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

Cousminer, D.L.*[1,2], Wagley, Y*.[8], Pippin, J.A.*[1], Way, G.P.[2], McCormack, S.E.[1,2], Chesi, A.[1,2], Mitchell, J.A.[1,2], Kindler, J.M.[1,2], Kalkwarf, H.J.[3], Lappe, J.M.[4], Johnson, M.E.[1,2],, Hakonarson, H.[1,2], Gilsanz, V.[5], Shepherd, J.A.[6], Oberfield, S.E.[7], Greene, C.S. [2], Voight, B.F.[2], Wells, A.D.[1,2], Zemel, B.S.*[1,2], Hankenson, K.D.*[8] and Grant, S.F.A.*[1,2]

1. Children's Hospital of Philadelphia, Philadelphia, PA, USA. 2. University of Pennsylvania, Philadelphia, PA, USA. 3. Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA. 4. Creighton University, Omaha, NB, USA. 5. Children's Hospital Los Angeles, Los Angeles, LA, USA. 6. University of Hawaii Cancer Center, Honolulu, HI, USA. 7. Columbia University Medical Center, New York, NY, USA. 8. University of Michigan, Ann Arbor, MI, USA.

Abstract

While many genetic loci are associated with adult areal bone mineral density (aBMD), less is known about genetic determinants of pediatric bone accrual. Moving beyond the standard GWAS approach using static phenotypes, we longitudinally modeled pediatric aBMD and bone mineral content (BMC) trajectories to identify novel loci. The 'Bone Mineral Density in Childhood Study' is a multi-ethnic cohort of healthy children and adolescents from five US clinical sites with up to seven annual bone scans. We modeled longitudinal bone gain across ~10,000 observations per skeletal site using 'SuperImposition by Translation and Rotation' (SITAR). 36 parallel GWAS were performed on SITAR parameters *a-size*, *b-timing* and *c-velocity* using linear mixed models for aBMD and BMC at the distal 1/3 radius, lumbar spine, femoral neck, total hip, total body less head and skull. We observed 27 genome-wide significant signals, plus 13 additional suggestive signals supported by more than one phenotype. 35 of the resulting 40 signals were novel, with only one previously reported in children. 15 signals reside near genes involved in Mendelian disorders of bone density and/or had functional annotations for osteoblast or osteoclast regulation. Since causal effector genes are uncertain at most GWAS loci, we aimed to physically implicate such genes in human mesenchymal stem cell (hMSC)-derived osteoblasts. We extracted proxy SNPs in LD with each sentinel that coincide with open chromatin determined by ATAC-seq. Leveraging high-resolution genome-wide promoter-focused Capture C data, we detected consistent contacts between open-proxy SNPs and candidate effector genes. At three such loci, we elected to perform siRNA knockdown for 12 implicated genes in hMSCs in six independent, temporally separated experiments using three hMSC donor lines, differentiated the cells into osteoblasts, and then assessed for metabolic and osteoblastic activity. Knockdown of *PRPF38A, KARS* and *TEAD4* (one gene at each locus) led to an absence of extracellular calcium deposition, providing new candidate genes for further functional follow-up. Given that five of our loci also yield suggestive association when queried against GWAS data for later-life fracture risk, our findings highlight that utilizing a longitudinal approach during the high bone turnover period of bone accrual combined with variant-to-gene mapping can lead to a greater understanding of the pathogenesis of bone loss and osteoporosis.