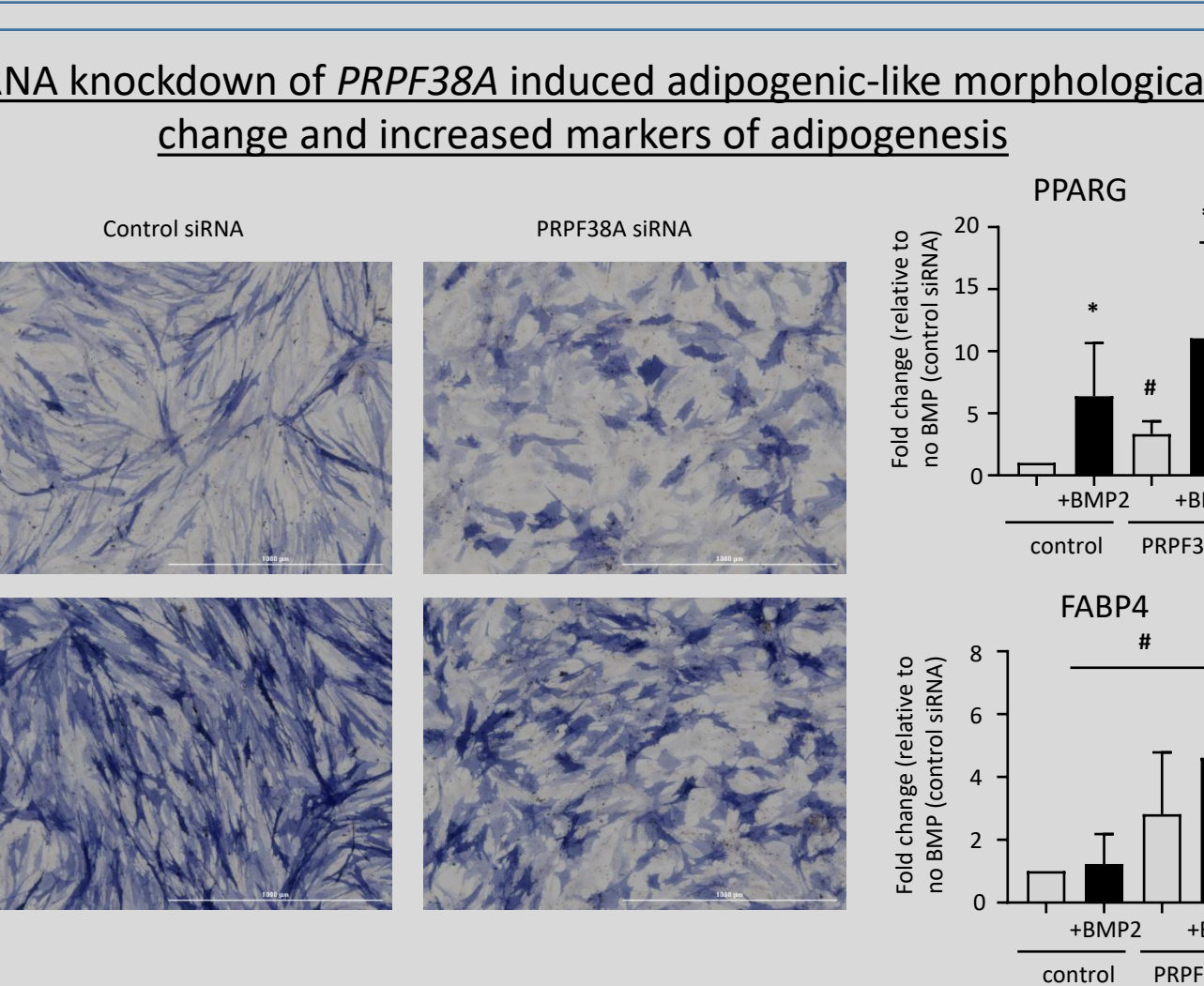
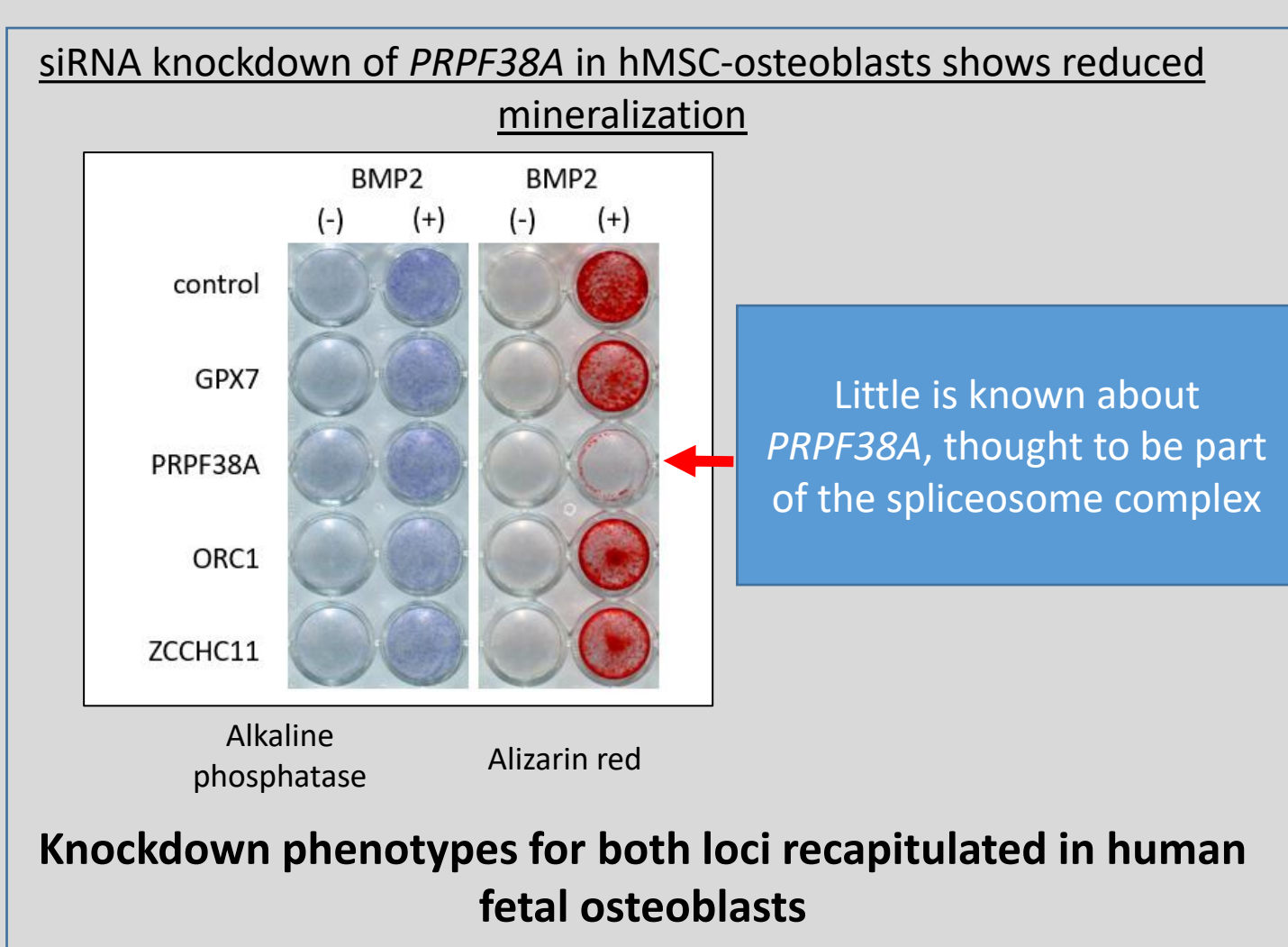
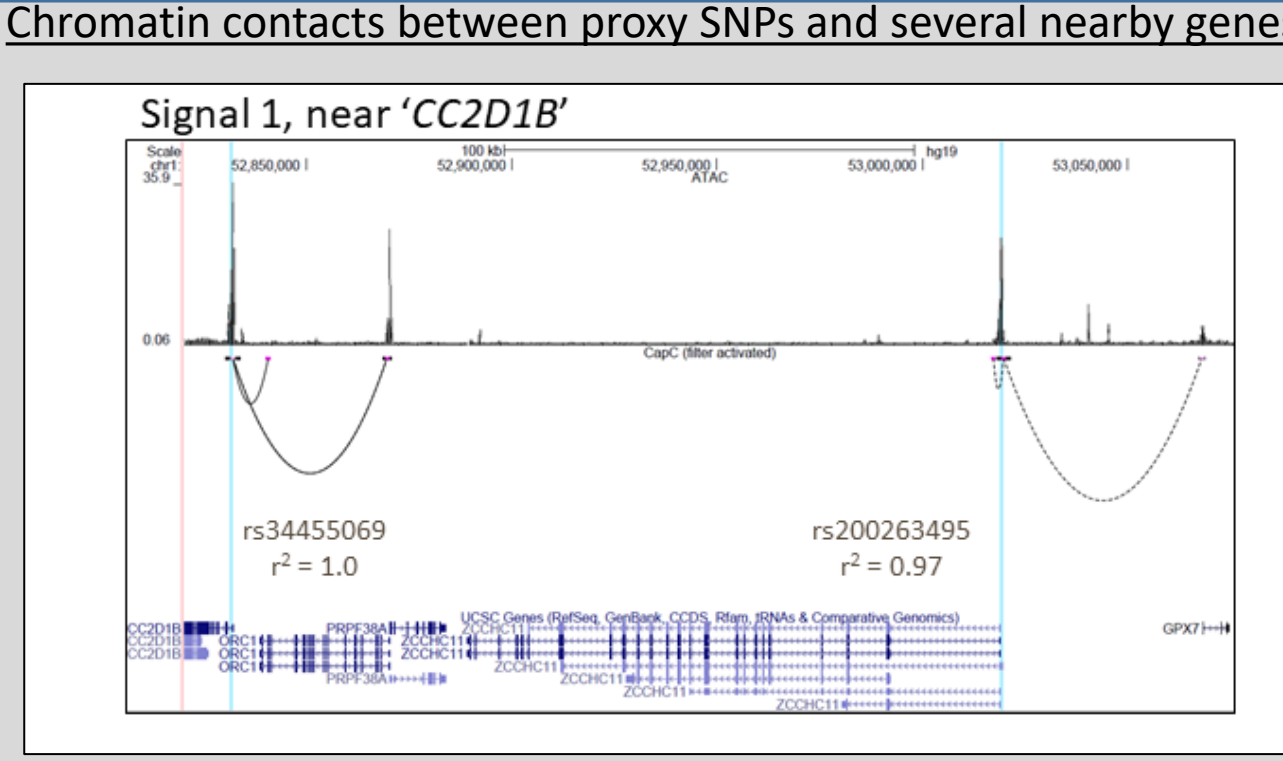
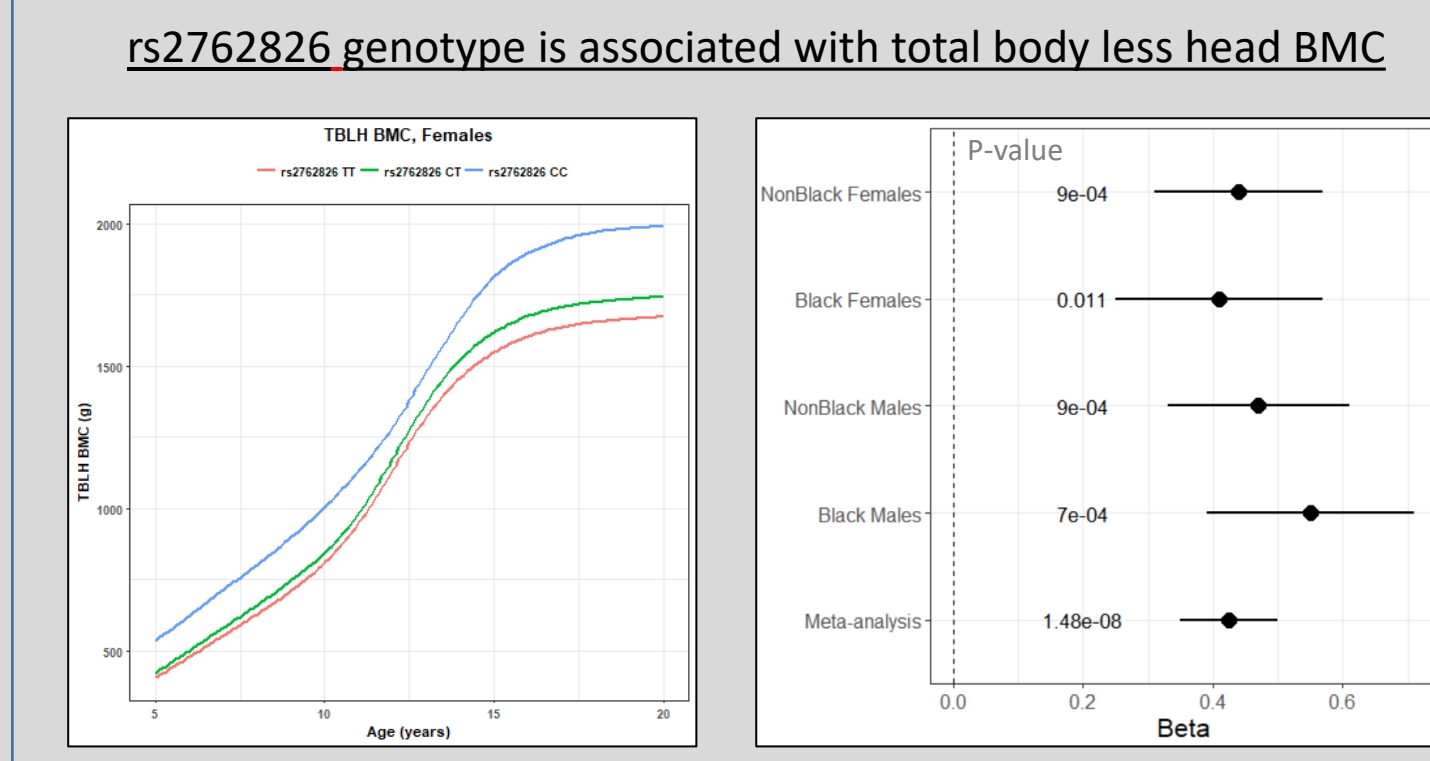
Multiple ontology and phenotyping resources highlight biologically plausible genes harbored in GWAS signal TADs

Signal	Gene	OMIM Mendelian disease	GO or KEGG term	Mouse phenotype
2	FOSL2		X	X
3	FOXP1		X	X
5	WWTR1 (TAZ)		X	X
8	IL17A		X	
9	TBXAS1	X		
15	BLNK		X	
18	SLC9A3R1 (NHERF)	X	X	X
19	GRB2		X	X
21	TGFB1	X	X	X
24	CITED1		X	
25-26	ATP7A	X		X
S2	HDAC4		X	X
S2	TWIST2	X	X	X
S6	TEAD4		X	
S6	TULP3		X	X
S7	PRKD1		X	X
S9	GNA11	X	X	X
S9	AES (TLE5)		X	
S11	SOX11	X	X	X

### 4: Functional mapping implicates KARS at 'TERF2IP' locus



### 5: PRPF38A knockdown at 'CC2D1B' locus favors adipogenesis



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**Platform Oral: PgmNr 144**  
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