# Graduate Research Plan Statement

Discrete changes in brain region size and shape in relation to other brain regions (structural covariation) are associated with various neurological disorders such as schizophrenia[6], bipolar disorder [3], and autism spectrum disorder[4]. Investigating how different brain regions change in size in relation to each other may provide insights into developmental and genetic programs, cognitive processes, sensory perception, motor control, and spatial analytical functions. **This proposal aims to identify genetic programs influencing changes in volume between pairs or groups of brain regions in zebrafish.**

Approximately 70% of zebrafish genes have at least one human ortholog due to a conserved genetic composition[8]. Therefore, they offer a promising avenue for exploring brain structural covariation that is translatable to humans. Similarities in covariation within homologous brain structures shared neurological diseases, and neurodevelopmental patterns[1, 2] between zebrafish and humans may lead to valuable insights into the role of a directed genetic programming of the brain that is generalizable. Additionally, their transparent embryos, rapid development, accessibility, manipulability, suitability for high throughput screening, and the existence of a brain atlas make zebrafish a great tool for studying brain development and understanding the mechanisms underlying brain structural covariation and neurodevelopmental processes[7].

Figure 1 shows preliminary data from 300 whole-brain images of 6-day-old zebrafish brains with pairwise brain region correlations represented as points, a trendline model (red line), and variability (grey area) showing that correlation between brain regions generally (y–axis) decreases as the distance between brain regions (x–axis) increases. This may suggest an interesting relationship between distance and correlation. A key goal of this research project is to build upon preliminary data from our lab by creating a standard for data collection by controlling for genetic diversity, environmental conditions during growth, and imaging parameters to ensure accurate measurement of volumetric relationships among wild-type zebrafish. Then, I plan to compare volumetric relationships obtained in wild-type zebrafish (Aim 1) to identify genes associated with these volumetric relationships (Aim 2).

**Aim 1: Identify anatomical volumetric relationships between brain regions.**

Hypothesis: By examining structural covariation, anatomical imbalance maps, and applying graph theory for brain volume analysis, I predict the discovery of significant functional relationships intricately associated with the brain's anatomical structure. Method: Zebrafish embryos from the national zebrafish database at the NIH will be raised in highly measured and standardized environmental conditions. High-resolution whole-brain confocal scanning will be conducted at a developmental stage in 6-day-old wild-type zebrafish where embryonic brain development has been completed. Next, the brain will be registered to a reference, and brain regions will be annotated using ANTS, a medical image registration tool kit[4]. I will use correlation analysis to measure the pairwise correlational relationships between brain regions (figure 1) and plot the resulting correlational values as a heatmap or "Structural Correlation Map." A new heatmap or “Anatomical Imbalance Map” will capture orthogonal residuals from the same linear model[5], and graph theoretical analysis will be applied to a set of zebrafish brains. Regional degree, clustering coefficient, and group differences in mean region volume's area under the curve via graph theoretical analysis will be used to address variability in data

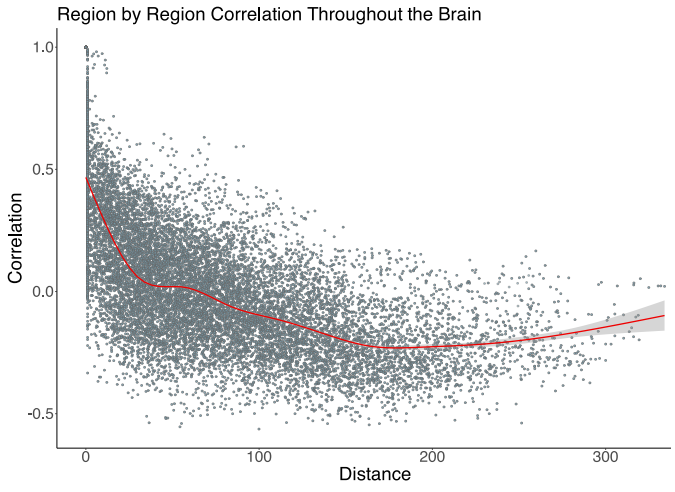


*Figure 1. Preliminary Data.* Correlation by Distance plot reveals levels of anatomical correlation throughout the brain. Where each point is a pair of regions, and the ‘r’ values of region-by-region combinations (y-axis) are plotted against edge-to-edge distances (x-axis).

standardization and environmental control[8]. Four primary relationships in structural correlation and anatomical imbalance maps will be characterized: 1) close-proximity positive correlations, 2) close-proximity negative correlations, 3) distant positive correlations, and 4) distant negative correlations, defined by the second standard deviation of the coefficient of variation. Exploration of advanced statistical methods or alternative brain region sets will occur as needed.

**Aim 2: Characterize strongly inversely- varied and co-varied brain regions to identify shared genes.** Hypothesis: If consistent patterns of structural co-variability exist between brain regions, genetic factors underlying the observed patterns of brain connectivity may be identifiable. Method: Methods from Aim 1 will be used to image brains and identify structural co-variability. Then, by analyzing the genotypes of individuals, PCA can reveal clusters or subgroups within the population, and k-means will reveal computationally clustered subgroups, allowing an understanding of how these groups are related to the observed anatomical patterns of covariation. To compare the genetic variation within the zebrafish population with the observed patterns of covariation. Single nucleotide polymorphism (SNP) analysis will be used for preliminary processing. I will then select zebrafish with differing SNP's *and* significantly different structural covariation maps. To employ genotyping by sequencing (GBS), and whole genome sequencing (WGS) to further identify candidate genes differing between the zebrafish populations[9,10]. Additionally, I will conduct a linkage disequilibrium (LD) analysis to evaluate the non-random association of alleles within the population. Anticipated Results**:** Anatomic relationships between brain regions may be associated with candidate genes wherever observed. Identified genes should be further investigated to understand their functional roles and potential mechanisms underlying their influence on structural development. These investigations will include analyzing gene expression, gene knockout, or knockdown experiments; other molecular biology techniques can be employed as needed to explore the genetic basis of these patterns, with the goal of shedding light on the connection between genetics and neuroanatomy. Pitfalls may include the complexities of pinpointing candidate genes and unraveling their functional roles and the challenges of data analysis and interpretation. An alternative approach could encompass supplementary analyses, such as gene expression experiments or further molecular biology techniques, to elucidate the mechanisms underlying the influence of identified genes on structural development. In addition, I could shift focus from structural co-variability to functional connectivity in the brain. Investigate how genes relate to functional networks and the dynamics of brain activity.

**Intellectual Merit:** The expected outcomes may enable the identification of genes influencing functional connectivity and their relationship with the anatomical organization of the zebrafish brain. This research may also unveil insights into the interactions and contributions of distinct brain regions to developmental patterns, behaviors, functions, or neurological disorders in zebrafish. Our current lack of genetic and molecular tools to manipulate the human brain necessitates a relatively easy-to-manipulate model organism whose brain shares homology with the human brain.

**Broader Impacts:** The proposed research has implications for understanding neural processes and by-proxy analysis of genetic programs underlying brain development, function, and disorder between a broad swath of living organisms sharing homology and neurological mechanisms as zebrafish. It offers a unique framework for comparative studies, opening up possibilities for innovative research in the broader field of translationally-focused neuroscience. I aim to continue my efforts from my undergraduate degree to personally train undergraduate students in microbiological, computational, systems thinking, general, and specific research methodology and provide a space for underrepresented and majority populations at UC Berkeley to interact, share, and learn in a diverse community. Computational pipelines and data will be communicated on open-access peer-reviewed articles and have open access on Github and protocols.io.

***References:***[1]Schilling et al. (2007) *Zoology Part B: Molecular and Developmental Evolution* 308, no. 5: 515-522.[2]Qu, Z., & Adelson, D. L. (2012). *PloS one*, *7*(12), e52275. [3]Cardon, G. J. et al. (2017). *Frontiers in Neurology*, *8*, 615. [4]Khundrakpam, B. S., et al. (2017). *Neuroimage*, *144*, 227-240. [5]Nadig, A., et al. (2021). *Proceedings of the National Academy of Sciences*, *118*(14), e2023860118. [6]Vijayakumar et al. (2021). *Scientific reports*, *11*(1), 9451[7]Vaz, R., Hofmeister, W., & Lindstrand, A. (2019). *International journal of molecular sciences*, *20*(6), 1296. [8]*Howe, K., et al. (2013). Nature, 496(7446), 498-503.* [9]Wu et al. *Neuron* (2017). 96:313-329. [10]Adam et al. *Development* (2017). 144:3625-3632.