Genetic Variation in Neuromedin U Influences Lean Body Mass and Bone Morphometry in Males

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Introduction: Neuromedin U (NMU) is a highly conserved hypothalamic neuropeptide that regulates food intake, body weight, glycemic control, energy homeostasis, and is thought to have an effect on the stress response, inflammatory diseases, and the biological clock1. NMU aids in the formation of bone by acting on osteoblast beta-2-adrenergic receptor (ADRβ2), which regulates cell proliferation in bones. Understanding genetic differences in how NMU affects bone development and metabolic functions may be important for creating strategies to maximize childhood bone development and prevent osteoporosis later in life. This study aimed to determine if there is a relationship between genotype for the chosen single nucleotide polymorphisms (SNP) of the NMU gene, rs6827359, rs12500837, and rs9999653, and bone health by examining their association with bone mineral density (BMD) and bone mineral content (BMC) in healthy individuals.

Methods: Study Cohorts
The Bone Health (BH) cohort included 142 healthy African American children aged 5 to 9 years, who had been previously recruited as part of a fracture risk in childhood study. The Muscle and Bone (MB) cohort included 116 healthy male and female college aged participants enrolled in the Bone and Muscle study from the University of Massachusetts-Amherst community.
Analysis was limited to individuals who were Caucasian by self-report and all analyses were performed in gender-stratified cohort due to large gender differences.
Height and weight were measured to determine BMI. Measurements were obtained by a radiology technician using standardized procedures. Case participants did not have cast/splint apparatus during measuring.
Dual Energy X-ray Absorptiometry Scan
Dual energy x-ray absorptiometry (DXA) scans were obtained using the Hologic QDR Discovery ADensitometer (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) and bone mineral content (BMC) (raw measurements in the M-B cohort; z-scores in the BH cohort), lean mass, and percent body fat. 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 and the Shapiro-Wilk normality test and visual inspection of histograms were used to assess normality of each quantitative trait. All SNPs were tested using a dominant genetic model where heterozygotes were combined with homozygous rare individuals for comparison to homozygous common individuals. Total body and lumbar (BMD, total body and lumbar BMC (raw and z-scores appropriate for each cohort), lean mass, percent body fat and body mass index (BMI) were compared among each SNP genotype using analysis of variance or covariance (ANOVA/ANCOVA) models as appropriate. In the M-B cohort all phenotypes were adjusted for age and body weight except for BMI, which was adjusted for age only. In the BH cohort, total body and lumbar BMD and BMC were un-adjusted. All other phenotypes were adjusted for age and gender.

Results: MB Cohort: Total body BMD, lumbar bone mineral density, total body BMC, and lumbar BMC were significantly associated with variations in rs6827359. Variations in rs12500837 were found to be significantly associated with lean mass in males. No significant associations were found with rs9999653. BMI was not significantly associated with any of the SNPs in males or females.

Ryan Cohort: Lumbar BMD without head (height adjusted z-score), lumbar BMC without head (height adjusted z-score), and total body BMD without head (height adjusted z-score) were found to be associated with variants in rs12500837. No significant associations were found between fracture status and variations in the NMU SNPs.

Discussion: Osteoporosis is the most common bone disorder in the western world and is a significant cause for morbidity and mortality. Bone mass increases throughout childhood and adolescence to reach its peak bone mineral density (BMD) around age 18 for women and age 20 for men. Studies have found that there is a strong correlation between childhood bone development and bone health in advanced age with poor childhood bone development being associated with higher rates of osteoporosis and fracture in old age. Therefore it is essential to understand the genetic factors affecting bone development and health in order to maximize potential bone development in childhood and prevent future bone health disorders.

It is known that NMU acts through the sympathetic nervous system through a poorly defined mechanism and is thought to be the first central mediator of leptin-dependent bone mass regulation. In addition, NMU aids in the formation of bone by acting on beta-2-adrenergic receptor (ADRβ2) located on osteoblasts, which regulates cell differentiation into osteoblasts in bones. We have demonstrated that variations in the NMU gene are associated with better BMD and BMC primarily in men. Further exploration into how these genetic variants influence bone development and metabolic functions may
be important for creating strategies to maximize childhood bone development and preventing osteoporosis.

**Significance:** This study demonstrates that genetic variations in the NMU gene are associated with higher BMD and BMC primarily in men. Future research into the influence of NMU on bone development may lead to more effective treatment strategies to prevent the debilitating bone diseases that affect millions of Americans such as osteoporosis.

### Significant Associations for MB Cohort

<table>
<thead>
<tr>
<th>SNP</th>
<th>Phenotype</th>
<th>Gender</th>
<th>Covariate(s)</th>
<th>P-value</th>
<th>N:adjusted mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMU (rs12500837)</td>
<td>Lean mass (g)</td>
<td>Male</td>
<td>Age, weight</td>
<td>0.0468</td>
<td>TT (N=31; 59253 ± 709) CT/CC (N=31; 57213 ± 709)</td>
</tr>
<tr>
<td>NMU (rs6827359)</td>
<td>Total body BMD (g/cm³)</td>
<td>Male</td>
<td>Age, weight</td>
<td>0.0499</td>
<td>CC (N=18; 1.261 ± 0.018) CT/TT (N=43; 1.217 ± 0.012)</td>
</tr>
<tr>
<td>NMU (rs6827359)</td>
<td>Lumbar BMD (g/cm³)</td>
<td>Male</td>
<td>Age, weight</td>
<td>0.0034</td>
<td>CC (N=18; 1.117 ± 0.022) CT/TT (N=43; 1.040 ± 0.014)</td>
</tr>
<tr>
<td>NMU (rs6827359)</td>
<td>Total body BMC (g)</td>
<td>Male</td>
<td>Age, weight</td>
<td>0.0126</td>
<td>CC (N=18; 3267 ± 85) CT/TT (N=43; 3034 ± 55)</td>
</tr>
<tr>
<td>NMU (rs6827359)</td>
<td>Lumbar BMC (g)</td>
<td>Male</td>
<td>Age, weight</td>
<td>0.0236</td>
<td>CC (N=18; 259 ± 8) CT/TT (N=43; 237 ± 5)</td>
</tr>
</tbody>
</table>

### Significant Associations for Bone Health Cohort

<table>
<thead>
<tr>
<th>SNP</th>
<th>Phenotype</th>
<th>Covariate(s)</th>
<th>P-value</th>
<th>N:adjusted mean± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMU (rs12500837)</td>
<td>Lumbar BMD w/o head, height adjusted z-score (g/cm³)</td>
<td>None</td>
<td>0.0126</td>
<td>TT (N=112; 0.259±0.104) CT/CC (N=14; -0.531±0.294)</td>
</tr>
<tr>
<td>NMU (rs12500837)</td>
<td>Lumbar BMC w/o head, height adjusted z-score (g)</td>
<td>None</td>
<td>0.004</td>
<td>TT (N=89; 0.242±0.083) CT/CC (N=13; -0.450±0.219)</td>
</tr>
</tbody>
</table>

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