Parental elevated Salt consumption in mice and the development of Autism Spectrum Disorder (ASD) – like behavior in the offspring
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Abstract

Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental disorder with no known etiology or cure. Recent studies have identified a possible causal relationship between maternal gut microbiome alterations (i.e., dysbiosis), mediated by an imbalance in the immune system, and ASD in the offspring. Interestingly, elevated dietary salt consumption has a significant effect on both the gut microbiota and immune system, amongst other well-known diseases. Considering these facts, the main objective of this project is to determine how parental high salt consumption can contribute to the development of ASD in the offspring. We hypothesize that high salt will result in gut dysbiosis and autoimmunity that will affect the normal development of the nervous system in the fetus. To test our hypothesis, we established an animal model in which we fed both male and female mice with food supplemented with 8% NaCl and 1% NaCl in their drinking water (HSD) for 8 weeks while Control groups (CD) were fed with 0.1% NaCl chow and regular water. Then, we paired HSD- or CD-fed males and females and their offspring were kept for behavioral analysis at 8 weeks-old. Additionally, fecal samples were collected from both parents and offspring between 2-3 weeks after birth. Using the samples, we conducted 16s RNA sequencing to determine gut microbiome diversity and abundance. Our results showed that only male mice from HSD-fed parents display less social interaction, hyperactivity, along with increased repetitive behaviors, all behaviors comparable to ASD mouse model. Moreover, our gut microbiome data showed a significant decrease in Lactobacillus genus in mice from HSD-fed groups and their offspring. Collectively, our data not only support the idea that parental HSD can potentially contribute to cause ASD-like behaviors in the offspring, but also supports the notion that environmental factors are involved in the etiology of neurodevelopmental disorders.

Background

Despite the growing number of ASD cases worldwide, the etiology of this disease is still unknown. The current consensus is that ASD is likely caused by the combination of genetic, environmental and neurodevelopmental factors. Amongst such environmental factors, HSD has been shown to increase the circulation of inflammation markers such as interleukin-17 (IL-17) along with the proliferation of T helper cells (Th17), both of which lead to autoimmunity diseases such as multiple sclerosis and rheumatoid arthritis. Therefore, we postulate that continuous rise in individuals affected with ASD could conceivably be associated with increased dietary salt intake, as it has been shown that HSD can alter gut microbiome and inflammation, both factors known to be associated with ASD. Various behavioral tests were conducted on two groups (control and HSD) of offspring mice in attempt to quantify ASD-like behaviors along with 16s RNA sequencing of fecal samples to allow gut microbiome characterization. The results obtained from this project could potentially lead to new mechanistic studies to establish a causal relationship between gut microbiota, autoimmunity, and ASD. Additionally, our results could also bring light to the latent detrimental effects of an unhealthy lifestyle (i.e. HSD) and its consequences for the neurodevelopment in the progeny.

Hypothesis

Parental high salt diet (HSD) is associated with maternal gut microbiome alterations and ASD-like behaviors in the offspring.

Methods

Control P Control F1 HSD P HSD F1

C57BL/6 Mice

1. Breeding after 8 weeks
2. Fecal samples collected from parents and 4-week old offspring (16s RNA sequencing)
3. Weaned at 4 weeks
4. Observation and testing at 8 weeks of age

Behavioral Data Analysis Results

Figure 1. Results from the open field test showed a significant increase in moving area between offspring mice from Control (C-C) and HSD-fed (H-C) parents. This difference was sex-dependent, only being observed in males. (*) P-value = 0.04, obtained using ANOVA. N.S (Not Significant)

Figure 2. Data from open field test showing the % of time exploring the center of the arena. We found no significant differences between all groups. N.S (Not Significant)

Figure 3. Marble burying test shows significant increase in the number of marbles buried between the offspring from Control (C-C) and HSD-fed (H-C) parents. Additionally, a significant sex-dependent differences was observed between offspring from HSD-fed parents. (*) P-value = 0.04 obtained using ANOVA.

Figure 4. Sociability test results showed a small but significant reduction in sociability in the group of mice from HSD-fed parents (H-C). (*) P-value = 0.006. Again, this difference was only found in male mice. N.S. (Not Significant)

Figure 5. Phenome test. After a two-tailed t-test was conducted, data shows no significance between any groups observed. N.S. (not significant)

Figure 6. Quantification of grooming behavior. Similar to the phenotype test, grooming did not show significant differences between groups. N.S. (Not Significant)

Microbiome Data Analysis Results

Figure 7. Relative abundance of organisms in gut microbiomes of control and HSD-fed mice. A notable decrease in Lactobacillus from the Control to HSD groups is observed. Additionally, an increase in abundance of Akkermansia is observed.

Figure 8. Gut microbiome OTU diversity. We observed a notable decrease in bacterial diversity from parents (Control P and HSD P) to offspring (Control F1 and HSD F1). No significant difference was observed in OTUs between Control and HSD groups in parents and offspring. (*** = 8.81E-5, ** = 0.006, one-way ANOVA).

Conclusions & Future Works

- The behavioral analysis of our mice indicates that offspring from HSD-fed parents (H-C groups) show hyperactivity, reduced sociability, and increased repetitive behaviors. Remarkably, these differences showed a robust sex-dependent effect, being observed in males only. These findings mirror the statistics in humans, where ASD is more prevalent in boys than girls.
- Microbiome analysis showed a significant decrease in Lactobacillus, a bacteria vital to neurodevelopment in neonatal mice suggesting a possible causal effect on the ASD-like behaviors observed in our study.
- Overall, we also observed a significant decrease in microbiome diversity from parent to offspring in both Control and HSD groups. In HSD groups there was an increase in the presence of Akkermansia.
- For future works we are planning to identify the causal relationship between parental high salt diets and offspring ASD.

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