Identifying and Characterizing Age-Associated Phenotypic Shifts in Mice

Aging is a complex biological process that affects numerous physiological traits over time. Understanding how these traits, or phenotypes, change naturally with age is essential for identifying patterns of decline, stability, or adaptation. By analyzing a wide range of phenotypes in mice across different ages, we can gain insights into how aging influences various biological systems.

This report focuses on identifying phenotypes that exhibit significant changes throughout a mouse's lifespan. By examining these patterns, we can determine whether certain traits increase, decrease, or follow more complex trends such as peaking at middle age. These findings provide a foundation for further research into the biological mechanisms of aging and potential interventions to promote healthy longevity.

This study analyzes 186 phenotypes, focusing specifically on their natural variation with age in an observational framework, without any experimental intervention. The data were collected through a cross-sectional study, in which each mouse was assessed at specific time points rather than being tracked longitudinally. Measurements were taken at six distinct ages, ranging from 3 to 26 months, with approximately 15 independent mice evaluated at each age. These phenotypes included both non-invasive measurements, such as heart rate, and post-mortem assessments, such as organ weight.

To identify phenotypes that exhibit significant age-related changes, a nonparametric one-way ANOVA procedure (Kruskal-Wallis) was applied, followed by a Benjamini-Hochberg (BH) correction to control the False Discovery Rate (FDR). The BH correction balances Type I (false positives) and Type II (false negatives) errors by controlling FDR rather than the Family-Wise Error Rate (FWER). This approach preserves statistical power while accounting for multiple comparisons. An FDR threshold of 0.05 was selected, meaning that, on average, 5% of the rejected null hypotheses are expected to be false positives. The BH procedure ranks p-values in ascending order and compares each to a calculated threshold: (rank of p-value / total tests) * FDR (0.05). Phenotypes with p-values below this threshold were considered to have significant age-related changes. Importantly, the Kruskal-Wallis test does not assume a linear relationship, making it well-suited for detecting a variety of change patterns, including increasing, decreasing, or non-linear trends across the lifespan. The phenotypes that are classified as having a significant change throughout a mouse's lifespan can be pictured, graphing their respective z-scores.

To visualize age-related phenotypic trends, Principal Component Analysis (PCA) was performed. A PCA biplot was generated, where the first two principal components served as axes, representing the variance in the dataset. This approach allowed for the simultaneous visualization of both phenotypes and ages, helping to identify whether significant phenotypes tended to peak at early, middle, or late life stages. Additionally, box plots of select phenotypes were analyzed to illustrate specific trajectory patterns and provide further insights into their age-related dynamics.

After applying the Kruskal-Wallis procedure with the Benjamini-Hochberg correction for an FDR of 0.05, 89 out of 186 phenotypes (47.8%) were identified as significant. This differs slightly from the previously published results, which reported 59% of phenotypes as significantly changing over time. The discrepancy is likely due to the FDR correction, which reduces false positives. Figure 1 illustrates the specific phenotypes classified as significantly changing over

the lifespan. Notably, all phenotypes exhibit positive z-scores, ranging from a z-score of 2 up to approximately 6.5.

To further investigate temporal trends in these phenotypes, a Principal Component Analysis (PCA) biplot was generated (Figure 2). In this visualization, green squares represent different age groups, while blue dots indicate phenotypes that demonstrate significant age-related changes. The x-axis corresponds to the first principal component, capturing approximately 66.5% of the variance, while the y-axis represents the second principal component, accounting for roughly 19% of the variance. Because these components are uncorrelated, the biplot effectively separates phenotypic patterns over time.

Phenotypes clustering near specific age points suggest that their expression or measurement values peak at those ages. The data reveal a clear pattern, where many phenotypes peak either at young ages (3–8 months) or later ages (20–26 months), with fewer phenotypes reaching their highest values at middle age. This clustering suggests distinct phases of physiological change throughout the lifespan.

To examine these trends further, individual phenotype trajectories were analyzed using Figures 3, 4, 5, and 6, which illustrate phenotypes peaking at middle age, no distinct peak, early age, and later age, respectively. The Kruskal-Wallis procedure identifies a range of change patterns, including U-shaped trajectories, sinusoidal trends, and linear progressions. These variations highlight the complexity of age-related phenotypic changes and provide insight into the potential biological mechanisms underlying different patterns of aging.

When interpreting the Kruskal-Wallis procedure results, it is essential to account for the False Discovery Rate (FDR), which necessitates applying a correction to control for false positives. Implementing this correction reduced the percentage of significant phenotypes from 59% to approximately 47.8%, meaning that, on average, 5% of rejected null hypotheses are expected to be false positives. The choice of FDR threshold influences the number of detected significant phenotypes, with stricter control reducing false positives but potentially overlooking true signals. The Benjamini-Hochberg (BH) procedure adjusts for multiple comparisons while preserving statistical power, allowing for a refined selection of phenotypes exhibiting age-related changes.

Further insights into phenotypic trends can be derived from the PCA biplot (Figure 2), which shows that most significant phenotypes cluster around early (3–8 months) or late (20–26 months) life stages. Some phenotypes, such as CD4 in Figure 5, peak at early ages and decline over time, consistent with immune system aging. Approximately half of the significant phenotypes exhibit a downward linear trend. Conversely, phenotypes such as PLT in Figure 6(platelet count) tend to increase with age, a pattern associated with a higher risk of clot formation in older animals. This suggests that while some physiological systems decline with aging, others may escalate due to compensatory mechanisms or pathological processes.

Beyond linear trends, certain phenotypes exhibit more complex trajectories. Some, as shown in Figure 3, peak around middle age (~14 months), which is expected for traits like fat mass that tend to accumulate before declining later in life. Others, like CI (Figure 4), display multiple peaks throughout the lifespan, suggesting non-monotonic variation. The PCA biplot provides a comprehensive visualization of these trends, highlighting clusters of phenotypes that follow distinct temporal patterns.

Overall, using the Kruskal-Wallis procedure with an FDR threshold of 0.05, we identified 89 out of 186 phenotypes exhibiting significant age-related changes. The majority follow a linear trajectory, either increasing or decreasing with age, while a subset demonstrates non-linear patterns such as middle-age peaks or multiple fluctuations across the lifespan. These findings underscore the diverse ways in which physiological traits evolve with aging, providing a foundation for future investigations into the biological mechanisms driving these changes.

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Figures

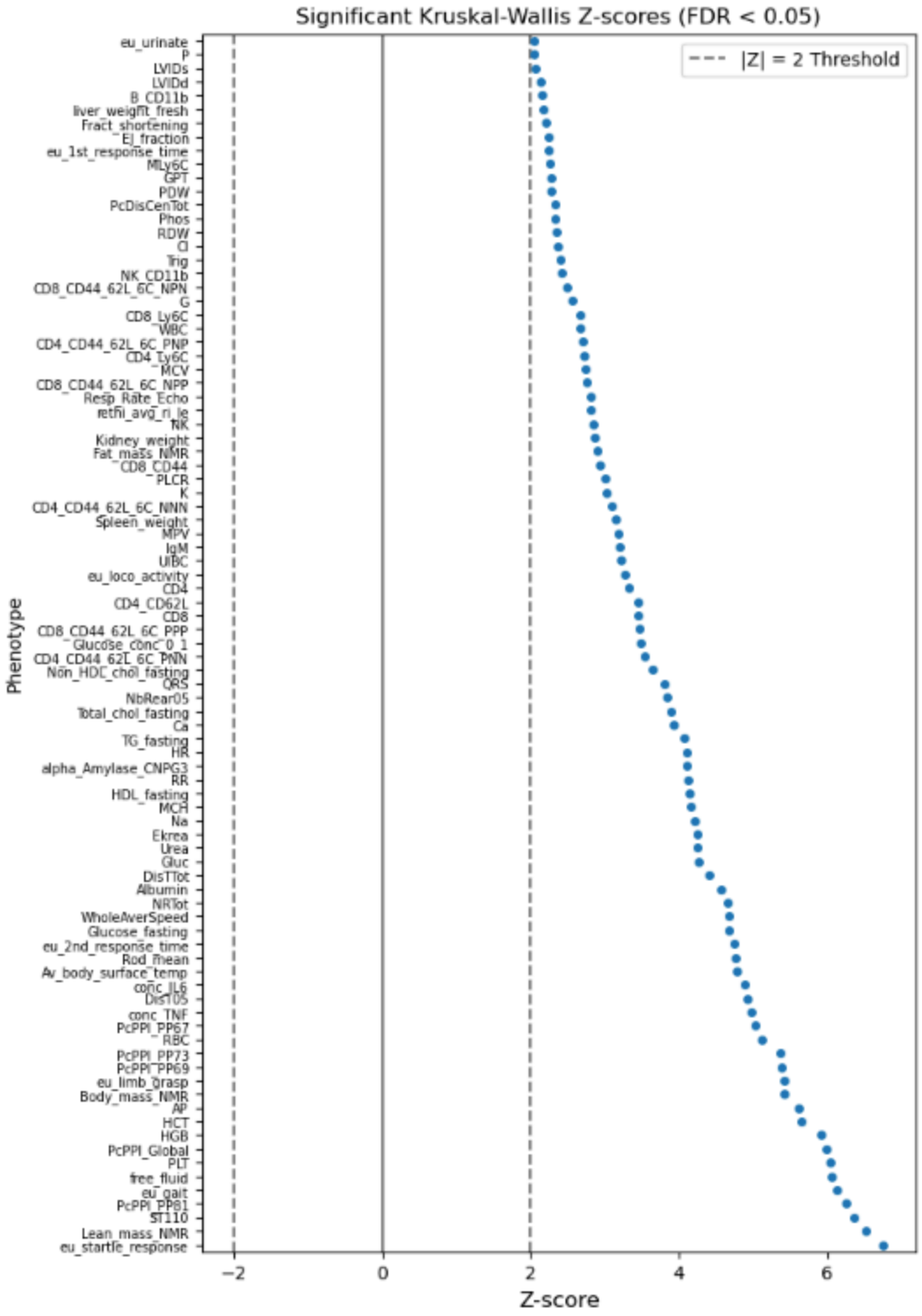

Figure 1: Z-scores for significant (magnitude above 2) phenotypes.

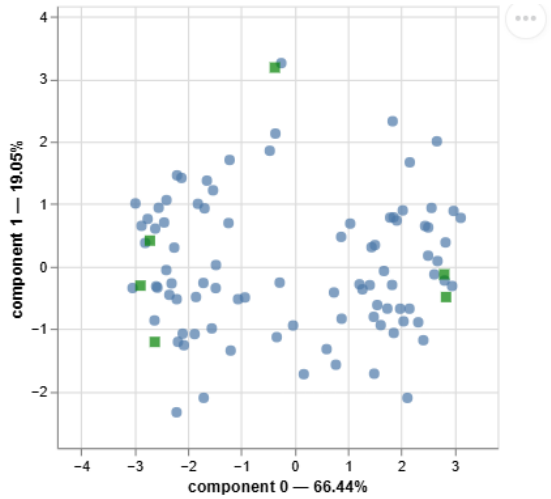

Figure 2: PCA biplot for phenotypes (blue circles) and ages (green squares). Phenotypes clustered together have similar age patterns. Phenotypes clustered around certain ages peak at that age.

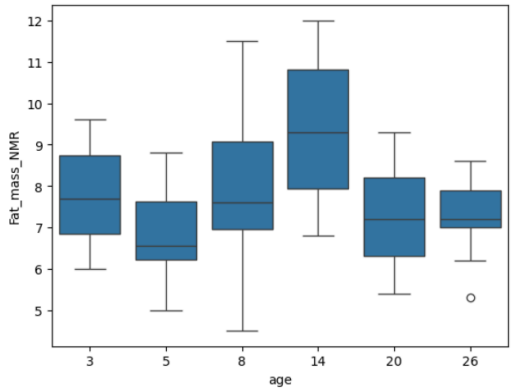

Figure 3: Phenotype change with age for fat_mass_NMR.

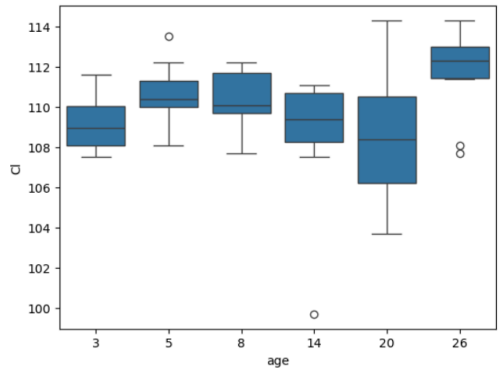

Figure 4: Phenotype change with age for CI.

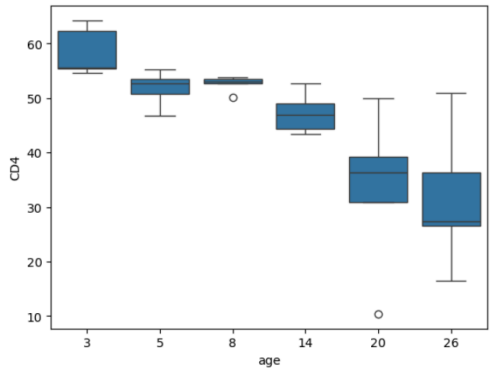

Figure 5: Phenotype change with age for CD4.

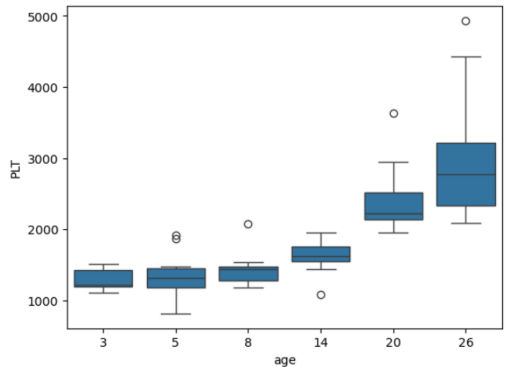

Figure 6: Phenotype change with age for PLT.