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Introduction:

Background:

- Osteoporosis is a significant burden for our aging population. Developing a better understanding of the genetic underpinnings of poor bone quality may assist in the future development of prevention strategies.
- Correa-Rodriguez et al. have identified a group of single nucleotide polymorphisms (SNPs) that were associated with both body composition and bone mineral density (BMD) in a population of Spanish Caucasians.3 In particular, they found that SNP rs3736228 in the low-density lipoprotein receptor related protein 5 (LRP5) gene had an influence on BMD.[1,2,3]
- While the role of LRP5 in the Wnt canonical pathway in regulating osteoblast growth and inhibiting apoptosis has been fairly well characterized, its association with phenotypic BMD and osteoporosis has only been explored in a limited fashion.[4]

Objective:

- The aim of this study is to expand existing knowledge of LRP5 and explore the association of rs3736228 polymorphisms and BMD to replicate the findings of previous studies in a cohort of healthy young adults.

Methods:

Study Cohorts:

- **Assessing Inherited Markers of Metabolic Syndrome in the Young (AIMMY)**
  - Healthy Caucasian young adults (Males: n=93,, Females: n=96, age range: 18-35 years)
  - Paticipants were recruited to identify genetic variants associated with risk factors for metabolic syndrome

Phenotyping:

- Height, weight, BMI, and total BMD.
- Phenotypes were adjusted for age, except for total BMD which was adjusted for both age and height.

Genotyping:

- Applied Biosystems Taqman allelic discrimination assays and the Applied Biosystems 7900HT Real-Time PCR were utilized to genotype the DNA samples in the AIMMY cohort per manufacturer's instructions.

Data Analysis:

- Hardy-Weinberg Equilibrium was assessed for LRP5 SNP rs3736228.
- Data was stratified by sex and run through analysis of covariance (ANCOVA).
- Where the f-tests were significant, post hoc pair-wise comparisons were performed and the p-values were adjusted for multiple comparison using the Sidak method.

Results:

- The genotype distribution for SNP rs3736228 in the AIMMY cohort was found to be in HWE. (See Figure 2)
- Using a dominant model, we found that females with one or more copies of the risk T allele of SNP rs3736228 had a significant negative association with total BMD (p = 0.0347). (See Figure 4)
- However, a similar association was not seen in males in this cohort.
- We did not find a significant association for this polymorphism and height, weight, or BMI.

Figure 1: AIMMY Demographics

Characteristic	Females		Males	
	N	Mean ± SD	N	Mean ± SD
Age (years)	96	22.3 ± 4.4	93	23.7 ± 4.2
Weight (kg)	96	62.4 ± 9.2	93	78.0 ± 12.3
Height (cm)	96	165.9 ± 5.8	93	178.8 ± 7.7
BMI	96	22.7 ± 2.9	93	24.3 ± 3.1

Figure 2: Hardy-Weinberg Equilibrium Calculations

	P(C allele)	P(T allele)	HWE p-value
NCBI ExAC [5]	0.8608	0.1392	-
AIMMY Cohort	0.841	0.159	0.50

Result (continued):

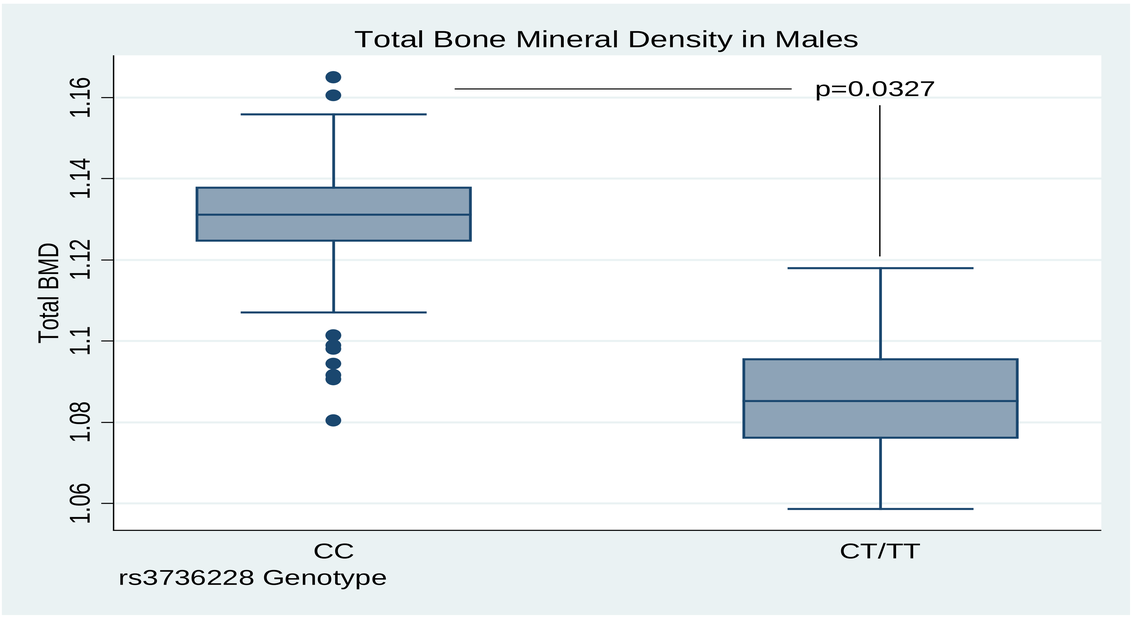
Figure 3: Phenotypes Run in AIMMY

	Covariates used	LRP5 (rs3736228)
Height	Age	X
Weight	Age	X
Total BMD	Age, height	X
BMI	Age	X

Figure 4: Significant Associations Found in AIMMY

SNP	Phenotype	Gender	N; Adjusted mean ± SEM	Significantly different genotypes
LRP5 (rs3736228)	Total BMD	Female	CC (N=57; 1.129 ± 0.011)* CT/TT (N=27; 1.085 ± 0.016)*	*p=0.0327

Figure 5: Plot of SNP Variations vs. Total BMD



Discussion & Significance:

- The rs3736228 CC variant of the LRP5 gene was found to be associated with BMD in females in the AIMMY cohort. The more common C allele encodes alanine, while the rarer T allele encodes valine. The LRP5 gene has now been shown in multiple studies to be associated with bone quality measures like calcaneal Qualitative Ultrasound (QUS) and BMD.
- Our study has expanded these findings and suggests that SNP rs3736228 also influences BMD in healthy young females. This supports the work of Correa-Rodriguez et al that found that when stratifying by sex, females only showed a trend towards significance (p = 0.092) in QUS measures.[1]
- While the development of BMD is polygenic, our work suggests that focus on LRP5 polymorphisms may be particularly helpful in defining genetic risk for low BMD. In addition, given that this polymorphism has shown to have pleiotropic effects, we plan to investigate the associations of SNP rs3736228 with other anthropomorphic phenotypes.
- This study expands our understanding of the importance of LRP5 rs3736228 polymorphisms in BMD by extending its relationship to a cohort of predominantly Caucasian college students. While the development of BMD is polygenic, this work broadened the role of SNP rs3736228 across the age span and underscores the sexual dimorphism seen in musculoskeletal traits.

References:

[1] Correa-Rodriguez, M., Schmidt-RioValle, J., & Rueda-Medina, B. (2016). The rs3736228 polymorphism in the LRP5 gene is associated with calcaneal ultrasound parameter but not with body composition in a cohort of young caucasian adults. Journal of Bone and Mineral Metabolism, 1-7.

[2] Falcon-Ramirez, E., Casas-Avila, L., Cerda-Flores, R. M., Castro-Hernandez, C., Rubio-Lightbourn, J., Velazquez-Cruz, R., . . . Valdes-Flores, M. (2013). Association of LRP5 haplotypes with osteoporosis in mexican women. Molecular Biology Reports, 40(3), 2705-2710.

[3] Funakoshi, Y., Omori, H., Yada, H., & Katoh, T. (2011). A1330V polymorphism of the low-density lipoprotein receptor-related protein 5 gene and bone mineral density in japanese male workers. Environmental Health and Preventive Medicine, 16(2), 106-112.

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