INTRODUCTION: Skeletal growth plays a critical role in determining adult bone mass and fracture risk. Classical studies examining the development of bone morphology reported typical growth patterns for boys and girls of different races [1], but did not show how growth patterns vary among individuals within these populations. Although Wolff's Law helps explain how typical growth patterns match bone size with applied forces [2], a "one-size-fits-all" paradigm may not be adequate for a population that shows tremendous inter-individual variation in body size and bone size. Earlier studies [4] have shown that the joint formation process (functional adaptation) works within the context of genetic variants affecting skeletal robusticity is not well understood. Robusticity, which defines the relationship between growth in width and growth in length of long bones, is a heritable trait that varies widely among individuals and predicts fracture risk throughout life [3]. Identifying the biological controls regulating robusticity is critically important because bone length and width contribute to bone strength in opposite ways [4]. Prior work showed that genetic and environmental variants leading to slender (narrow relative to length) adult mouse long bones were strengthened by compensatory changes in morphology and mineralization during ontogeny [5]. This compensatory response means that bone strength can arise from different sets of traits, implying that a population is better predicted by genetic or environmental factors. A population's response, a population would be expected to show random combinations of traits, ranging from under-designed (weak) to over-designed (bulky). Our goal was to translate the systems biology concepts learned from the mouse directly to the human skeleton. We tested the hypothesis that a slender bone relative to body size is compensated morphologically during post-natal growth by coordinated changes in endosteal expansion.

METHODS: We tested the hypothesis by examining longitudinal changes in the structure of the second metacarpal diaphysis for Caucaisan children growing up in Cleveland, Ohio circa 1930. A total of 330 hand-radiographs were obtained from the Bolton-Brush collection, which is a longitudinal database curated by the School of Dental Medicine at Case Western Reserve University. Digitized radiographs (512 dpi) of the non-dominant hand and wrist for 24 girls and 31 boys were examined at 3 months, 9 months, 2 years, 4 years, 6 years and 8 years of age. In addition to bone length (Le), outer and inner diameters of the diaphysis were measured at minimum shaft thickness (~mid-shaft) and used to calculate total area (Tt.Ar), cortical area (Ct.Ar), and cross-sectional area (Ma.Ar), and polar moment of inertia (J) assuming a circular cross-section. Robusticity (Tt.Ar/Le) was correlated with relative cortical area (RCA = Ct.Ar/Tt.Ar) at each age to test whether variants leading to slender bones were compensated by proportional changes in endosteal expansion. Variation in RCA is a measure of the relative expansion of the sub-periosteal and endosteal surfaces. Further, we developed a novel analysis to further examine the relationship between bone surface movements using data collected at all time points simultaneously. Theoretically, the relationship between J and Ct.Ar varies with bone size (Tt.Ar/Le) if endosteal expansion is coordinated with sub-periosteal expansion across the study population. J versus Ct.Ar curves were fitted using a power-law regression, J = A x Ct.Ar^B. The relationship between robusticity and the constant A (a measure of the structural efficiency of growth) was determined using linear regression analysis.

RESULTS: Robusticity (Tt.Ar/Le), which was normally distributed for boys and girls at each age (p<0.10, Kolmogorov-Smirnov), varied widely, showing an average coefficient of variation of 16.4%. For each individual, robusticity increased rapidly during the first year, then plateaued after 2 years of age (Fig 1a,b). Robusticity at 2 years explained 57% (p<0.001) and 76% (p<0.001) of the variation in robusticity at 8 years for boys and girls, respectively, indicating that the inter-individual variation in robusticity was established largely by 2 years for both sexes. Boys had significantly greater Tt.Ar, but not length, compared to girls at all ages, indicating that sex-differences in robusticity were apparent post-natally and resulted from differences in sub-periosteal expansion and not longitudinal growth. This dimorphism in robusticity was accompanied with greater RCA in girls compared to boys by 2 years of age. RCA correlated negatively with robusticity for boys and girls at each age (Fig 2), as hypothesized. The regressions were significant for boys at all ages except 2 years and significant for girls at 4 and 8 years of age. The inter-individual variation in growth patterns resulted in widely varying J versus Ct.Ar curves for both sexes, as expected. The exponent, B (1.62±0.18), calculated from the power-law regression J = A x Ct.Ar^B showed little variation among individuals and was similar for boys and girls (p=0.57, t-test). When B was fixed to 1.62, the average value for A was significantly (p<0.006) greater for boys (0.91±0.14) compared to girls (0.81±0.11), indicating that the metacarpal diaphyses of boys grew on average in a more structurally efficient manner compared to girls. A correlated positively with robusticity measured at 8 years for both sexes (R²_Girls = 50%, R²_Boys = 54%, p<0.0001) (Fig 3), indicating that the relationship between expansion of the sub-periosteal and endosteal surfaces was not random but highly coordinated across the study population, as hypothesized. Slopes and intercepts were not significantly different between boys and girls (p>0.2, ANCOVA). All correlations remained significant after taking body weight into consideration by partial regression analysis.

DISCUSSION: The results showed that variability in a critically important fragility trait, robusticity (Tt.Ar/Le), was determined by 2 years of age, indicating that the biological (i.e., genetic) factors controlling the relationship between sub-periosteal expansion and longitudinal growth were established during post-natal growth. The significant correlations between Tt.Ar/Le and RCA examined for individual ages and between A and Tt.Ar/Le examined across growth confirmed the hypothesis that the inter-individual variation in sub-periosteal expansion was highly coordinated with narrow expansion. For this study population, individuals with slender bones had a proportionally greater amount of cortical tissue (RCA) to maximize strength, whereas individuals with robust bones had proportionally thinner cortices presumably to minimize bone mass [6]. Thus, the data indicated that humans do not acquire random sets of skeletal traits, but rather build functional skeletal structures using a narrow range of trait sets. Finding that growth patterns were consistent across a genetically heterogeneous population provided important new insight into the system properties of bone, suggesting that individuals within this population share a common biological control that buffers variants by allowing certain trait sets to exist without loss of function. Importantly, finding a consistent growth pattern across this population and finding that morphological compensation depended on robusticity may provide novel clinical traits to better quantify how genetic and environmental variants impact the development of bone strength.

ACKNOWLEDGEMENTS: NIH AR44927, Doris Duke Charitable Foundation, Dr. Mark Hans and Dr. Martin Palomo.