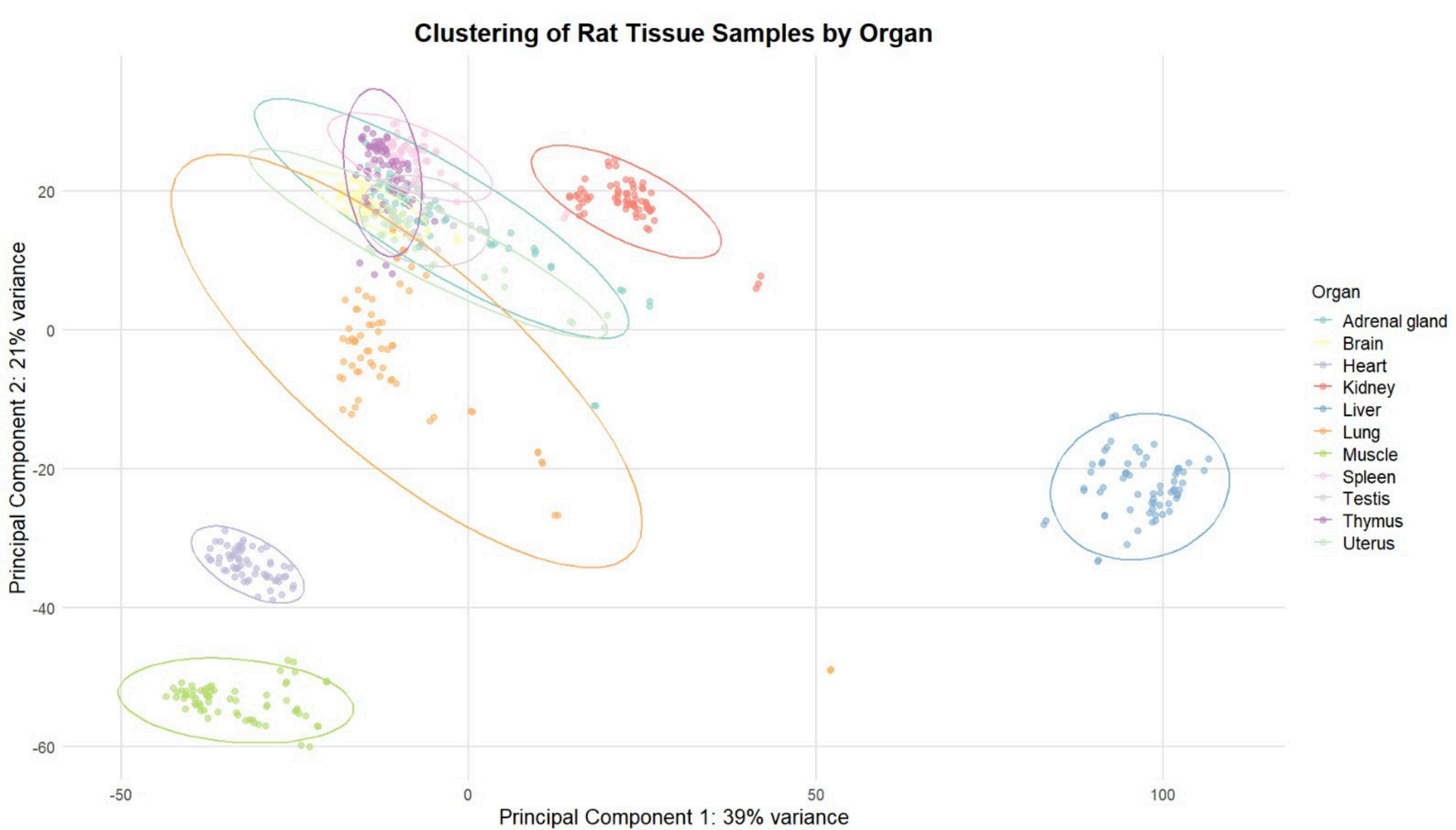


SEX- AND AGE-DEPENDENT GENE EXPRESSION IN RAT HEART TISSUE

Introduction

Gene expression profiles change over time as organisms grow, develop, and age (Glass et al., 2013). Other sources of variation in gene expression are caused by sex differences and hormones (Oliva et al., 2020). By studying the most up- and down-regulated genes across age for each gender, we can investigate how these two biological factors interact and alter gene expression. Sex and age are two major risk factors in cardiovascular disease (Rodgers et al., 2019). Differences in heart disease onset patterns and response to treatment are observed between men and women. Identifying the most up- or down-regulated genes can show which pathways are activated or suppressed in cardiac diseases, and how these pathways differ between sex and developmental stage. Unravelling how sex and age influence gene expression can aid in understanding when certain therapies work best and in developing more targeted therapies.

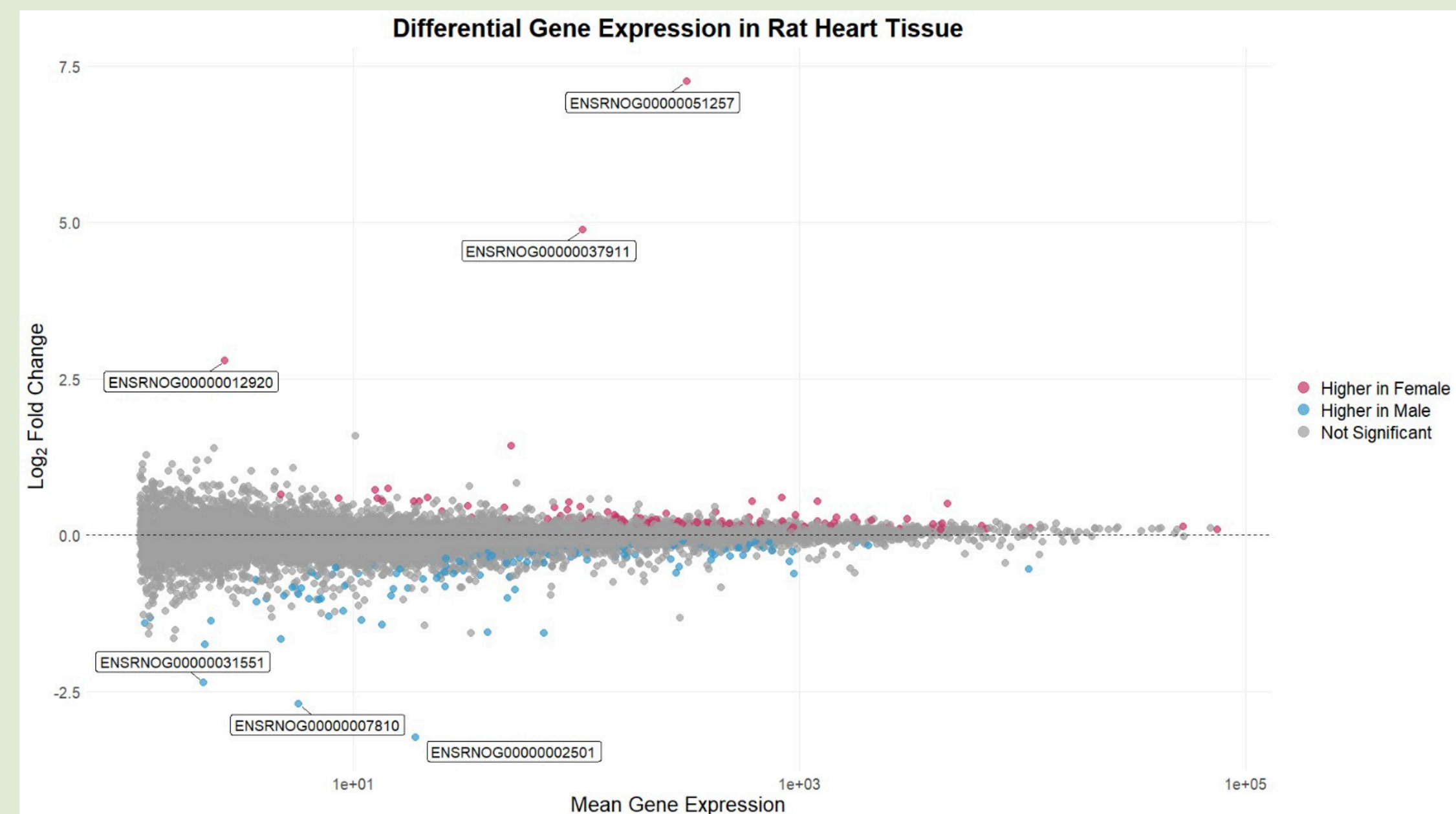
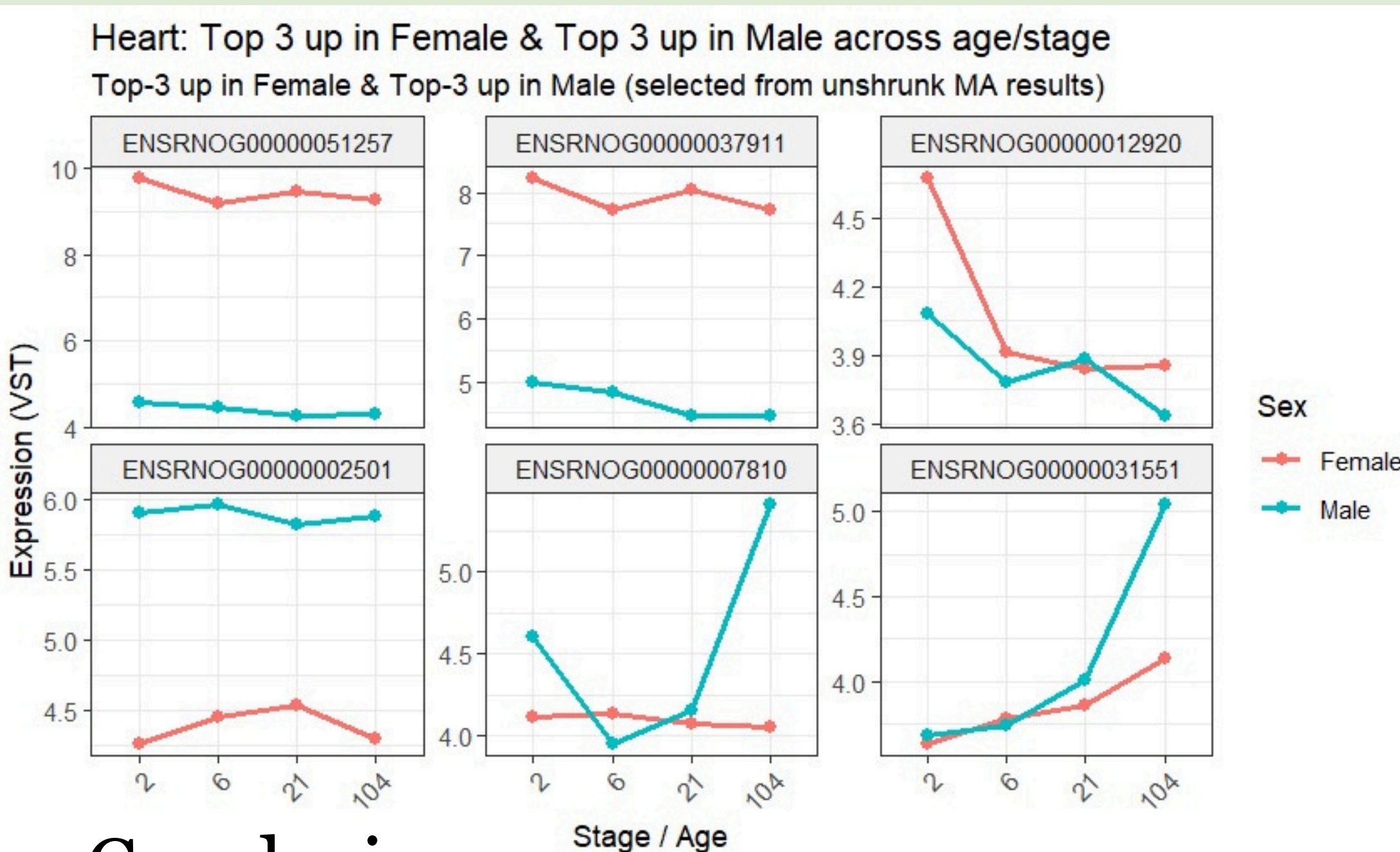


Which organ shows the most distinct and consistent gene expression pattern?

Figure 1. Principal component analysis of gene expression in different rat organ tissues. Data was normalised with variance stabilizing transformation (VST) method. A principal component analysis (PCA) plot was made to visualize the different tissue gene expression, with PC1 (39% variance) and PC2 (21% variance) explaining 60% of the variance. The plot shows close clustering of the data for the adrenal gland, brain, kidney, lung, spleen, testis, thymus and the uterus in the top left corner. The liver shows close clustering of data points, but shows more variation from the other organs. The muscle and the heart also show clustering further away from the other organs, with the heart samples showing the closest clustering of all organs.

Which genes are differentially expressed between male and female rat hearts?

Figure 2. Differential gene expression between male and female rats in heart tissue. Gene expression counts were normalized using the variance stabilizing transformation (VST). The log2 fold change of mean expression in females relative to males is plotted against the log10 of the average gene expression. The 143 genes expressed significantly higher in females (adjusted $p < 0.05$) are shown in red, and the 156 genes expressed significantly higher in males (adjusted $p < 0.05$) are shown in blue. P-values were adjusted for multiple testing using the Benjamini-Hochberg method. The three genes most highly expressed in females relative to males, and the three genes most highly expressed in males relative to females, are labeled.



How do sex-biased genes in the heart change across the lifespan?

Figure 3. Differential gene expression analysis in heart samples using DESeq2 (female vs. male). From the MA plot results, the three genes with the largest positive log2 fold change (higher in females) and the three with the most negative log2 fold change (higher in males) were selected. For each selected gene (one facet per gene), lines and points show the mean variance-stabilized expression (VST) per stage/age for each sex; y-axes are free per panel, and stages are ordered chronologically. Colors indicate sex: female = red and male = blue. Top row facets correspond to genes upregulated in females; bottom row facets correspond to genes upregulated in males.

Conclusion

- Gene expression in rat heart tissue differs between sexes, with 156 genes higher expressed in males and 143 in females
- Gene expression patterns differ between sexes and change with age: in females, some genes remain stable while others decrease with aging, reducing sex differences; in males, several genes vary over time, with some maintaining consistently higher expression or showing stronger age-related increases compared to females.
- The most sex-biased genes identified are not involved in heart disease, indicating that while sex influences gene expression, disease-related genes show no major sex differences under normal conditions.

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