Population-Level Genetics Human Gene-Muscle-Bone Associations

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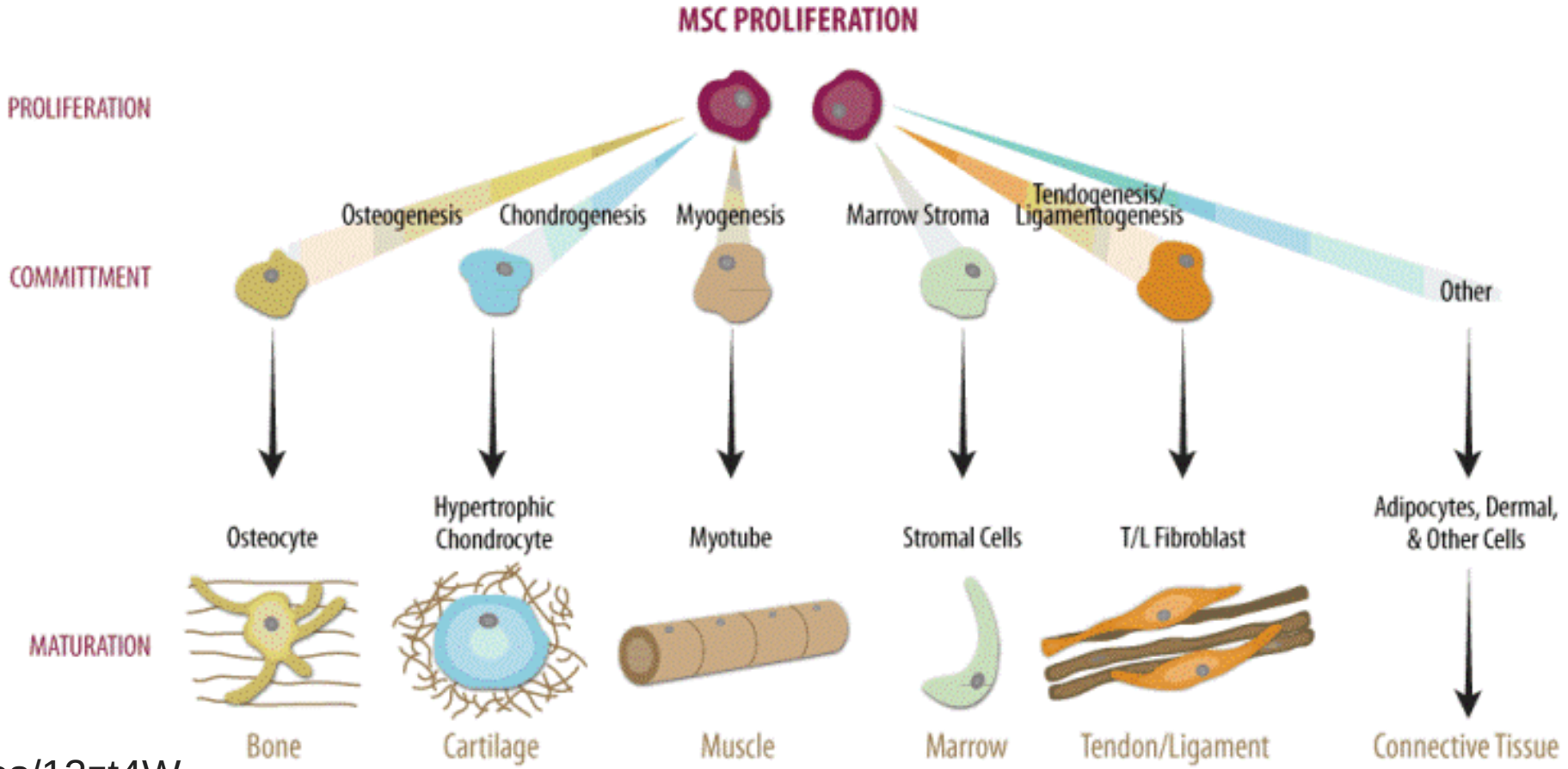
Stem Cell Differentiation Pathways in the Bone Marrow

- Muscle, bone and fat develop from somites and share a common mesenchymal precursor



https://goo.gl/images/12zt4W

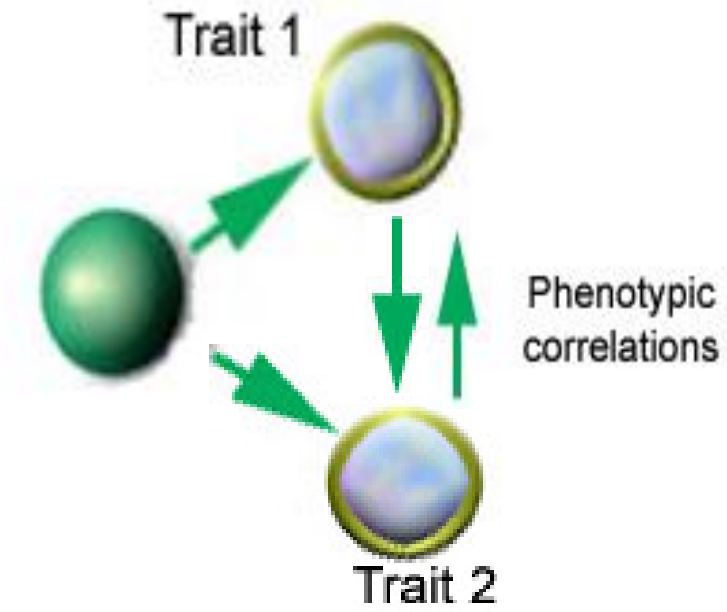
Pleiotropy

A genetic locus (a gene or a single variant within a gene) affects more than one trait or disease

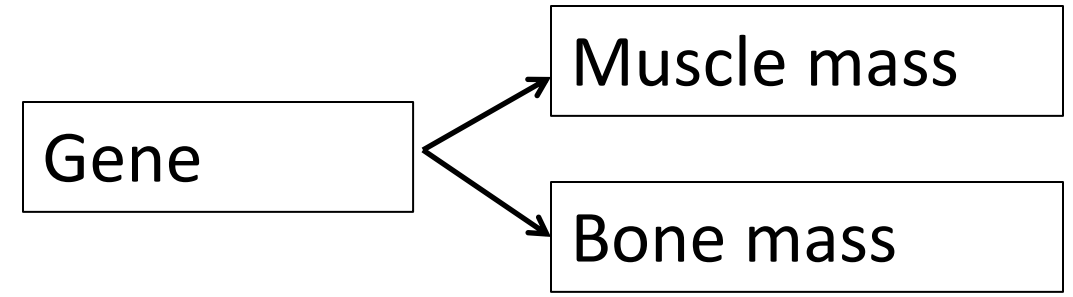
- Biological – when a gene has a direct biological effect on more than one trait or biomarker
- Mediated – where a gene has a biological effect on one trait which lies on the causal path to another trait and thus the gene affects both traits;
- Spurious - when different forms of biases can lead to false-positive findings

Genetic Pleiotropy is Measurable

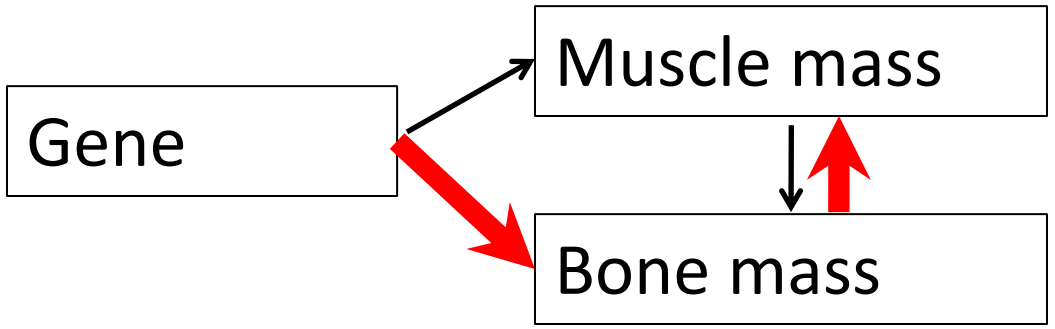
Genetic variant



Pleiotropy and “Mediation” May Be Difficult to Disentangle



Pleiotropy



Mediation

Candidate Genes for Bone-Muscle Pleiotropy

Example of a Potentially Important Pleiotropic Gene for Bone and Muscle

- Myostatin (*GDF-8*)
  - First identified in 1997 as being expressed during development
  - KO mouse dramatic phenotype of myofiber hypertrophy and hyperplasia
  - Naturally occurring mutations in the myostatin gene observed in cattle, sheep dogs and a German child

Myostatin (*GDF8)* Mutation

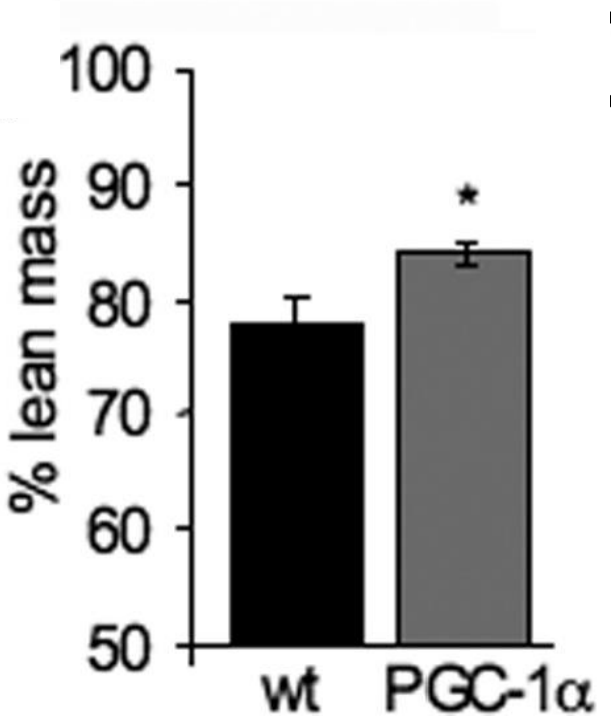
Myostatin Mutation Affects Bones

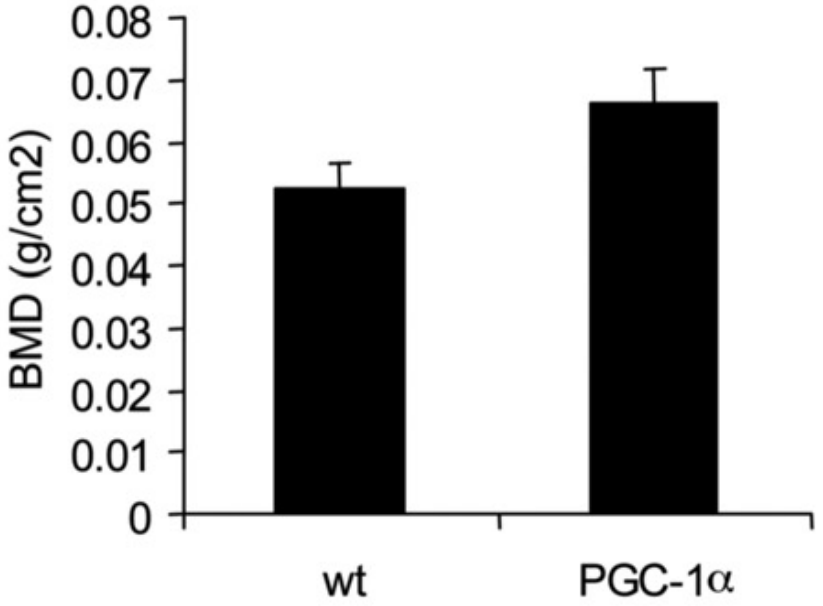
- Myostatin-null mice (*GDF8-KO*) differ from wild type mice
  - Larger entheses on both the humerus (deltoid crests) and the femur (third trochanter)
  - Significantly greater cortical thickness and cortical BMD of femur
  - Significantly larger spinous processes on L1–L4 and broader transverse processes on L1–L2 (Hamrick et al. 2002, 2003)
  - Two SNPs have been associated with peak hip BMD variation in Chinese women (Zhang et al. 2008)

Another Example – *PPARGC-1A* (alias *PGC-1α*)

- Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PGC1-α*), a key player controlling mitochondrial function
  - Peroxisome master regulator of mitochondrial biogenesis
- In skeletal muscle, *PGC-1α* can also prevent muscle wasting by regulating autophagy
- Levels of PGC-1α in skeletal muscle decrease with aging

PGC-1α Transgenic Mice Have More Lean Mass and Preserved Bone Density





Wenz PNAS 2009

Using Genome Wide Association Studies to Search for Genetic Pleiotropy Between Bone and Muscle

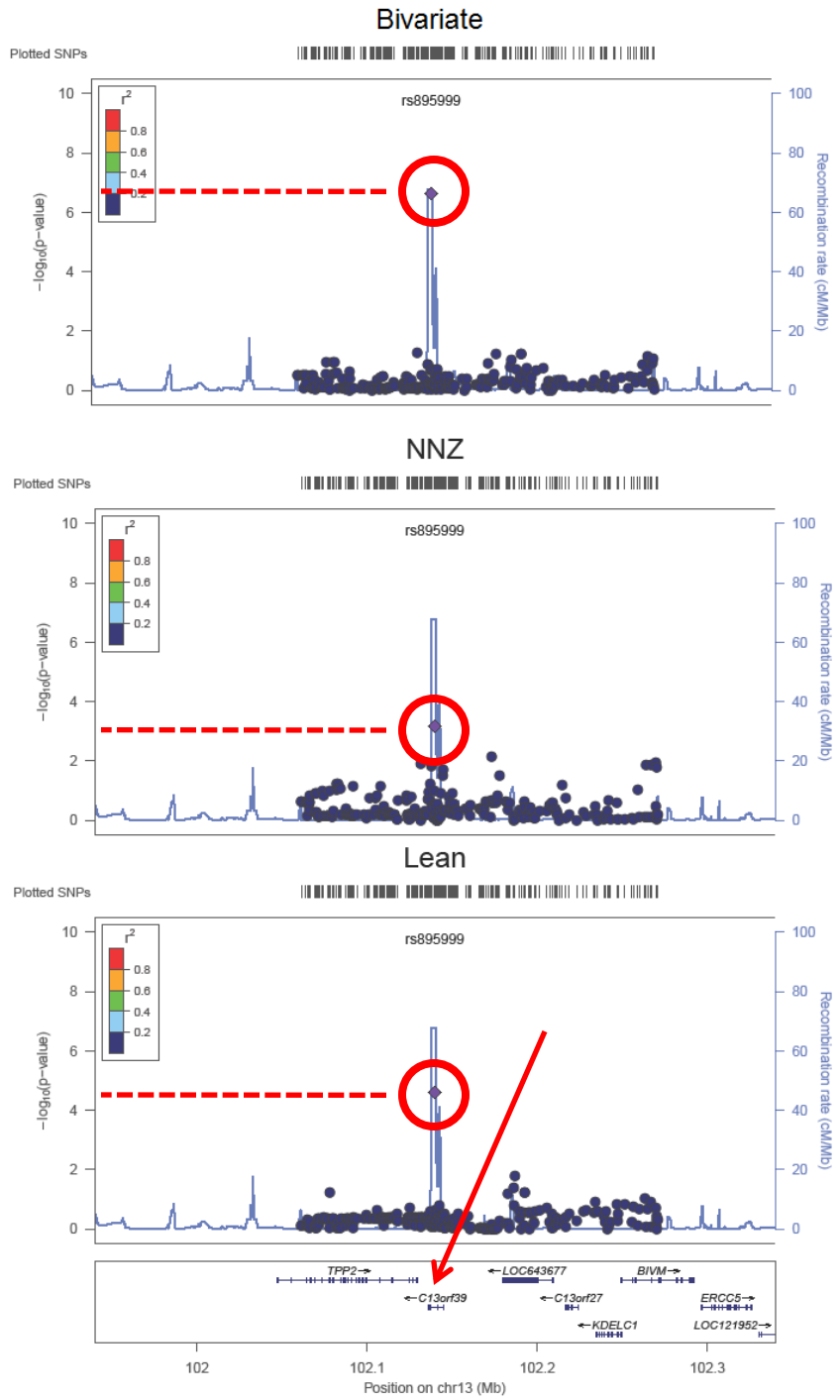
Using Genome Wide Association Studies to Study Bone and Muscle

- Perform genome-wide association studies (GWAS) for bone and muscle phenotypes among multiple cohorts
- Combine results of individual GWAS using meta-analysis
- Ultimately combine the results from bone and muscle meta-analyses themselves to detect *potentially* pleiotropic effects

Overlapping Genes from GWAS of Bone and Muscle Traits

Muscle-related trait	Bone mineral density			
	Gene	eBMD P-value	Muscle related traits P-value	Reference
TBLM	*MC4R*	2.0×10^{-15}	4.2×10^{-13}	Karasik et al 2019
TBLM	*FTO*	1.6×10^{-26}	1.4×10^{-09}	Zillikens et al. 2017
Grip strength	*IRS1*	4.7×10^{-08}	1.5×10^{-11}	Zillikens et al. 2017
Grip strength	*MGMT*	2.3×10^{-22}	1.0×10^{-13}	Tikkanen et al. 2018
Grip strength	*TCF4*	9.4×10^{-10}	5.9×10^{-15}	Tikkanen et al. 2018
Grip strength	*TMEM18*	2.0×10^{-11}	5.4×10^{-22}	Tikkanen et al. 2018
Grip strength	*LINC01104*	7.9×10^{-11}	3.1×10^{-09}	Tikkanen et al. 2018
Grip strength	*MC4R*	2.0×10^{-15}	2.1×10^{-19}	Tikkanen et al. 2018
Grip strength	*PEX14*	6.7×10^{-13}	5.6×10^{-11}	Willems et al. 2017
Grip strength	*SLC8A1*	7.4×10^{-38}	7.7×10^{-09}	Willems et al. 2017
Grip strength	*TGFA*	9.3×10^{-19}	4.8×10^{-13}	Willems et al. 2017

Bivariate GWAS of Hip Section Modulus and Appendicular Lean Mass



Huang JBMR 2014

Potential Pleiotropic Locus on Chrom 13

- Contained a gene *LOC196541* (a.k.a*. METTL21C – a* methyltransferase like 21C*)*
- Since *METTL21C* appears to be important for embryonic development and is over-expressed in muscles compared to other tissues, it was selected for “knock-down” studies

Huang JBMR 2014

METTL21C – Chromosome 13q33.1

- Belongs to the *METTL2* family of the methyltransferase superfamily and has protein-lysine N-methyltransferase activity
- Highly expressed in normal human muscle and over expressed in muscle compared to other tissues
- Important for meat quality traits in cattle
- *METTL21* family of proteins methylates valosin containing protein (VCP) chaperones, which themselves can harbor specific mutations that are causal to both Inclusion Body Myositis and Paget's Disease of bone

Huang JBMR 2014

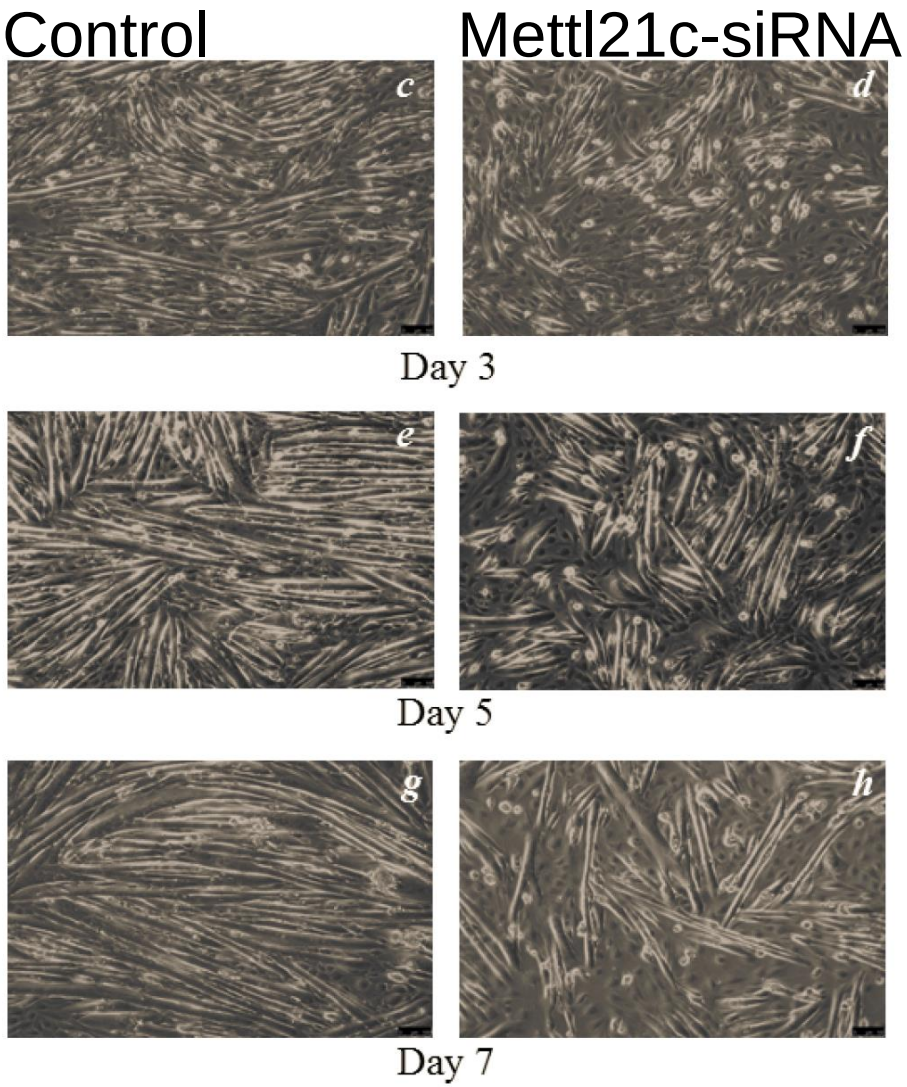
Validating Function of Mettl21c in Muscle

- Designed targeting siRNA sequences
  - Transfection protocols were optimized for C2C12 myoblasts to study phenotypic changes in differentiation
- Observed control and siRNA treated cells daily for potential phenotypic changes and systematically analyzed them at days 3 and 5 of differentiation
- After transfection, cells were allowed to differentiate for 7 days
  - Transfection efficiency reached 70.8 ± 7.6%
  - After 24h of transfection, *METTL21C* expression was reduced by ~36% in the siRNA-treated cells (r-t PCR).

Huang JBMR 2014

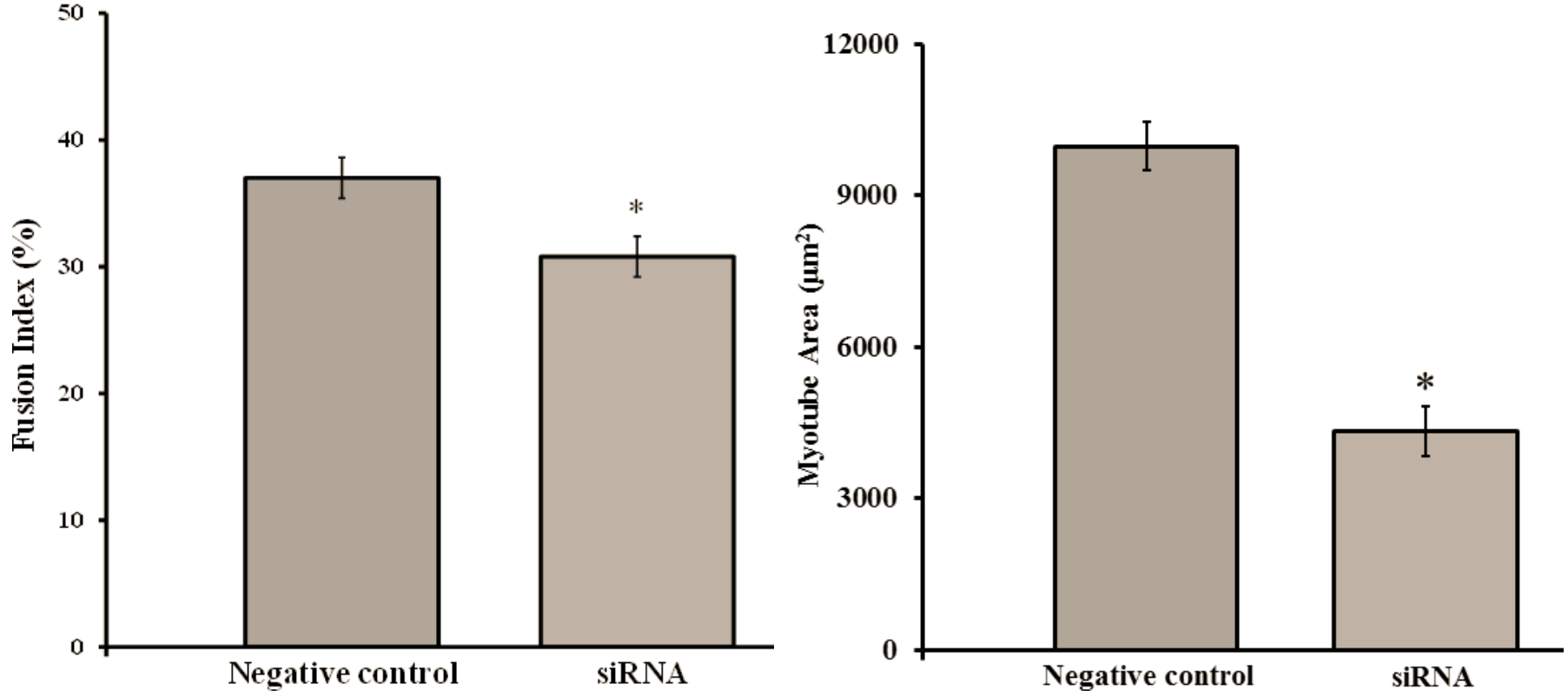
Fewer and Smaller Myotubes Formed From C2C12 Cells Exposed to Mettl21c siRNA During Differentiation

- C2C12 myoblasts cultured under conditions favoring proliferation without differentiation into myotubes
- siRNA transfection
- Cells cultured in differentiation medium for seven days
- *At day 3 of differentiation, reduced number of myocytes for fusion was* observed in the *Mettl21c-siRNA-treated group compared to both negative control and vehicle control groups,*



Huang JBMR 2014

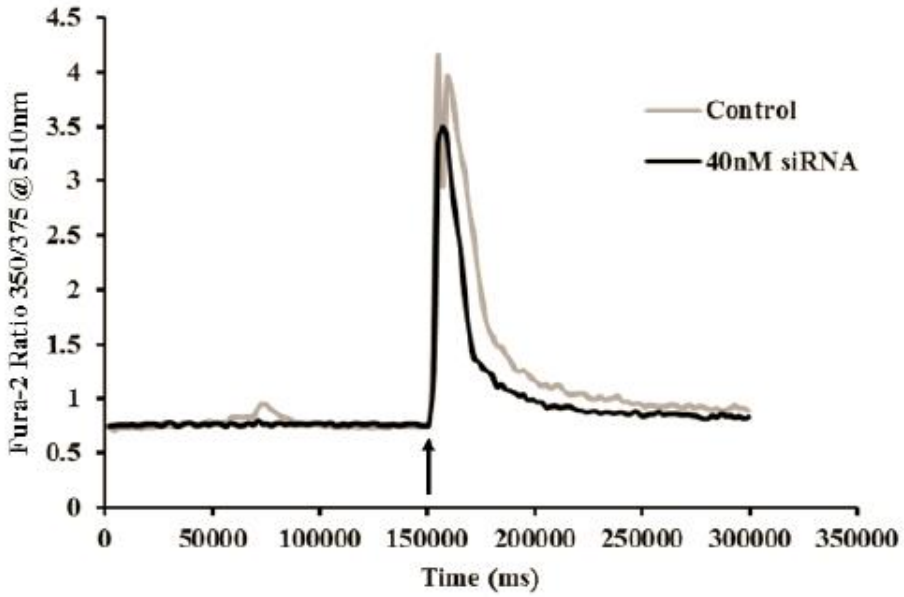
Fusion Index and Myotube Area in Mettl21c siRNA treated C2C12 Cells Significantly Decreased Compared to Control



Huang JBMR 2014

Amplitude Peak Calcium Response to Caffeine Significantly Decreased and Relaxation Phase Shorter in siRNA Transfected C2C12 Cells

- Determined if SR calcium release influenced by partial silencing of *Mettl21c by monitoring Fura-2 intracellular calcium transients in response to caffeine-induced* SR Ca2+ release
- Amplitude peak Ca2+ response to caffeine decreased 16.1% (2.78 ± 0.03 vs. 3.31 ± 0.03)
- Relaxation of caffeine-induced calcium transients was 23.0% shorter (110802 ± 597ms vs. 85298 ± 395ms)

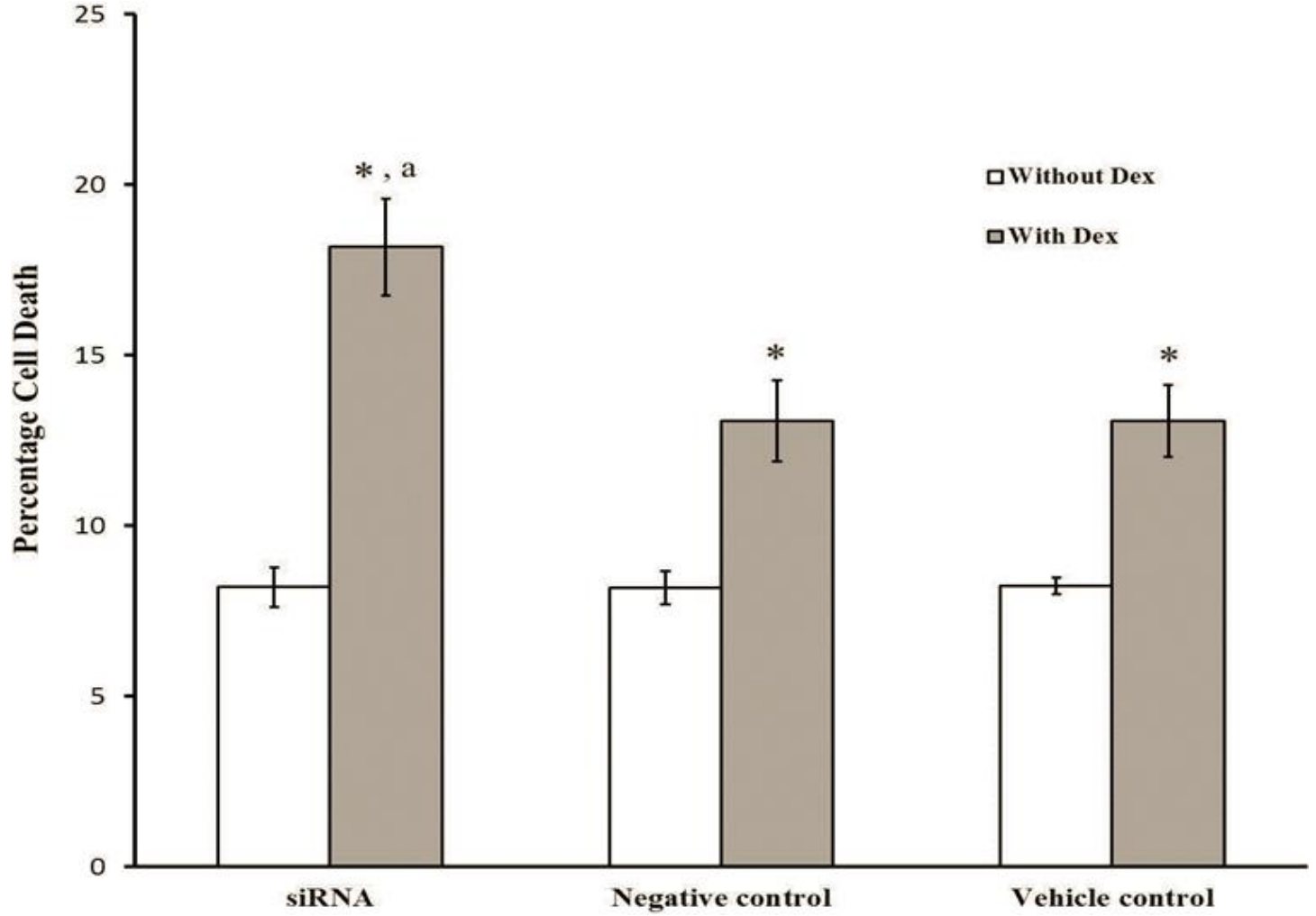


Huang JBMR 2014

Validating Function of Mettl21c in Osteocytes

- MLO-Y4 cells were plated and allowed to attach and grow overnight
- Transfection of osteocyte cells with either control siRNA or with*Mettl21c-siRNA + 6µl the transfection reagent, “oligofectamine”*
- *Oligofectamine-only vehicle treated cells were* used as a vehicle control

Partial Knockdown of Mettl21c mRNA in MLO-Y4 Osteocyte Cells Increased Cell Death Induced by Dexamethasone



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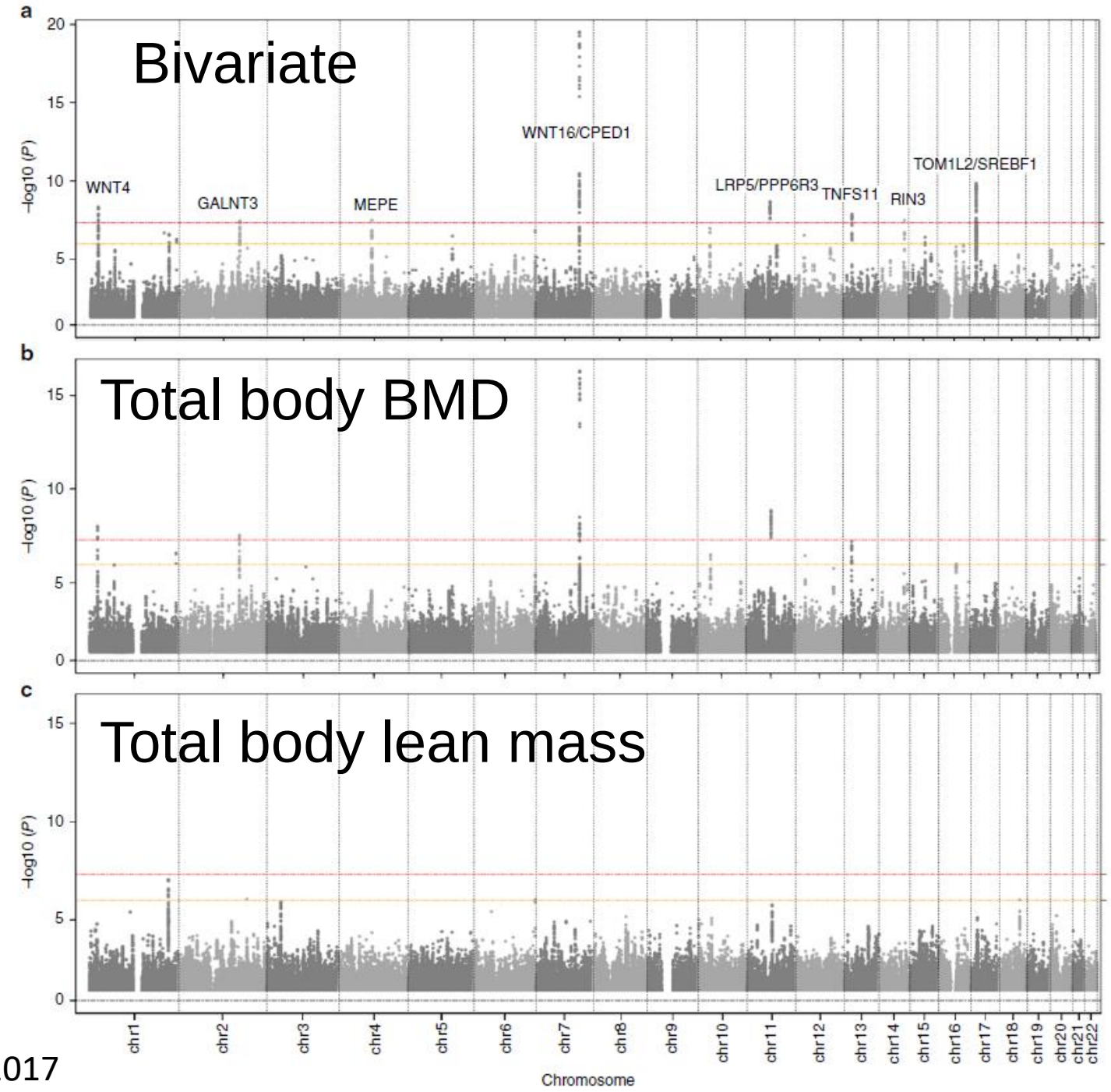
A Bivariate GWAS of Total Body Lean Mass and BMD

Bivariate analysis yields more loci

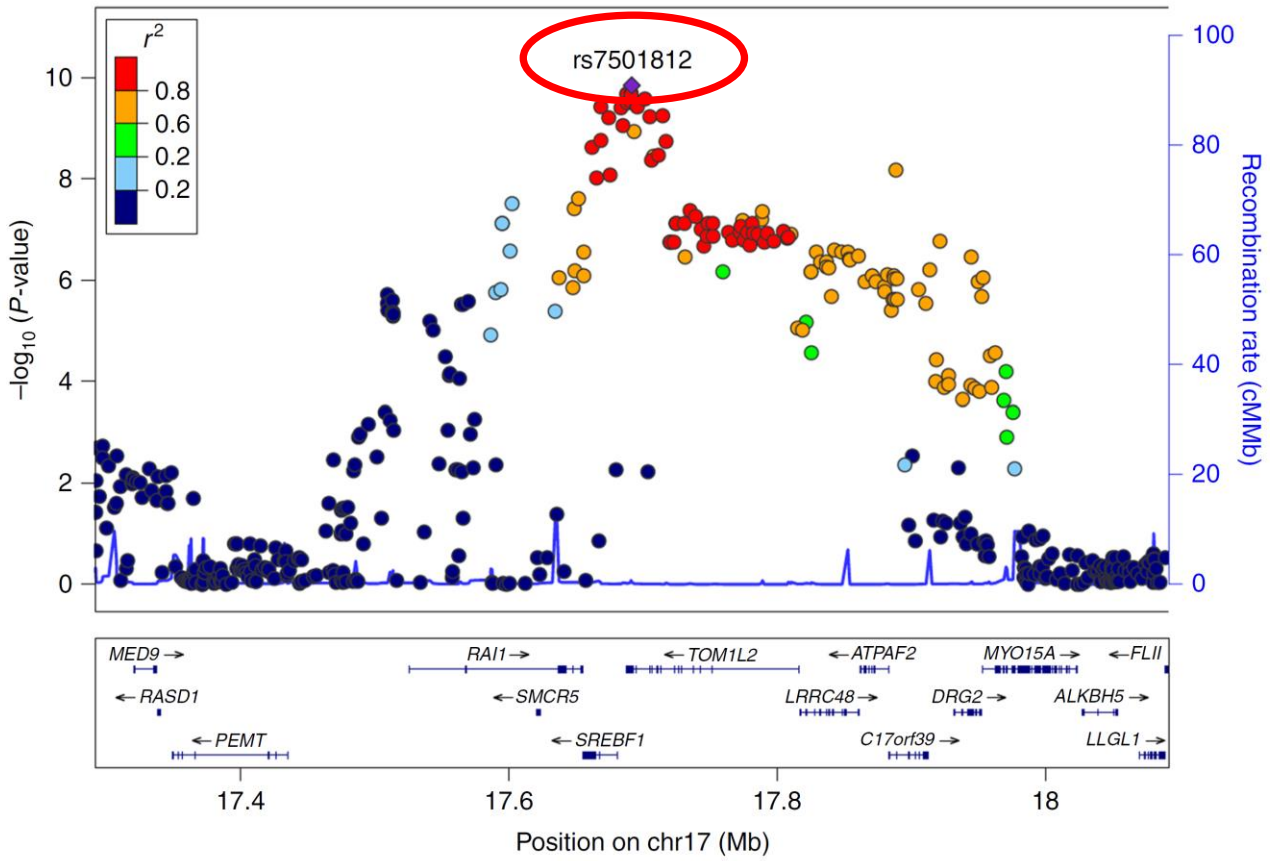
All the BMD univariate loci were known

No loci were significant in the univariate lean mass GWAS



Medina Gomez, et. al., Nature Communications 2017

The Chromosome 17p.11.2 Bivariate Locus



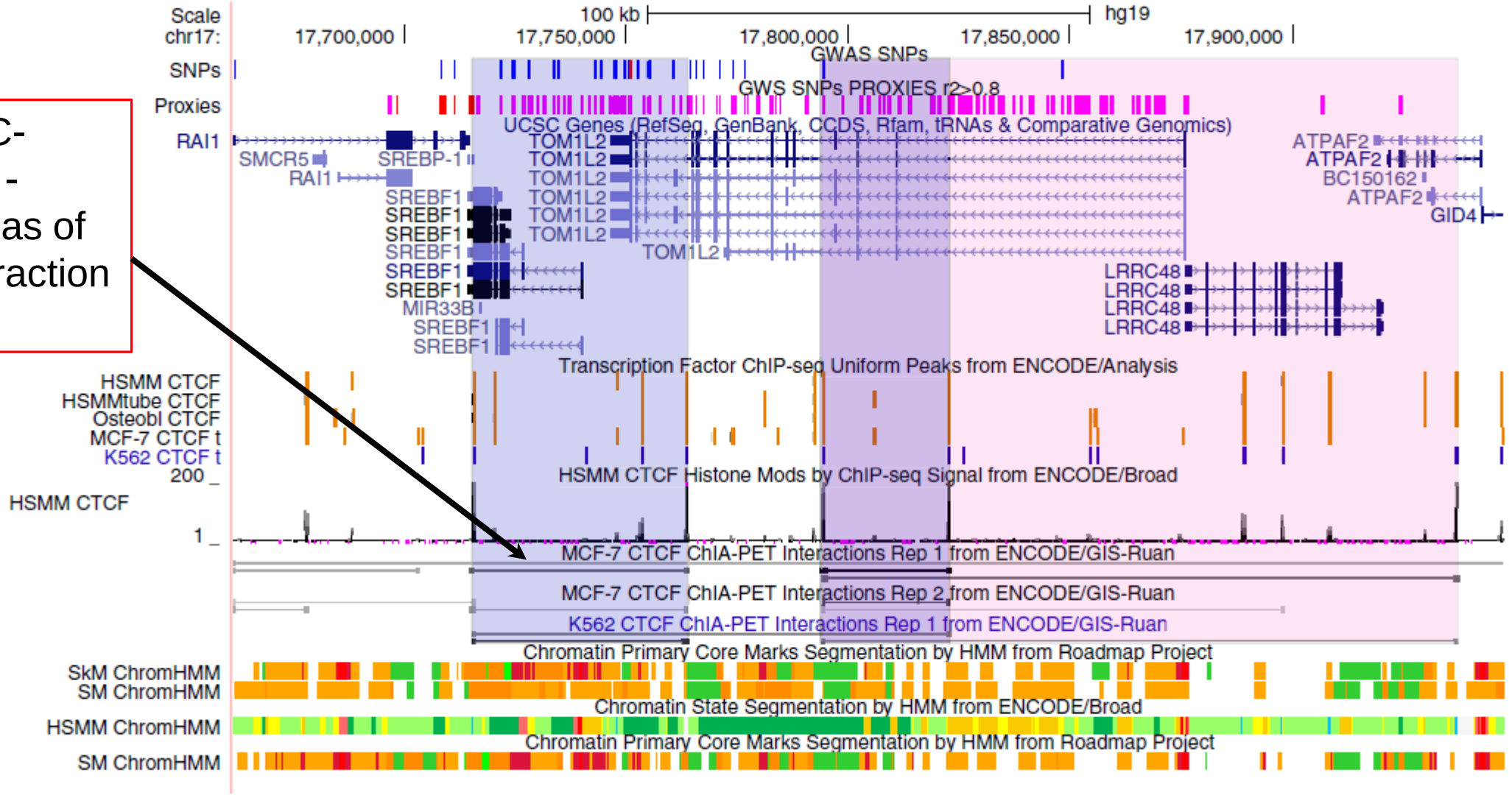
- The only associated region where the signal was stronger for TB-LM than for TBLH-BMD
- LD-block harboring several genes including MYO15A, LRRC48, MIR33B, C17orf39 [GID4], DRG2, RAI1, SREBF1, TOM1L2, ATPAF2,
- Latter seven all shown to be expressed in skeletal muscle

The 17p11.2 Locus

- Most of the association signal in 17p11.2 arises from non-coding variants
- Reviewed possible regulatory annotation of these SNPs using data from ENCODE and ROADMAP EPIGENOMICS projects

Regulatory Landscape in the Region of the Bivariate GWAS Association – *SREBF1, TOM1L2, and ATPAF2*

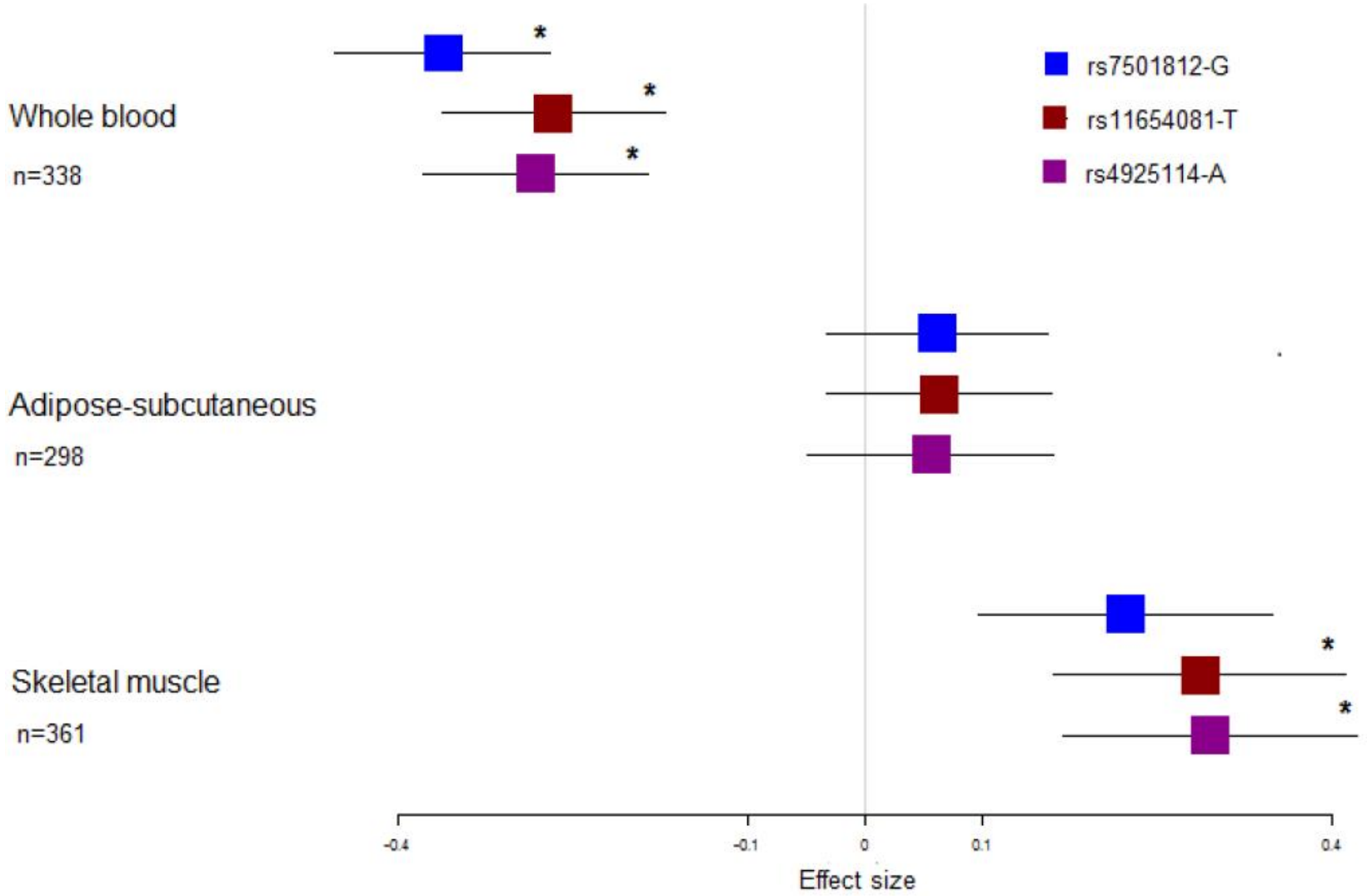
CTCF (CCCTC-binding factor) - associated areas of chromatin interaction by looping.



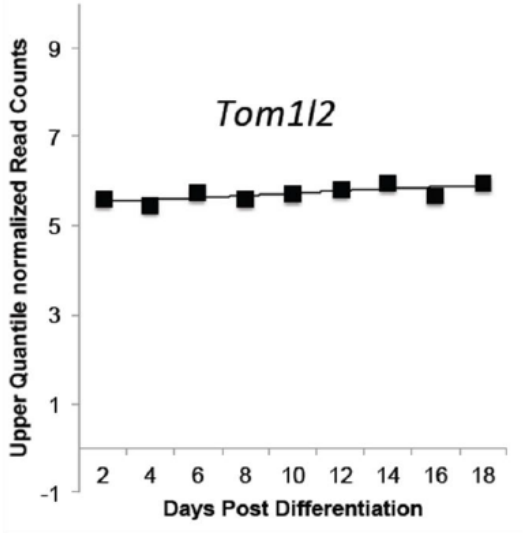
SREBF1 is a Leading Candidate Gene

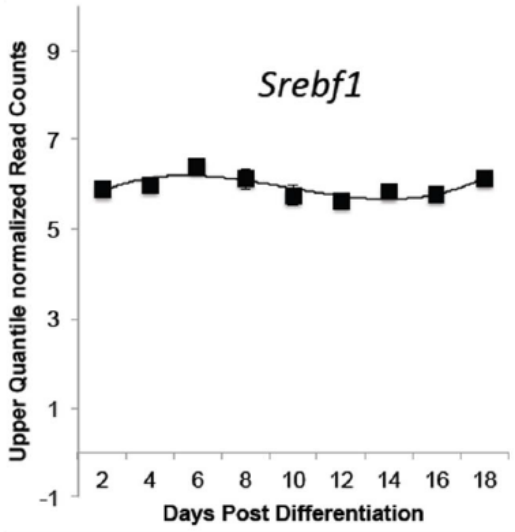
- rs7501812 had the strongest bivariate association with TBLH-BMD and TB-LM in this locus, and is also a cis-eQTL variant found to regulate the expression of *SREBF1*, *C17orf39* [*GID4*], *TOM1L2* and *ATAPF2*
- *SREBF1* expression, represented by two probes in data set, showed highest correlation with rs7501812
- Alleles from the bivariate GWS SNPs associated with higher TBLH-BMD and lower TB-LM in the region, associated with decreased expression of *SREBF1*, *TOM1L2*, and *C17orf39* [*GID4*] but increased expression of *ATPAF2* in whole blood
- *SREBF1* is most likely gene driving the associations with TB-LM and TBLH-BMD

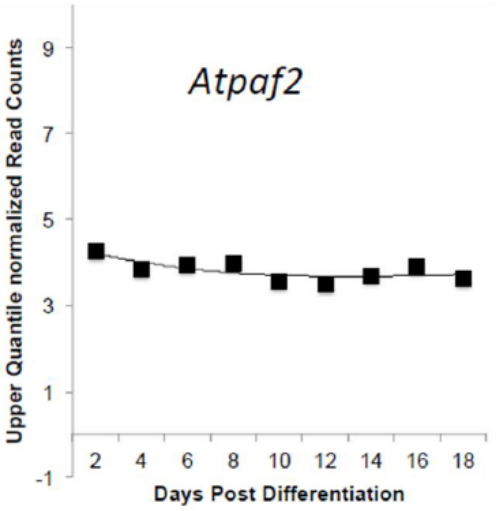
Gene Expression of 3 SNPs in the Bivariate GWAS Locus at 17p11.2

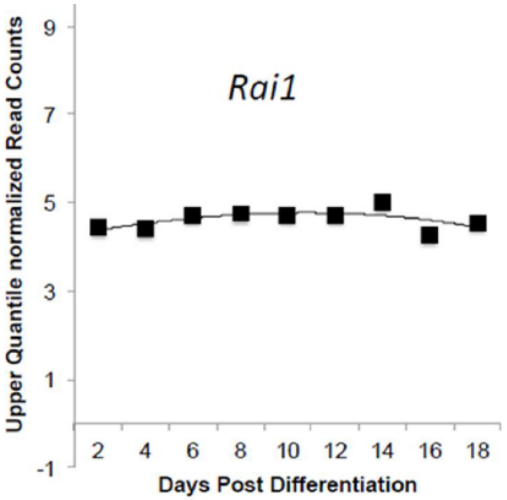


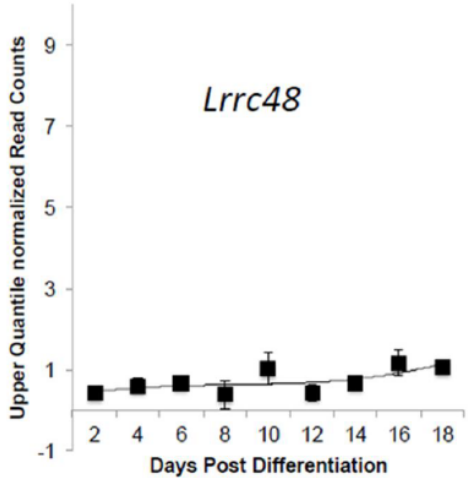
Gene Expression Profiles in Mouse Calvarial Osteoblast Maturation











SREBF1

- An adipocyte differentiation factor that produces SREBP-1, a transcription factor ubiquitously expressed (more strongly in lipogenic tissues) and directly regulating the transcription of over 200 genes involved in the de novo synthesis of fatty acids, triglycerides, and cholesterol.
- Active form important for the mineralization of osteoblastic cultures in vitro
- In skeletal muscle SREBP-1 protein indirectly downregulates *MYOD1*, *MYOG*, and *MEF2C*, acting as a key regulator of myogenesis.
- Overexpression of *SREBP-1* inhibits myoblast-to-myotube differentiation, reduces cell size and leads to loss of muscle-specific proteins in differentiated myotubes

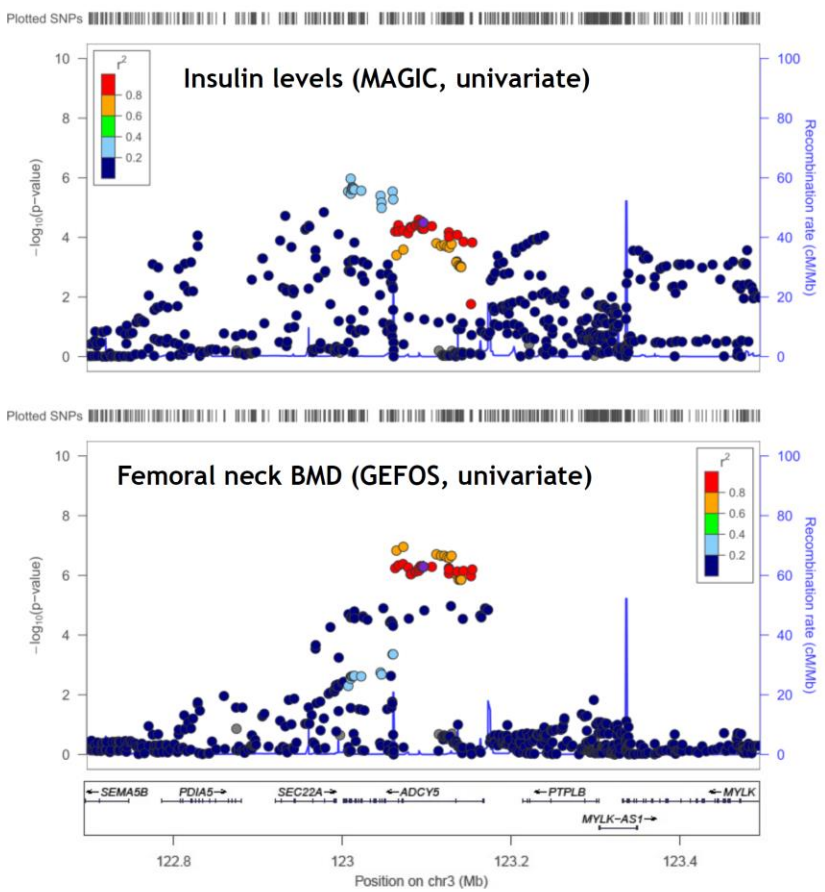
Knowledge Gaps

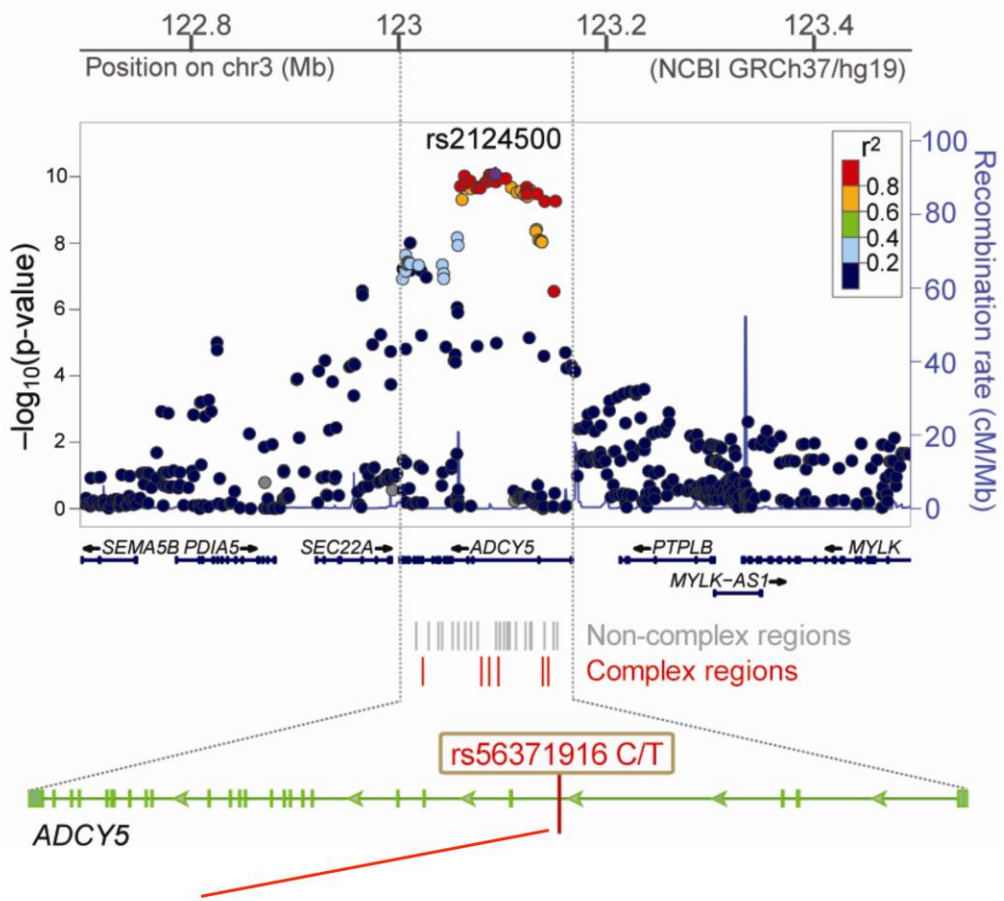
- With growing number and size of genome wide association studies, there are likely to be novel potentially pleiotropic loci
- Lack of high throughput cell and animal models to use in validation experiments
- The challenge of moving from drug targets derived from genetic studies to actual drugs with beneficial effects on bone and muscle

Research Opportunities

- Collect and analyze all relevant musculoskeletal phenotypes from genome wide association studies using bivariate methods
- Develop collaborations between basic and applied laboratories to test potentially pleiotropic genes in cellular and animal models
- Anticipating the availability of drugs that have joint effects on bone and muscle, there is a need to plan the future clinical trial methods and outcomes that will be required to test new therapies

A Bivariate GWAS of BMD and Metabolic Traits





T allele: risk for T2D, protective for BMD
C allele:protective for T2D, risk for BMD

Bivariate GWAS for Total Body Lean Mass and BMD

- Bivariate GWAS meta-analysis in four pediatric cohorts (n=10,414) identified 8 variants associated at the genome-wide significant level with TBLH-BMD (total body BMD not including the head) and TB-LM
  - Map to seven loci all of which have been previously associated with BMD in adults and/or children

7q31.31 WNT16/CPED1	13q14.11 TNFSF11
11q13.2 LRP5/PPP6R3	14q2.12 RIN3
1p36.12 WNT4	4q22.1 MEPE
2q24.3 GALNT3	

- Univariate GWAS meta-analysis of TB-LM yielded no GWS associations