Susceptibility to Obesity and Bone Mineral Density in Young African American Populations

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Introduction: In the United States in 2014, 31.8% of children and adolescents were obese, a leading public health problem in the US, as studies have found that overweight and obese children are at a greater risk to become obese adults. This disease further disproportionately affects minority populations including African American (AA) children. Studies show that 20% of AA children ages 2-19 are obese compared to 14% in Caucasians. Furthermore, the obesity rate in AA adult’s increases as they age, nearly 50% of AA adults classified as obese compared to only 35% of Caucasians. Affected individuals are more likely to suffer from many chronic diseases including type 2 diabetes, cardiovascular diseases, and several types of cancers.

Furthermore, the environmental and lifestyle factors complicates the susceptibility to obesity. To date, most genome-wide association studies (GWAS) have identified various loci associated with BMI within European origin. However, given the notable disparate effects of obesity on Europeans and African Americans, it is crucial to generate a better understanding of the genetic influences on obesity and how they may be modulated by environmental factors within the AA pediatric population. Understanding these factors will likely offer the possibility of better intervention and treatment options in the future. Monda et.al recently published a GWAS identifying six independent SNPs associated with BMI. These 6 SNPs were selected for genotyping in our pediatric African American cohorts to examine the relationship between genetic risk variants for obesity and bone mineral density.

Methods: Study Cohorts

This study includes African American children, ages 5 to 9 years. Case patients had an isolated and radiographically demonstrated forearm fracture (radius, ulna or both bones) and control patients had no self-reported history of a prior bone fracture. A convenience sample of patients was enrolled between December 2005 and July 2010. The study was conducted in Washington, DC, at Children’s National Medical Center (CNMC), a large, urban pediatric hospital.

Patients were studied in the CNMC General Clinical Research Center. The CNMC Institutional Review Board approved this study (#3682). All guardians provided informed consent and children between the ages of 7 and 9 provided assent.

Dual Energy X-ray Absorptiometry Scan

Dual energy x-ray absorptiometry (DXA) scans were obtained using the Hologic QDR Discovery A Densitometer (Hologic, Inc, Bedford, MA). DXA scans were performed on case participants without cast/
splint apparatus. Participants received whole body and lumbar spine scans because these are most accurate and reproducible in pediatric patients. DXA scans were interpreted by a radiology attending who was blinded of participants’ fracture status. DXA scores were reported as real BMD (minus head) z-scores according to the International Society for Clinical Densitometry. Phenotypes analyzed here included total body and lumbar bone mineral density (BMD). All measurements in the BH cohort were calculated without inclusion of the head.

Genotyping
DNA was isolated from blood samples using the Gentra Puregene Blood DNA purification kit (Qiagen, Valencia, CA). Genotyping was performed using the Taqman allele discrimination assay (Applied Biosystems) using standard thermal cycling conditions. Genotypes were called using the Applied Biosystems 7900HT Real-Time PCR system (Clarkson PM 1992).

Statistical Analysis
Each phenotype was tested for an association with each SNP. Hardy-Weinberg equilibrium was tested for each SNP via chi-squared test. All SNPs were testing using a dominant genetic model where heterozygotes were combined with homozygous rare individuals for comparison to homozygous common individuals. The six obesity associated SNPs and bone mineral density height adjusted z-score was analyzed using analysis of variance or covariance (ANOVA/ANCOVA) models as appropriate. In the BH cohort, total body and lumbar BMD and BMC z-score were un-adjusted because z-scores are already adjusted for gender, ethnicity, and age. All other phenotypes were adjusted for age and gender.

Results: BH Cohort: Lumbar BMD without head (height adjusted z-score) were associated with variants in rs974417 and rs10261878. No significant associations were found between obesity related SNPs and total body BMD z-score, and total lean mass.

Discussion: In the analysis of the obesity-related SNPs, we found a statistically significant association between Lumbar BMD (height adjusted z-score without head) and SNPs rs974417, and rs10261878. Though these obesity associated SNPs were identified through GWAS, we were unable to detect significant associations with BMI for most of them. Our study of the Bone Health cohort, with a sample size of 142 participants has limitations. With the small sample size, our statistical power was lower, therefore it is plausible that certain associations were not able to be detected. Yet even with a small sample size, we were still able to show a significant association between the SNP rs974417, SNP rs10261878 and Lumbar BMD z-score (height adjusted without head). Participants with the T allele (n=75) near the KLHL32 gene had a lower Lumbar BMD z-score than those with the risk C allele (n=50). Furthermore, participants with the C allele near the NFE2L3 gene had a lower Lumbar BMD z-score than those with the risk A allele. Our studies demonstrate that the participants with the risk alleles for each of these obesity related SNPs have higher lumbar BMD z-score (height adjusted without head), which suggests that young African-American populations susceptible to obesity have initially higher lumbar BMD z-scores. In literature, obesity has been associated with increased bone mass in some, but conflicting results exist, and mechanisms poorly understood. In our young healthy Bone Health cohort we have shown those children with susceptibility to obesity to have already possessed higher lumbar BMD z-scores. This may indicate that increase BMD associated with obesity is more than just the mechanical loading of bone through excess weight, since the risk alleles confer both susceptibility to obesity and increased BMD z-scores.
Significance: This study demonstrates that certain African American related obesity SNPs have significant associations with increased lumbar BMD z-scores. BMD is the best predictor of fragility fractures and is an important criterion for osteoporosis. Our study is one of few that have reported genetic studies of BMD determination in childhood, especially in AA pediatric population. Given the disparate effects of obesity on non-whites, it is crucial to generate a better understanding of the genetic influences on obesity and how they may be modulated within the African America population. Understanding these factors will likely offer the possibility of better intervention and tailored treatment options in the future.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Phenotype</th>
<th>Covariate(s)</th>
<th>P-value</th>
<th>N (adjusted mean SEM)</th>
</tr>
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<tbody>
<tr>
<td>KLHL22 (rs9324117)</td>
<td>Lumbar BMD z-score (kg adjusted; w/o head)</td>
<td>Age, Gender</td>
<td>0.0494</td>
<td>CC (N=50); 0.396 ± 0.184</td>
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<td>CT; TT (N=75); 0.179 ± 0.156</td>
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<tr>
<td>2FEB2L2 (rs10261972)</td>
<td>Lumbar BMD z-score (kg adjusted; w/o head)</td>
<td>Age, Gender</td>
<td>0.0465</td>
<td>AA (N=36); 0.512 ± 0.264</td>
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<td>AC; CC (N=89); 0.130 ± 0.162</td>
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