Introduction: Prior studies in young adult mouse and human skeletons suggest that long bones possess biological processes which co-adapt morphological and compositional traits in a particular way to satisfy mechanical demands [1,2]. The disadvantage to this co-adaptation is that the tissue-quality factors that tend to make bone stiff also tend to make bone less ductile, less tough, and more damageable. However, it is not known whether similar co-adaptive mechanisms exist in more complex corticocancellous sites such as vertebrae. Previous work in inbred mouse vertebrae showed that individual strains do not inherit a single bone trait, but a set of trabecular, cortical, and compositional traits that reflect the organ-level functional and mechanical functionality of adult vertebrae [3]. Thus, improved understanding of the biological processes that contribute to phenotypic variation may provide a novel approach to identifying sets of traits that better predict fracture risk.

The goal of this study was to test whether trabecular, cortical, and compositional bone traits are functionally related, as this would imply there is a strong biological process in bone that co-adapts traits. We postulate that the cortical and trabecular bone traits will co-vary with matrix composition contributing to bone stiffness and strength. We tested this hypothesis using a panel of AXB/BXA Recombinant Inbred (RI) mouse strains. The unique pattern of randomization of A/J and B6 genomic regions among the RI panel provides a powerful tool that can be used to measure the tendency for different traits to co-vary and to study the biology of complex traits [4].

Materials and Methods: We examined L4 vertebrae from 20 female AXB/BXA RI strains (n=10/genotype; age=16wks) with IACUC approval. Micro-architectural traits were determined using a desktop micro-CT (GEMS) with a voxel size of 16μm³. Traits included vertebral body total cross-sectional area (TtAr), cortical area (CtAr), trabecular bone volume fraction (BV/TV), cortical and trabecular tissue mineral density (TMD), and total vertebral body volume (TtBV = bone vol + marrow vol). Intact L4 vertebrae (n=5/genotype) were compressed axially such that load was only applied to the vertebral body. Mechanical properties measured were stiffness and failure load.

A Path Analysis, which allows for testing how multiple traits co-vary simultaneously, was conducted using the mean Z-scores of each RI strain. Path Analysis favors the a priori, theory-based model such that models are rejected if the observed data and the expectations derived from the model do not match (p<0.05). A Path model (Fig 1) was constructed by specifying directed paths among select bone traits that could be related to biological processes involved in the formation and resorption of bone tissue (CtAr/TtAr – periosteal and endosteal activity; BV/TV – endosteal activity; TMD – mineralization; TtV – growth plate biology) rather than complex traits that have to be decomposed to reveal the underlying biology. The Path Model tested whether phenotypic variation in bone size and architecture arises from genetic variants affecting body weight and vertebral size (TtV), and constraints imposed on the amount of tissue that can be used to construct functional vertebral trabecular bone (TtBV). The model also tested whether the genetic variants affecting these variables are answered by functional interactions among the set of cortical (CtAr/TtAr), trabecular (BV/TV), and compositional (TMD) bone traits that collectively define whole bone stiffness.

Results: Despite similar phenotypes, randomization of A/J and B6 genomic regions resulted in wide variation in vertebral size, composition, and architecture among the RI strains. A correlation analysis using the mean values for each RI strain revealed that 30% of the correlations examined were significant. Thus, many bone traits co-vary after genetic randomization. The Path Model (Fig 1) fit the data well (p>0.8). Path coefficients reflect the strength of association among traits (line thickness). The analysis showed that body weight, TtV, and TtBV explained 61-85% of the variation in adult TMD, CtAr/TtAr, and BV/TV. The Path Analysis also confirmed that cortical and trabecular bone traits were functionally related. In particular, the proportional amount of trabecular bone (BV/TV) was negatively related to the proportional amount of cortical bone (CtAr/TtAr) and TMD. The structural equations derived from the Path Model showed the functional interactions among TMD, CtAr/TtAr, and BV/TV explained 67% of adult stiffness. The path coefficient between body weight and stiffness suggests an unmeasured variable in addition to these functional interactions may also contribute to vertebral stiffness.

Discussion: The data show that functional interactions among trabecular, cortical, and compositional traits play a key role in achieving organ-level mechanical functionality. Assessment of physical bone traits that determine mechanical strength of corticocancellous bone largely centers on bone mineral density (BMD) or BV/TV. However, few studies are conducted with knowledge of the relationships among genes, cellular processes, physical traits, and mechanical functionality. The Path Model provides insight into the genetic basis of skeletal fragility by suggesting that the co-variation observed among adult traits is a result of biological processes that co-adapt traits. Based on the RI analysis, smaller vertebrae relative to body size achieve stiffness sufficient for mechanical functionality by increasing TMD and the relative amounts of cortical and trabecular bone. Without this co-adaptation, smaller vertebrae relative to body size would be unable to support daily loads (i.e., not functional). However, because mineralization is involved, the co-adaptation of traits may lead to a set of traits that is functional for daily loading, yet susceptible to fracture under extreme loading (i.e., fall) [1-3]. Therefore, understanding of the genetic basis of skeletal fragility can be improved by understanding the genetic variation in the interaction among sets of traits and how this variation defines complex traits like BMD, strength, and fragility.

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Fig 1. Path Model showing positive (solid) and negative (dashed) relationships among functionally interacting bone traits.