Gain of function mutations in LRP5 regulate bone mass via peripheral serotonin levels
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**Background:** LRP5 is a gene that encodes a widely expressed cell surface receptor that, together with the receptor Frizzled, forms a receptor complex for the family of Wnt ligands. LRP5, a member of the low density lipoprotein receptor (LDLR) family, has an important role in regulating bone mass. Loss-of-function mutations in LRP5 cause osteoporosis pseudoglioma, characterized by low bone mass and blindness. Gain-of-function mutations in LRP5 cause high bone mass, a thickened mandible, and torus palatinus. Recently, it was proposed that LRP5 regulates osteoblastogenesis via a non-canonical Wnt signaling pathway. In mouse models, LRP5 was shown to control bone formation by inhibiting serotonin synthesis in the enterochromaffin cells of the duodenum. Gut-specific LRP5 knock out mice had low bone mass and increased peripheral serotonin levels. Consistent with this, gut-specific LRP5 activation led to increased bone mass and low serum serotonin level.

**Aims:** 1) To measure plasma serotonin levels and bone mineral density (BMD) in subjects with and without a gain-of-function mutation in LRP5. 2) To explore the relationship between serum serotonin level and BMD in these subjects.

**Hypothesis:** In subjects with a gain-of-function mutation in LRP5 and high bone mass, peripheral blood serotonin levels will be significantly lower than in unaffected members of the same kindred.

**Methods:** **Subject enrollment:** Patients in kindreds with a gain-of-function mutation in LRP5 and known to either Dr. Karl Insogna or Dr. Joseph Belsky were invited to participate in this study. **Serotonin measurement:** Venous blood samples were collected into EDTA tubes and processed within 1-2 hours of collection. The platelet pellet and platelet poor plasma (PPP) were separated using differential centrifugation. Platelet pellets were resuspended in PBS and both platelet pellets and PPP were stored at -70°C until the serotonin ELISA (Labor Diagnostika Nord GmbH, Nordhorn Germany) was performed. **BMD measurement:** If not done previously, BMD was measured by DXA using either an Hologic 4500C machine or a Lunar Prodigy machine. **Statistical analysis:** An unpaired student’s t-test was used for all comparisons.

**Results:** Nine affected subjects and 7 control subjects within these kindreds were recruited. However, results for only 5 in each group are available because the serotonin measurements were not yet available for 4 affected subjects and 2 controls. The mean L-spine T-scores in affected individuals and controls were 6.4±0.5 vs. 0.3±0.3 (p<0.01) respectively, and for the femoral neck, 4.5±1.0 vs. 0.5±0.4; (p=0.02). The mean PPP serotonin levels in affected subjects and controls were 15.8±1.9 vs. 17.6±3.8, respectively (p = 0.7). The difference between the means was 1.9±4.2 (95% CI : 7.9 - 11.6). The mean serotonin levels in the platelet pellets in subjects with the LRP5 mutation vs. controls were 901.4±412.1 vs. 671.7±174 (p= 0.6). The difference between the means was -229.7±447.4 (95% CI: -1261 - 801.9). These data were not normalized for platelet number and we are currently measuring protein content of the pellet as an index of platelet number. The mean serum serotonin levels in affected subjects vs. controls were 282.3±86.4 vs. 178.4±62.0 (p= 0.4). The difference between the means was -103.9 ± 106.4 (95% CI -349.3 - 141.5).

**Conclusions:** In subjects with a gain-of-function mutation in LRP5, serotonin levels in platelet poor plasma appear to be slightly reduced compared to controls but this difference was not statistically significant. In affected subjects, serotonin levels in the serum and platelet pellet were slightly higher in comparison to controls but these differences were also not significant.