# Using epigenomic data to inform genome-wide association studies of bone mineral density

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Osteoporosis is primarily an aging-related disease, characterized by decreased bone strength and increased fracture risk. The incidence of osteoporosis is increasing worldwide due to aging populations and is a major healthcare burden. For instance, osteoporosis-related healthcare costed China $9.45 billion USD in 2010, will likely double by 2035, and increase to $25 billion USD by 2050 (1). Bone mineral density (BMD) is used to predict fracture risk and is the primary clinical measurement used to diagnose osteoporosis. Genome-wide association studies (GWAS) of BMD have successfully identified many genetic loci that influence osteoporosis (2-12), but like most GWAS, these studies used stringent statistical significance thresholds to limit false positive results at the expense of false negatives.

Large collaborative efforts such as ENCODE (13) and the Epigenome Roadmap (14) projects have come to completion in recent years, generating genome-wide epigenetic data from hundreds of cell lines and primary tissues. This resource is immensely useful, particularly to help investigate whether non-protein coding single nucleotide polymorphisms (SNPs) associated with a disease are functional (e.g., via perturbation of regulatory elements such as transcription factors). Guo *et al*. (15) integrated epigenomic data from ENCODE and BMD GWAS data from GEFOS (2) to identify a novel gene, brain-derived neurotrophic factor (*BDNF*), demonstrating how large epigenomic datasets can be utilized to explore putative false negative GWAS results and inform experimental follow-up studies.

Guo *et al*. (15) reasoned that enriched regulatory elements for known BMD genes can be used to predict which genes from a pool of putative false negative GWAS results may have a role in osteoporosis etiology. Guo *et al*. (15) identified 259 previously reported BMD and osteoporosis-related trait associated genes (16) and performed pathway analyses to identify which biological pathways were enriched, resulting in several known pathways such as the Wnt, Hedgehog, MAPK, and osteoclast differentiation pathways. Guo *et al*. (15) then integrated data from the ENCODE project; using data from multiple cell lines to determine which transcription factor binding sites (TFBSs), chromatin states, or histone marks were enriched within the 259 known genes. By developing a scoring system and prioritizing genes with the highest rankings for gene set enrichment analysis, Guo *et al*. (15) validated their gene list by identifying similarly enriched pathways with the known pathways, and further performed network analyses to evaluate shared pathways between their gene list and the known genes.

The high scoring genes, *BDNF* and phosphodiesterase 4D (*PDE4D*), both demonstrated evidence to be implicated in osteoporosis etiology. GWAS data showed that *BDNF* and *PDE4D* had suggestive association with BMD measured at the lumbar spine, with association P values of approximately $5\times10^{-4}$ (2). In addition, the SNPs mapping to *BDNF* were found to have suggestive association with fracture

(rs11030119: P=0.024; rs7124442: P=0.042) in the Chinese Fractures Study (CFS) of 350 cases and 350 controls. However, these SNPs have relatively low minor allele frequencies (MAF) in cases (rs11030119: MAF =0.037; rs7124442: MAF =0.062) and controls (rs11030119: MAF =0.062; rs7124442: MAF =0.089), thus limiting the power of the study, resulting in a confidence interval of the odds ratio for rs7124442 encompassing the null [OR =0.67 (0.45–1.00)]. As such, fracture results of rs11030119 and rs7124442 would require replication in a larger sample size to conclusively state their association with fracture.

To functionally link the *BDNF* SNPs to *BDNF* function, Guo *et al*. (15) performed eQTL analyses and short interfering RNA (siRNA) knock-down experiments. Cis-eQTL analysis of *BDNF* SNPs in 462 unrelated lymphoblastoid cell line (LCL) samples identified proximal SNPs in high linkage disequilibrium significantly associated with *BDNF* mRNA, suggesting that the effect of these SNPs on BMD are through *BDNF*. siRNA knockdown assays of *BDNF* in mouse-derived osteoblasts provided evidence that *BDNF* interacts with osteoblast differentiation factors, and its inhibition may stunt bone formation.

Guo *et al*. (15) have therefore shown that genes with suggestive association to BMD from population-based studies may still be relevant to osteoporosis etiology, and have identified *BDNF* as one such candidate for a novel BMD gene. Studies using epigenomic data are not without caveats, and it is important to consider these points when conducting integrative analyses. Data from the ENCODE project were entirely derived from cell lines, many of which many not be directly relevant to the trait or disease under study. ENCODE project data did include a human osteoblast cell line, but in the study of bone biology, there is a clear deficit of epigenomic data from other important bone-related cells such as osteoclasts and osteocytes. Studies integrating epigenomic data and GWAS of BMD will be greatly improved by using primary tissues or cell cultures directly related to bone biology, once these become available. Whether or not *BDNF* becomes clinically utile in diagnosing osteoporosis or predicting fracture risk in humans will be of interest, as Guo *et al*. (15) have presented intriguing functional evidence in murine osteoblast.

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## Footnote

*Conflicts of Interest:* The author has no conflicts of interest to declare.

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