Parental elevated salt consumption and the development of Autism Spectrum Disorder (ASD)-like phenotype in the offspring

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1. Background

Neurodevelopmental disorder refers to a range of syndromes caused by the abnormal development of the central nervous system. These disorders are mostly characterized by abnormal motor function, reduced social interaction, impaired learning, etc. ASD is one of the most common forms among neurodevelopmental disorders, affecting approximately 1% of the population worldwide. Despite being highly prevalent, the exact mechanisms underlying the abnormal nervous system development in ASD are not fully known. Several recent studies have shown a potential effect of maternal gut microbiome alteration on offspring neurodevelopment in animal models as well as in humans. While the offspring's brain and immune system development depends on the initial microbial population obtained from the mother, depletion of beneficial microbes such as Lactobacillus, can hamper it. To strengthen the association, a very recent study showed that probiotic treatment (with Lactobacillus and Bifidobacterium strains) on female mice subjected to maternal immune activation (MIA, a well-known animal model for the study of ASD) prevented the development of ASD-like symptoms in their offspring. As the gut microbial population is heavily modulated by dietary habits, offspring ASD has also been associated with maternal diet. For instance, consumption of high fat diet in female mice has been associated with ASD in the offspring and the underlying mechanisms involved maternal gut microbiota alteration.

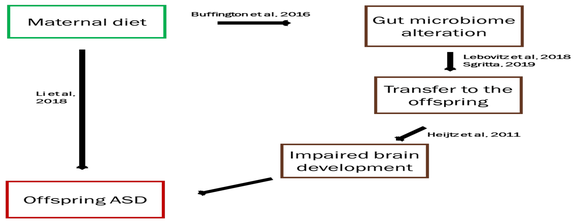


Fig 1.A: Effect of maternal diet on offspring ASD

Similarly, salt, another very common dietary component, has been associated with altered gut microbiota. Furthermore, overconsumption of salt is a matter of concern as the majority of people worldwide consume more salt than what the World Health Organization (WHO) recommends. This excessive salt comes primarily from processed foods. Studies suggest that beside causing heart attack, stroke, and hypertension, elevated dietary salt can significantly alter the gut microbial composition specially by depleting Lactobacillus spp. Our preliminary data shows that offspring from high salt fed parent mice elicits ASD-like behavior. Therefore, we hypothesize that offspring from high salt diet (HSD) parents shows ASD-like behavioral phenotypes due to gut microbiome alteration and the result can be reversed by shearing the microbiome with the offspring from control-fed parental group

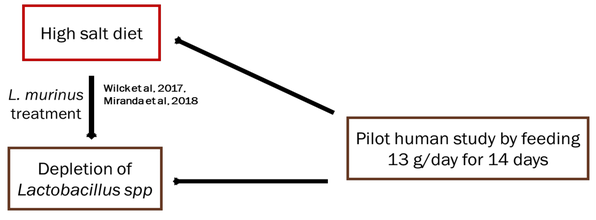


Fig 1.B: Effect of high salt diet on gut microbiome alteration

2. Hypothesis

High salt diet (HSD) consumed by the parents (P) can lead to ASD-like behavioral phenotypes in the offspring (F1) through gut microbiome alteration

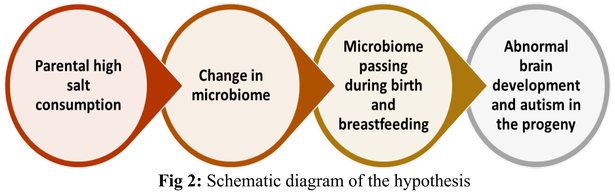


Fig 2: Schematic diagram of the hypothesis

3.1 Research Design

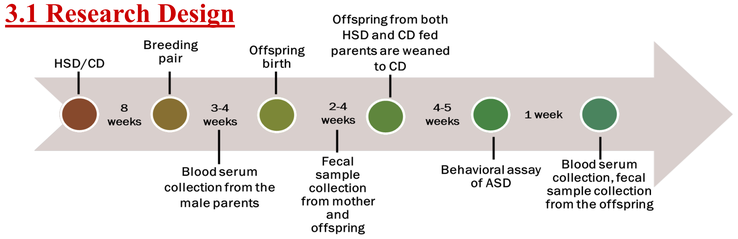
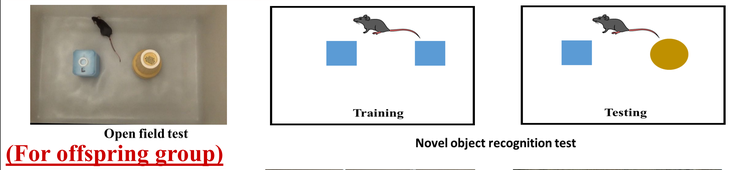


Fig 3.A: Research design

3.2 Behavioral tests (for parental group)



(For offspring group)

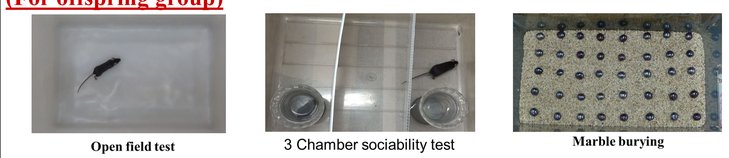


Fig 3.B: Behavioral tests

4. Results

4.1 Parental generation

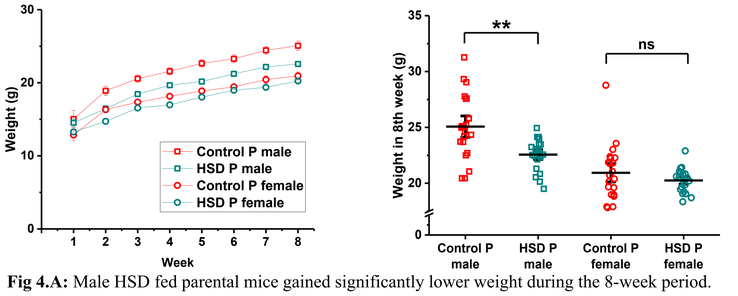


Fig 4.A: Male HSD fed parental mice gained significantly lower weight during the 8-week period. Female weight were similar in HSD and control (n= 20-21 males, 20-21 females).

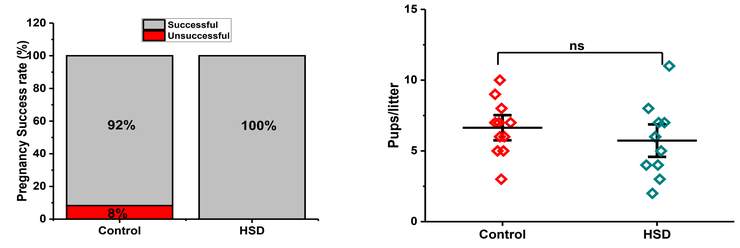


Fig 4.B: HSD parental generation doesn't show any reproductive abnormality compared to the control group (n=10-11 breeding pairs).

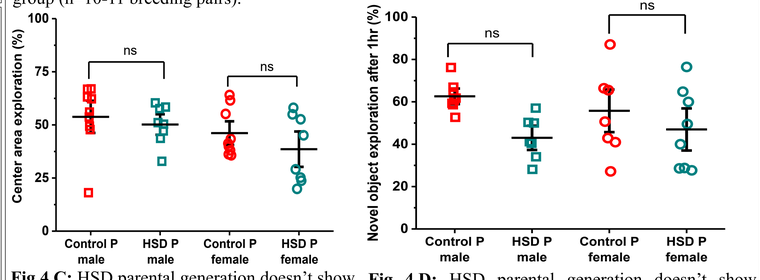


Fig 4.C: HSD parental generation doesn't show anxiety behavior in open field test compared to the control group (n=8-9 males, 8 females).

Fig 4.D: HSD parental generation doesn't show memory impairment novel object test compared to the control group (n=8-9 males, 8 females).

(*) p<0.05, t-test, 1-way ANOVA

4.2 Offspring generation

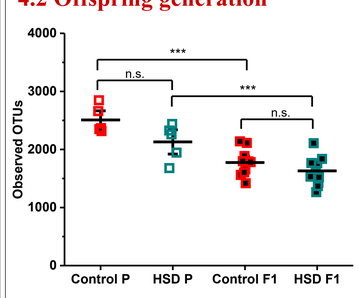


Fig 4.E: Gut microbiome analysis showed no difference in the observed OTU numbers (alpha-diversity) between the parental groups but a significant difference between parental and offspring generation (n=parental group=5, offspring=10).

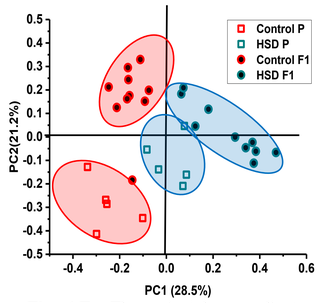


Fig 4.F: The principle coordinate analysis (PCoA) plot shows a significant difference in the beta diversity between the two-diet group and two generation (n=parental group=5, offspring=10).

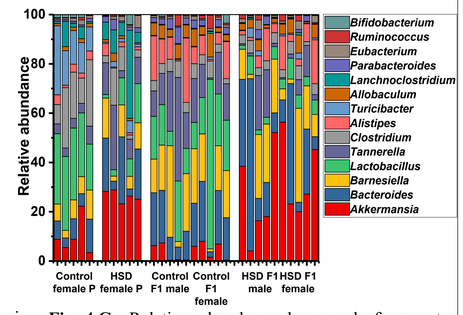


Fig 4.G: Relative abundance bar graph for most abundant bacterial genera in the parental and offspring mice (n=parental group=5, offspring=10).

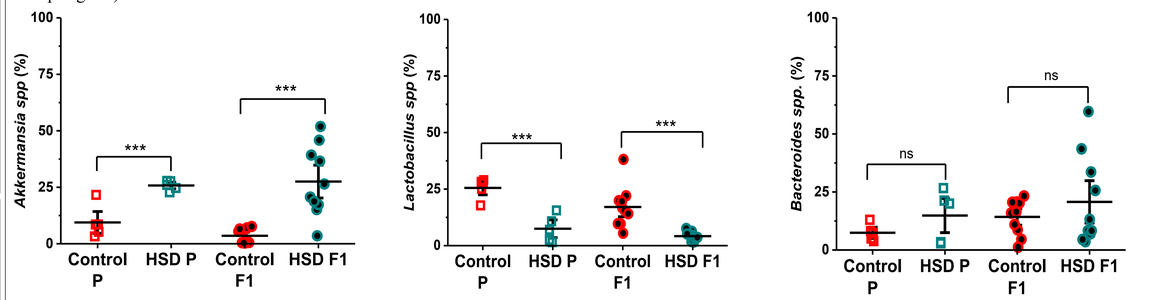


Fig 4.H: Abundance percentage of specific bacterial genus. *Akkermansia* (the most abundant genus) is significantly increased in the HSD fed parents and in their offspring. *Lactobacillus* (2nd most abundant genus) is significantly reduced in the HSD fed parents and in their offspring. *Bacteroides* (3rd most abundant genus) is not changed significantly (n, parental group=5, offspring=10).

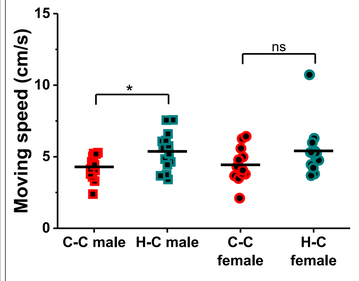


Fig 4.I: Male offspring from HSD fed parental mice showed increased moving speed in the open field test. The females showed no difference (n, male=12, female=11-13)

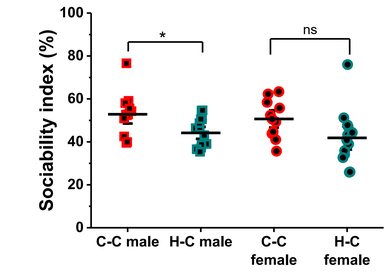


Fig 4.J: Male offspring from HSD fed parental mice showed reduced social interaction in the 3-chamber sociability test. The females showed no difference (n, male=12, female=11-13)

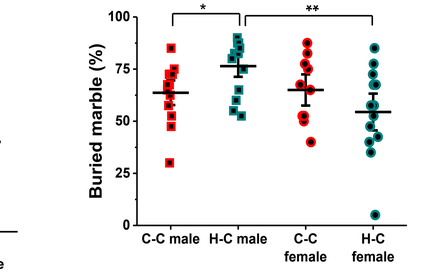


Fig 4.K: Male offspring from HSD fed parental mice showed increased marble burying behavior in the open field test. The females showed no difference (n, male=12, female=11-13)

(*) p<0.05, 1-way ANOVA, ADONIS

5. Conclusion and Future Direction

- No significant physiological or reproductive changes in the parental generation due to the consumption of HSD.
- No significant change in the exploratory behavior and memory tasks after 8 week of HSD consumption in the parents.
- Gut microbiome of the offspring generation from the HSD fed parents is significantly similar to the mother.
- Offspring from HSD fed parents has very low abundance of a vital bacterial genus Lactobacillus spp.
- HSD offspring showed hyperactivity, social deficit and repetitive behavior in the behavioral assay.
- Manipulation for establishing causal effect of parental HSD and offspring ASD

6. Acknowledgement

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7. References

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