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Presented by Elnaz Ghorbani
Ph.D. Student of Medical Bacteriology
Mashhad University Of Medical Sciences

genes

MDPI

Article

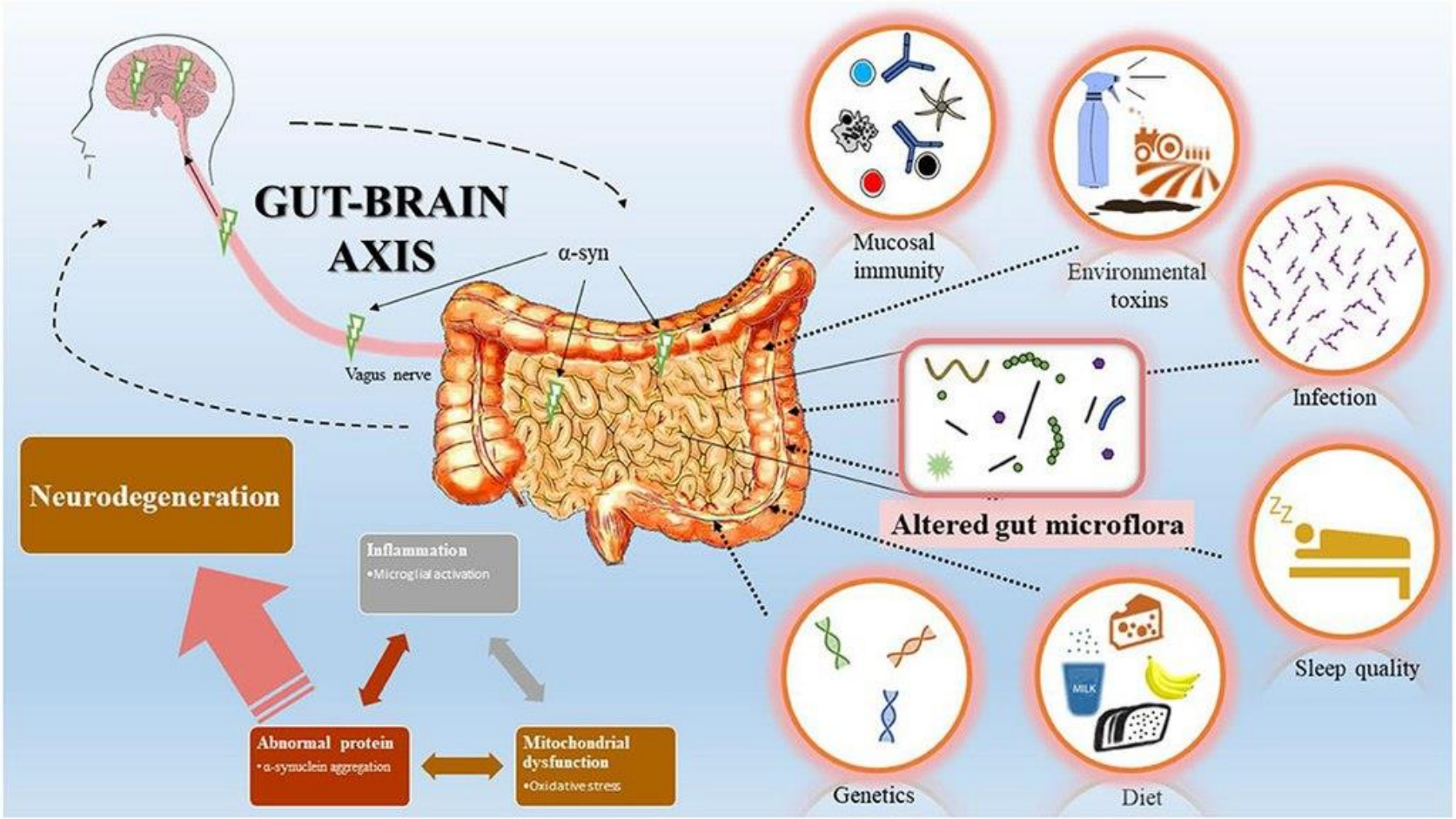
Maternal Microbiota Modulate a Fragile X-like Syndrome in Offspring Mice

Bernard J. Varian [1], Katherine T. Weber [1], Lily J. Kim [1], Tony E. Chavarria [1], Sebastian E. Carrasco [1], Sureshkumar Muthupalani [1], Theofilos Poutahidis [2], Marwa Zafarullah [3], Reem R. Al Olaby [4], Mariana Barboza [3], Kemal Solakyildirim [3], Carlito Lebrilla [3], Flora Tassone [3,5], Fuqing Wu [6,7], Eric J. Alm [6,8] and Susan E. Erdman [1,*]

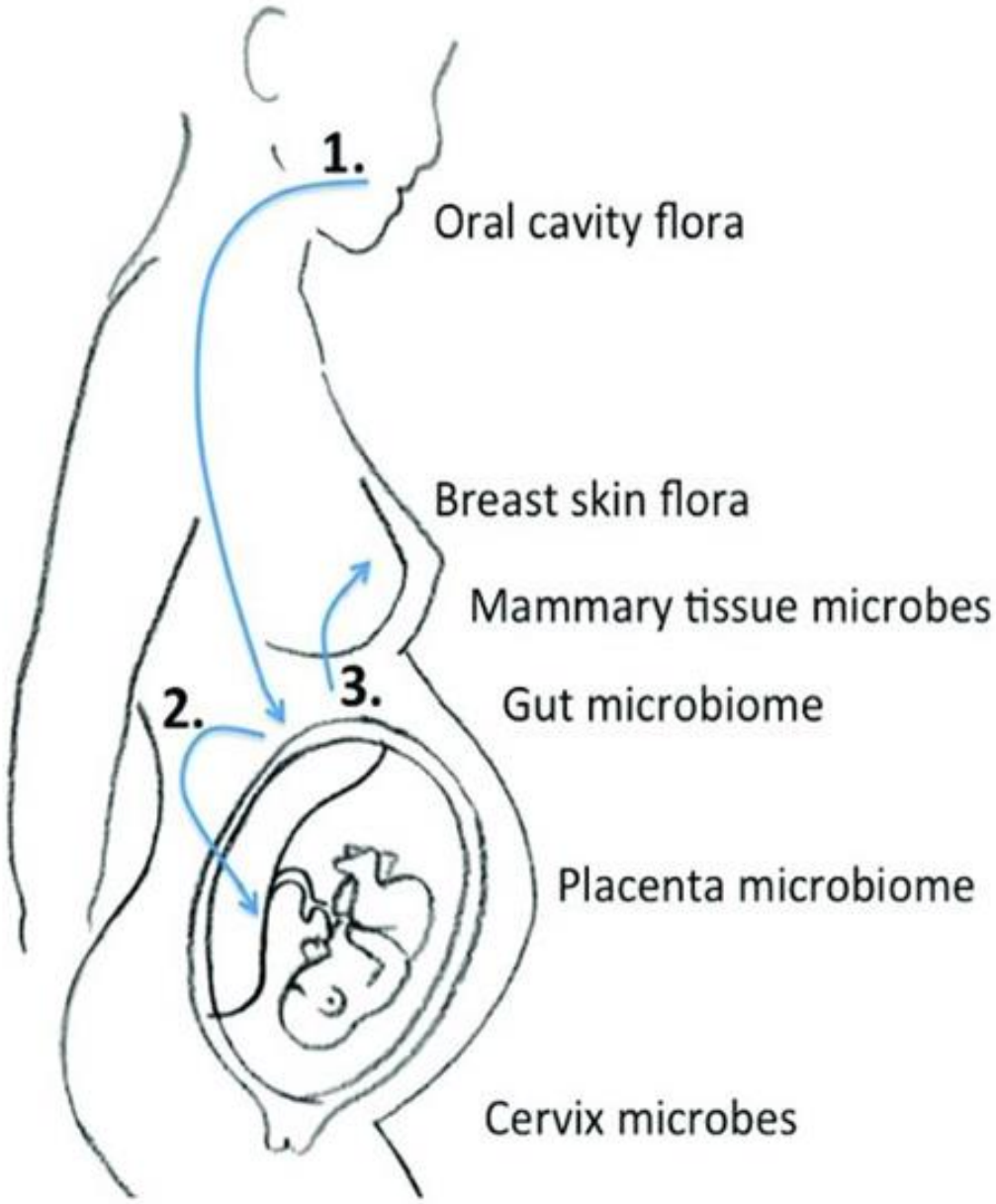
1 Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
2 Laboratory of Pathology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
3 Department of Biochemistry and Molecular Medicine, School of Medicine, University of California Davis, Sacramento, CA 95817, USA
4 College of Health Sciences, California Northstate University, Rancho Cordova, CA 95670, USA
5 MIND Institute, University of California Davis, Sacramento, CA 95817, USA
6 Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
7 Center for Infectious Diseases, School of Public Health, The University of Texas Health Science Center,

Bidirectional signaling exists between the gastrointestinal tract and the brain. The **gut** has been claimed as our **second brain**, thus rise the term

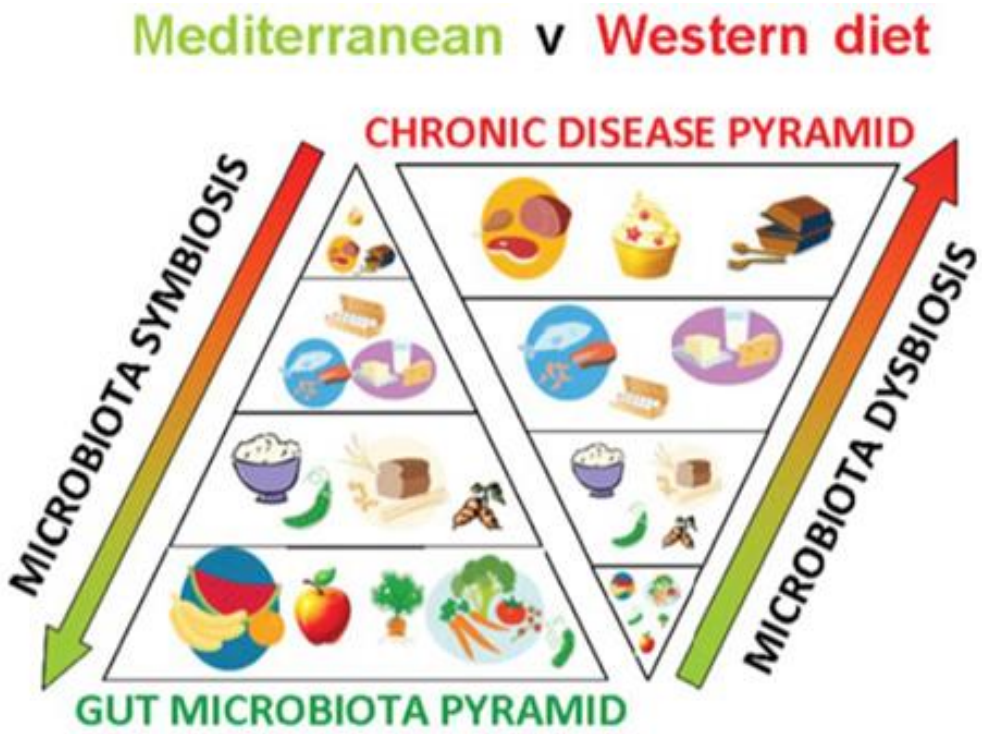
“gut-brain axis”

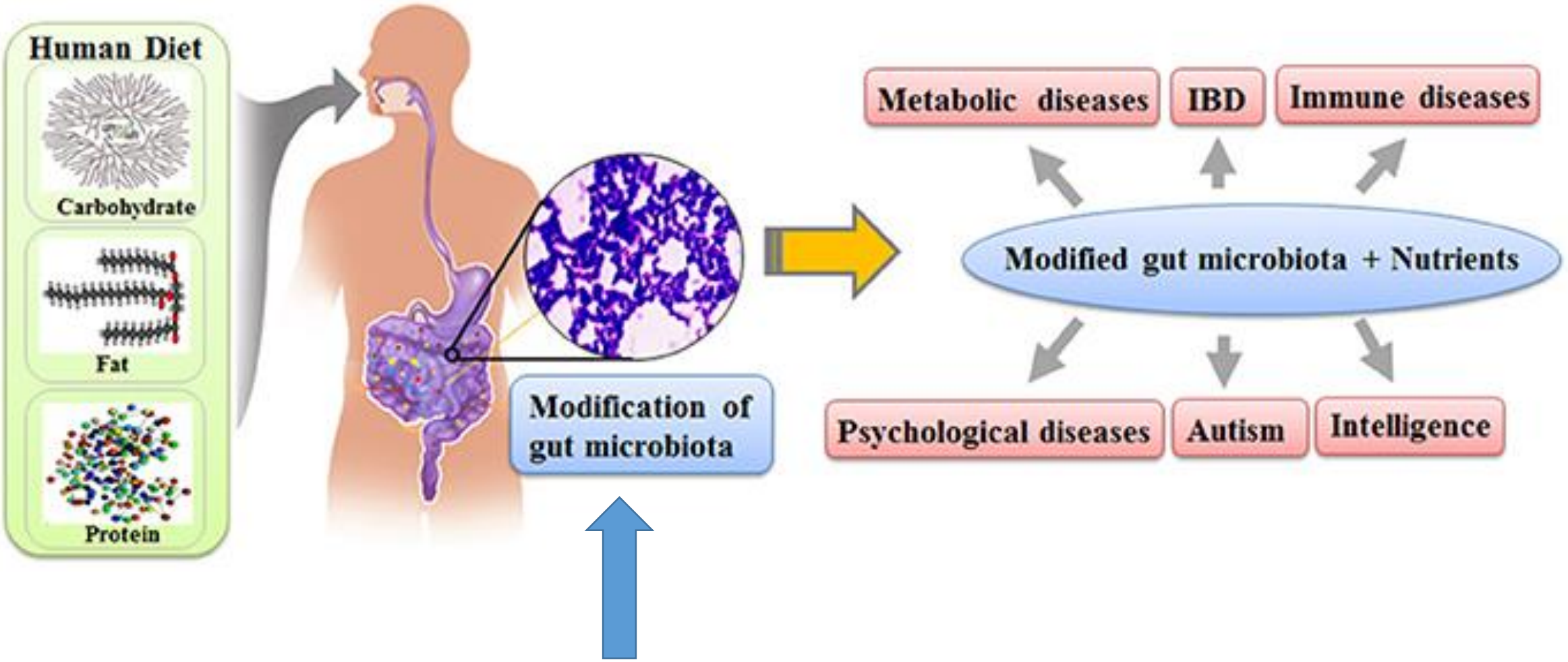


- A person’s gut microbiome is first established in utero and is influenced by their **mother’s own gut microbiome.**



- A mother’s microbiome can enter a state of dysbiosis, defined as an “imbalance” in the gut microbial community, when consuming a high-fat diet, such as a Westernized diet (WD) .





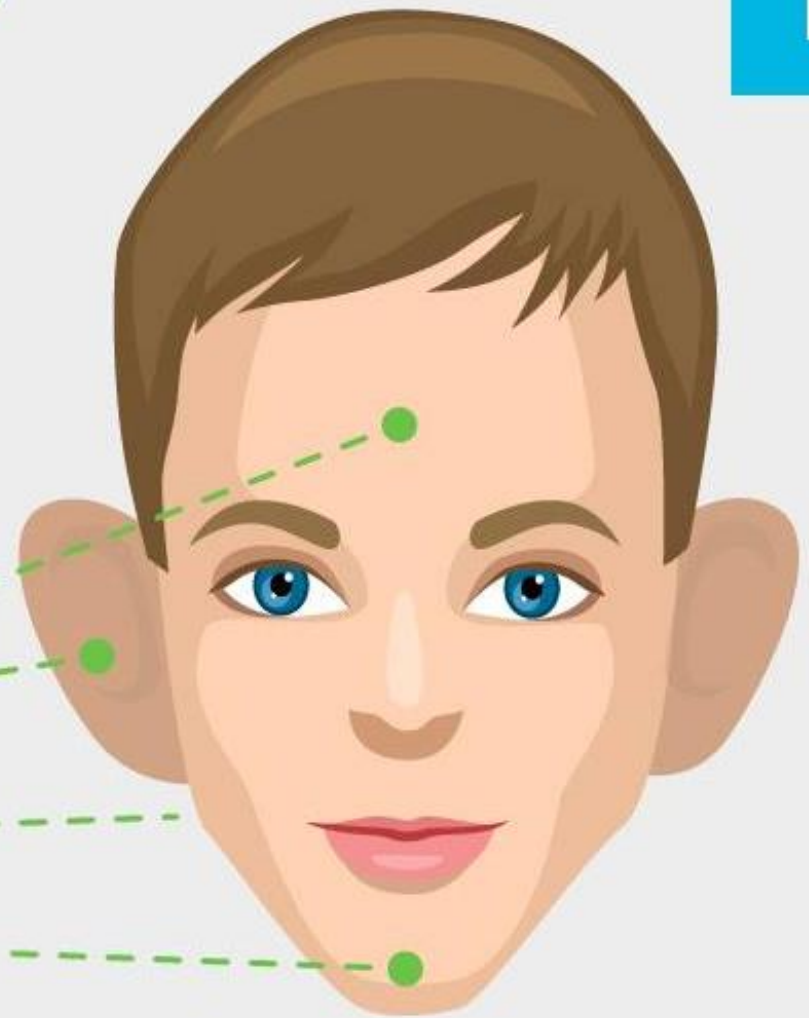
- Supplementation with **probiotics** that modulate host inflammation and hormones has been shown to reverse the adverse effects of maternal gut dysbiosis, and in some cases erase the aberrant phenotype altogether.

RAREDISEASEDAY

Fragile X syndrome

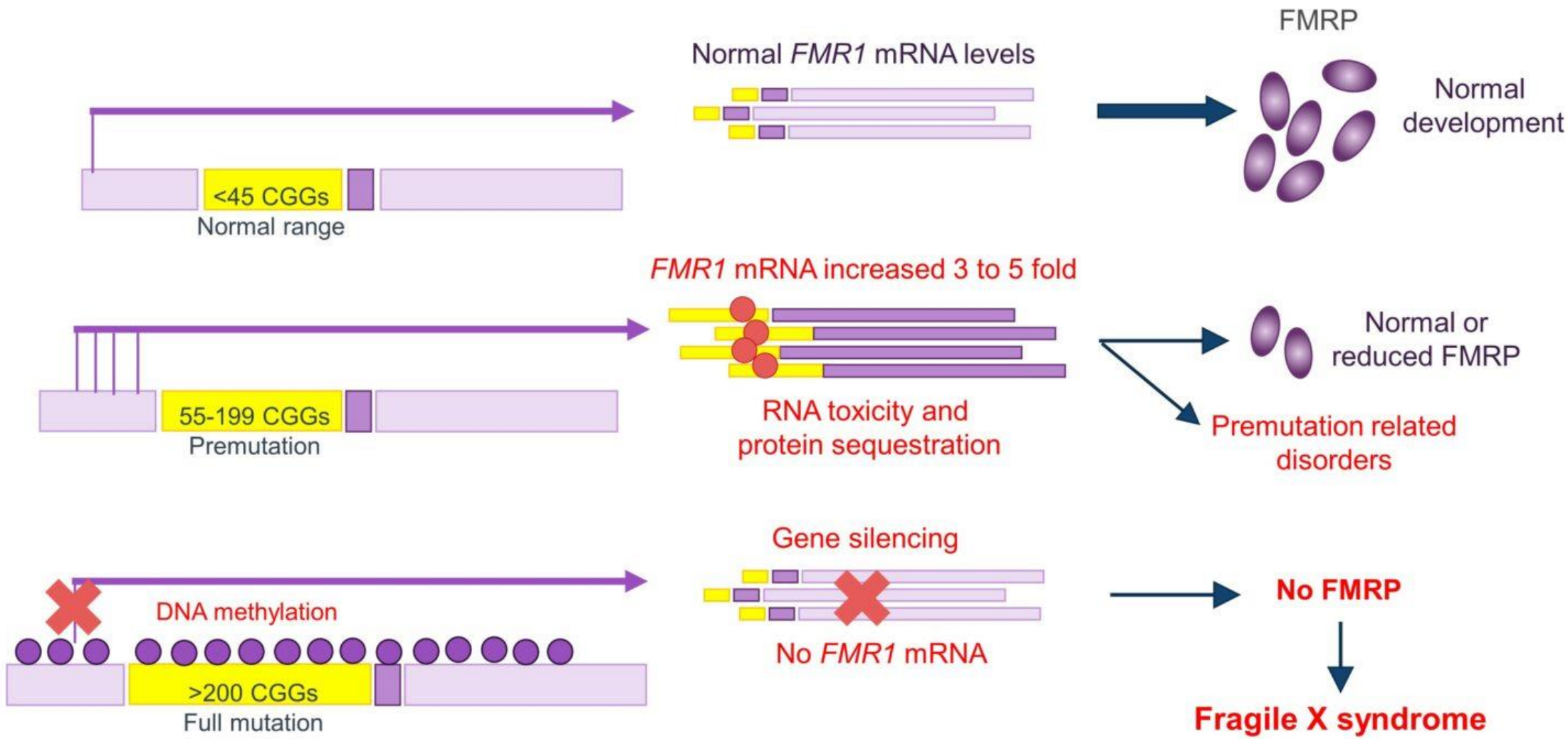
SYMPTOMS

- Autism spectrum disorders
- Intellectual disability
- Abnormal facial features



INCIDENCE

1 in 4,000 males
1 in 8,000 females


<45 CGGs
Normal range
Normal *FMR1* mRNA levels
FMRP
Normal development
55-199 CGGs
Premutation
FMR1 mRNA increased 3 to 5 fold
RNA toxicity and protein sequestration
Normal or reduced FMRP
Premutation related disorders
DNA methylation
>200 CGGs
Full mutation
Gene silencing
No *FMR1* mRNA
No FMRP
Fragile X syndrome

Hypothesis

- Gut dysbiosis and inflammation during pregnancy influenced the prenatal uterine environment, leading to abnormal phenotypes in offspring

Aims

- To explore the relationship between FXS and the gut microbiome and discovery of a link between maternal gut dysbiosis and FXS development

Materials and Methods:

1- Animals and Experimental design

- Outbred conventional CD-1 Swiss stock mice

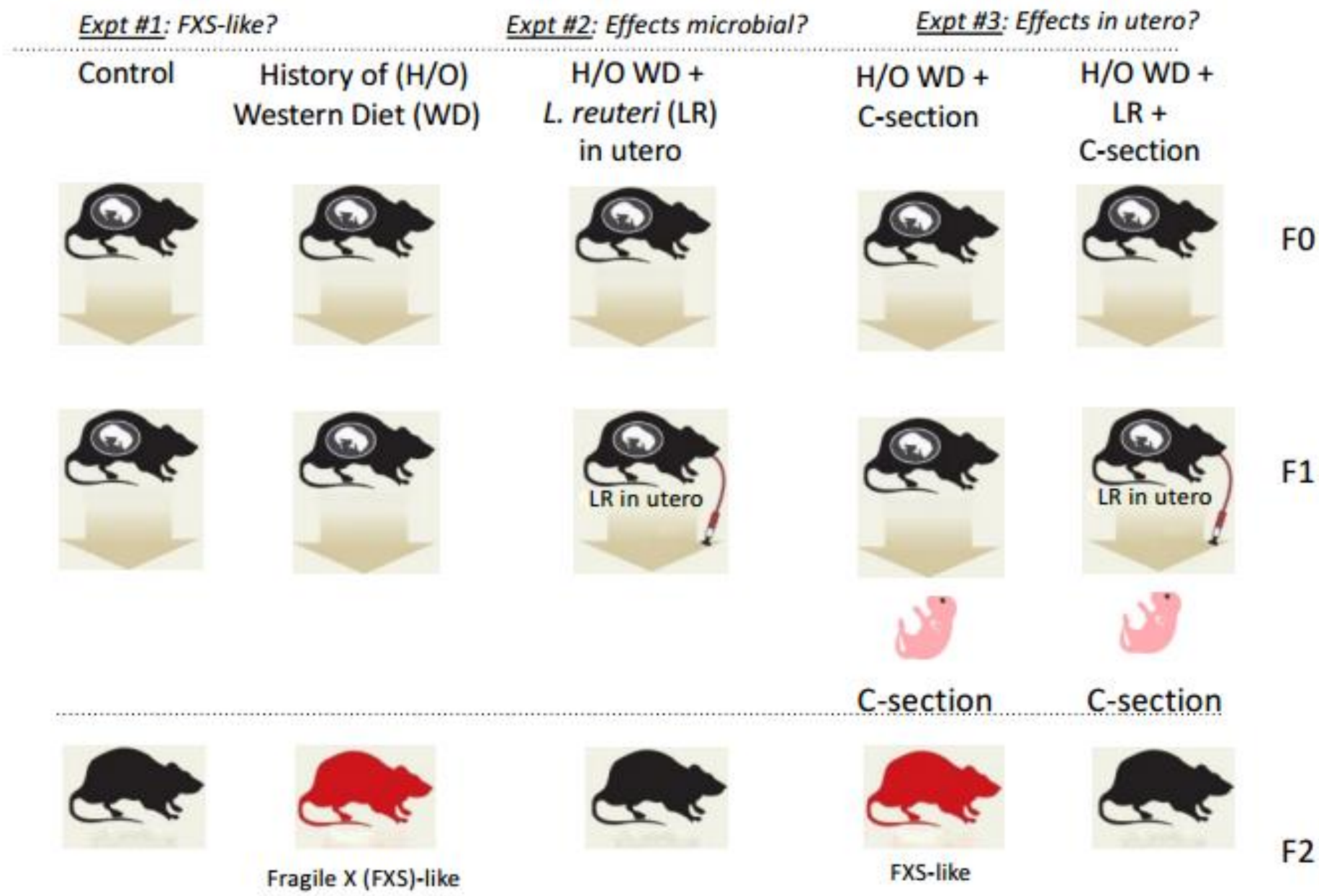
- To test the impact of dietary microbes on progeny, the experimental design was to expose F0 (grandmother) mice to **special diets**, starting with mating at the age of 8 weeks. Special dietary treatment continued until the birth of their pups.

- The present study focused on offspring with a syndrome of **behavioral atypia and features** resembling FXS in humans. Progeny mice were characterized using videotape analyses of behaviors and post-mortem evaluation of tissues, displaying a neurodevelopmental phenotype with Fragile X-like features.

- To test a microbe-driven hypothesis, pregnant mice with a history of (h/o) WD (n = 12) were then randomly subdivided with half receiving a probiotic ***L. reuteri (LR)*** in their drinking water.

- These animals underwent testing of inflammatory cytokines, and their progeny was examined for FXS-like phenotypes and FMRP expression levels.

- Finally, pregnant mice underwent Caesarian (C-section) rederivation to test our hypothesis that in utero microbial events rather than vaginal or post-partum transfer of microbes were leading aberrant behavioral phenotypes in offspring.


Expt #1: FXS-like?
Expt #2: Effects microbial?
Expt #3: Effects in utero?
Control
History of (H/O) Western Diet (WD)
H/O WD + L. reuteri (LR) in utero
H/O WD + C-section
H/O WD + LR + C-section
LR in utero
LR in utero
C-section
C-section
F0
F1
F2
Fragile X (FXS)-like
FXS-like

2-Microbial Treatments

- Live organisms were supplied at a starting dosage of **3.5×10^5** organisms/mouse/day in drinking water. Control mice received regular drinking water.

3-Phenotyping Mice

- Dysmorphia of head and ears are characteristics of FXS in humans. To examine these characteristic features in our mice, ear pinnae morphology was examined by measuring the height and width of each ear

- Behavioral scores : number of times the cage midline was crossed for hyperactivity, and the number of times the rostrum crossed a horizontal plane for head bobbing stereotypy

4- Caesarian Rederivation of Offspring

Pregnant female CD-1 mice (n = 12) who experienced the various treatments underwent terminal sterile C-section rederivation procedures of e20 offspring to test post-partum effects of microbiota.

5- Histopathology

Brain structural, or inflammatory abnormalities, necrotizing polyarteritis of blood vessels, intranuclear inclusions in neurons and astrocytes, and Purkinje cells

6- FMRP Expression

A clear marker of FXS in humans and animal models is the decrease in Fmr1 protein (FMRP) production following a trinucleotide CGG expansion and hypermethylation of the Fmr1 gene.

7- Inflammatory Cytokines

Circulating Levels of Pro-Inflammatory Cytokine Interleukin -17A

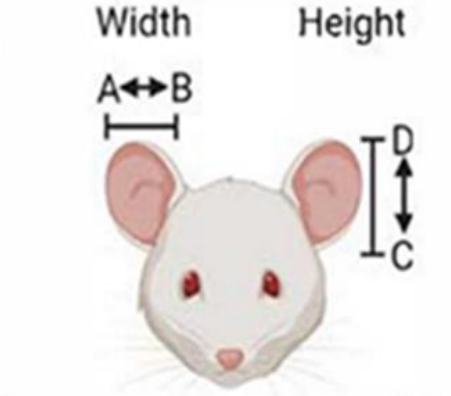
Results

1- Offspring Mice Exhibit a Fragile X Syndrome (FXS)-like Phenotype including Elongated Head, Enlarged Ears, Head Bobbing, and Hyperactivity

2- Utero L. reuteri Supplementation in Drinking Water Counteracts FXS-like Phenotype in Offspring Mice

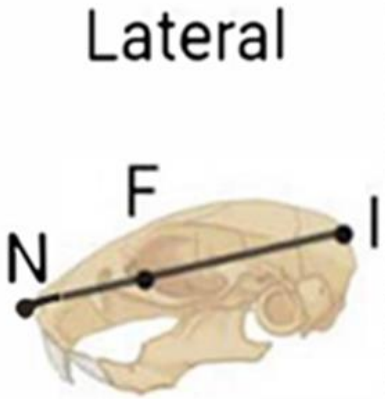
3- Fragile X-like Phenotype in Mice Is Retained after Caesarian-Section Rederivation

A

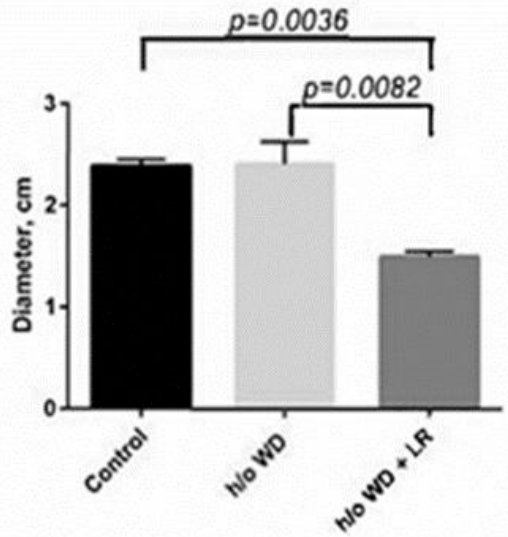


Diameter = SqRt (AB x CD)

D

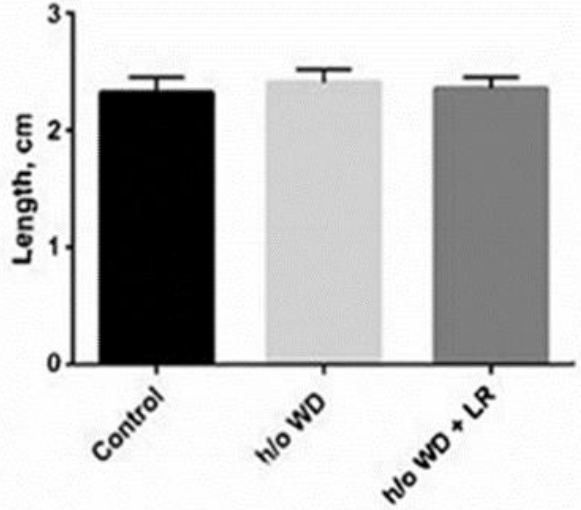


B



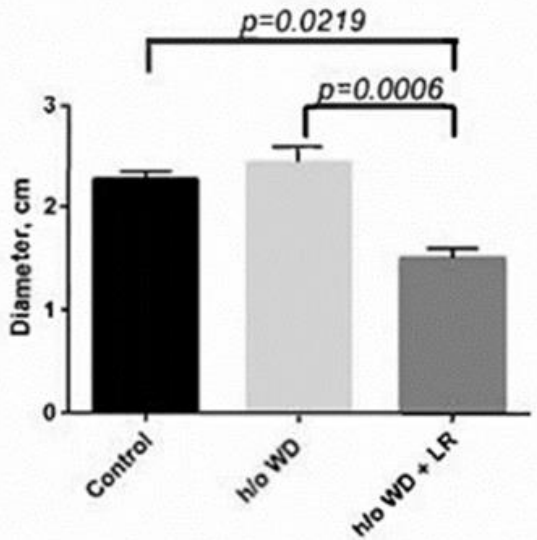
Diameter of left ear

E



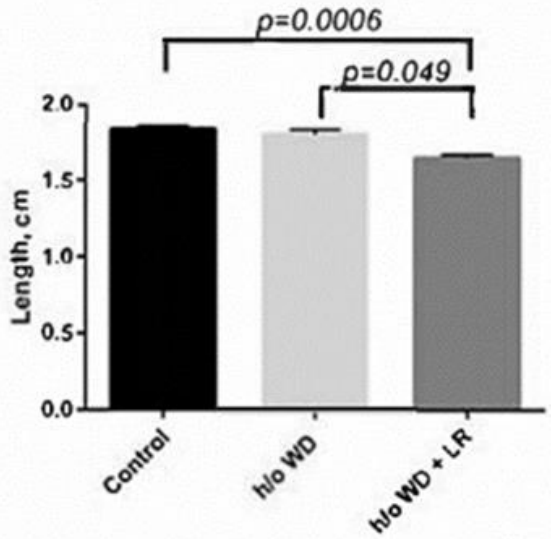
Length of interparietal bone to nasal bone in lateral vie

C



Diameter of right ear

F



Length of interparietal bone to facial bone in lateral view

Results

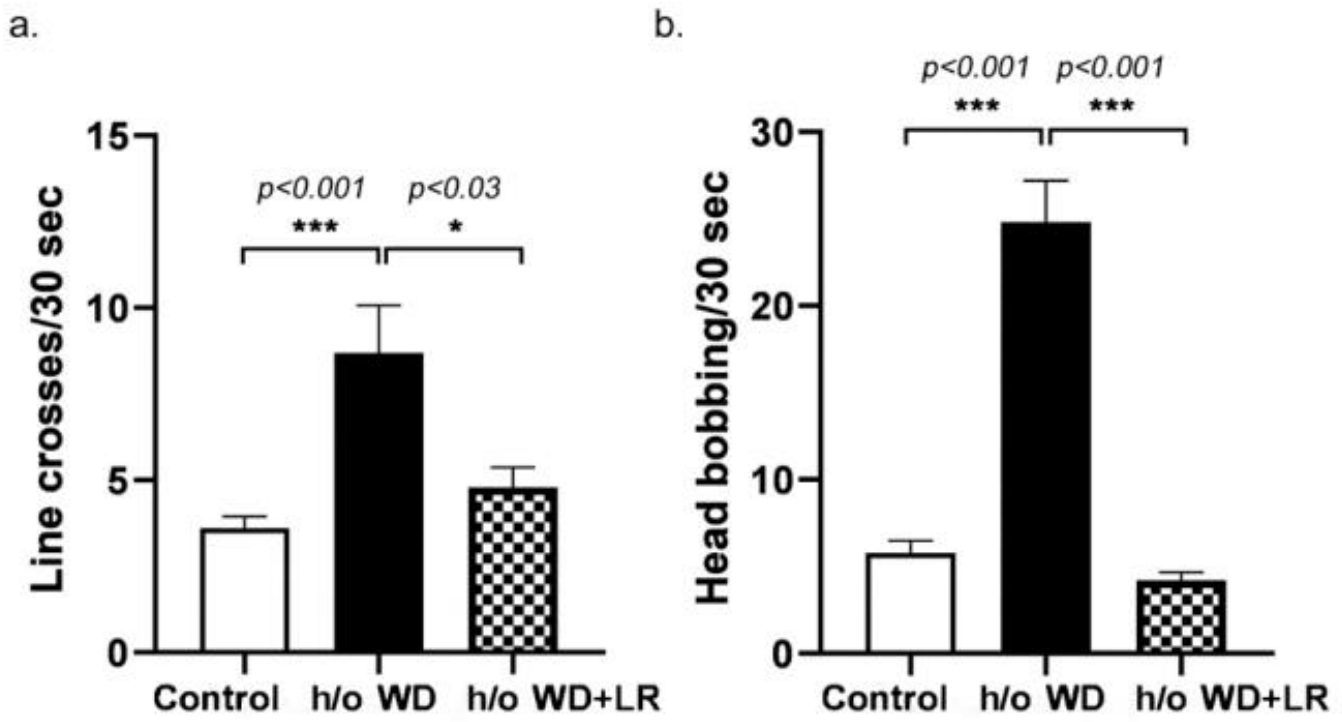


Figure 3. Hyperactivity (**a**) and **stereotypic head bobbing** (**b**) in mice with FXS-like phenotype. We examined video footage of sham control animals (n = 6), animals with a history of (h/o) WD (n = 6), and animals with h/o WD + LR (n = 6). The animals were measured at 30 s intervals in home cages under standardized conditions. Significant differences were found between treatment groups (* $p < 0.05$ and *** $p < 0.001$).

Results

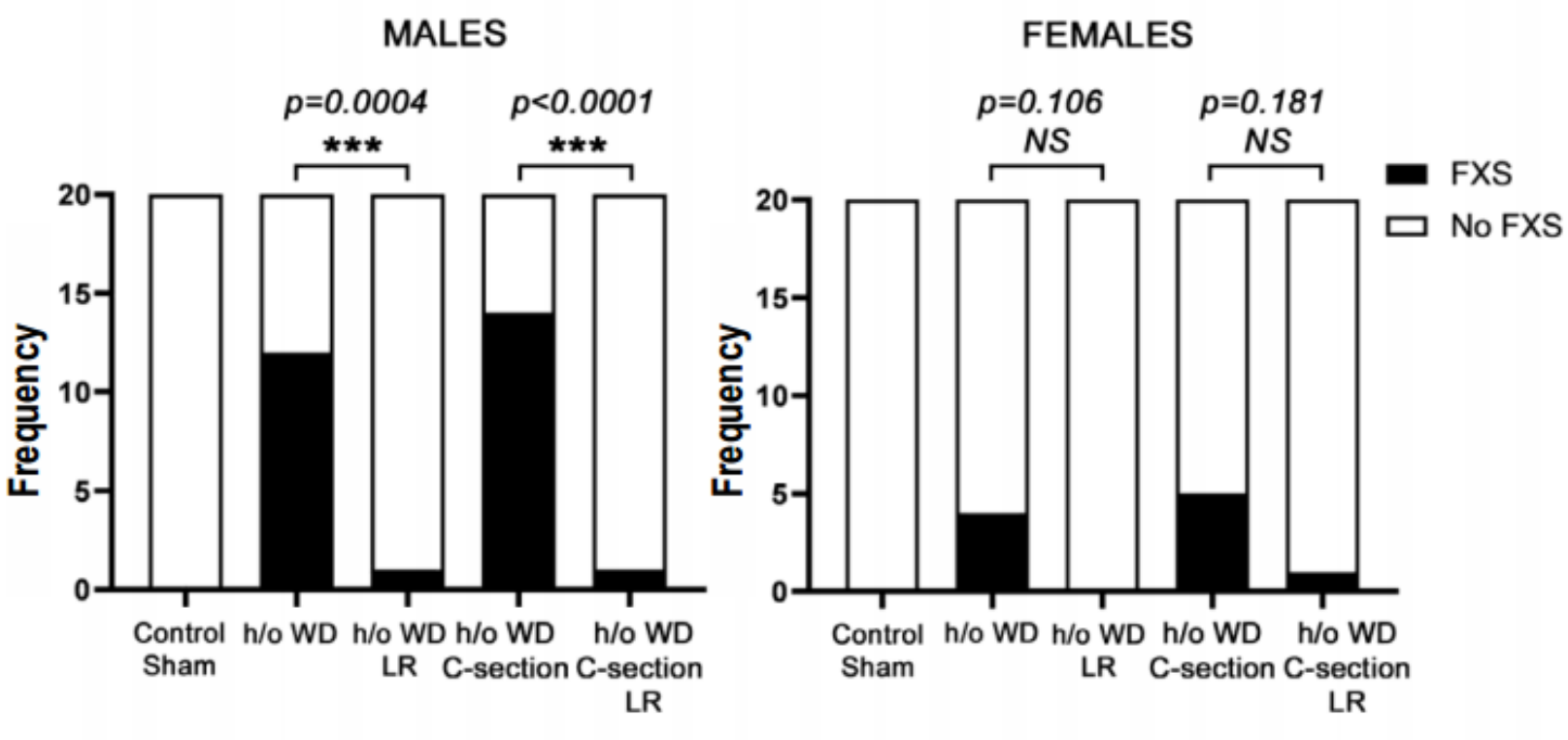


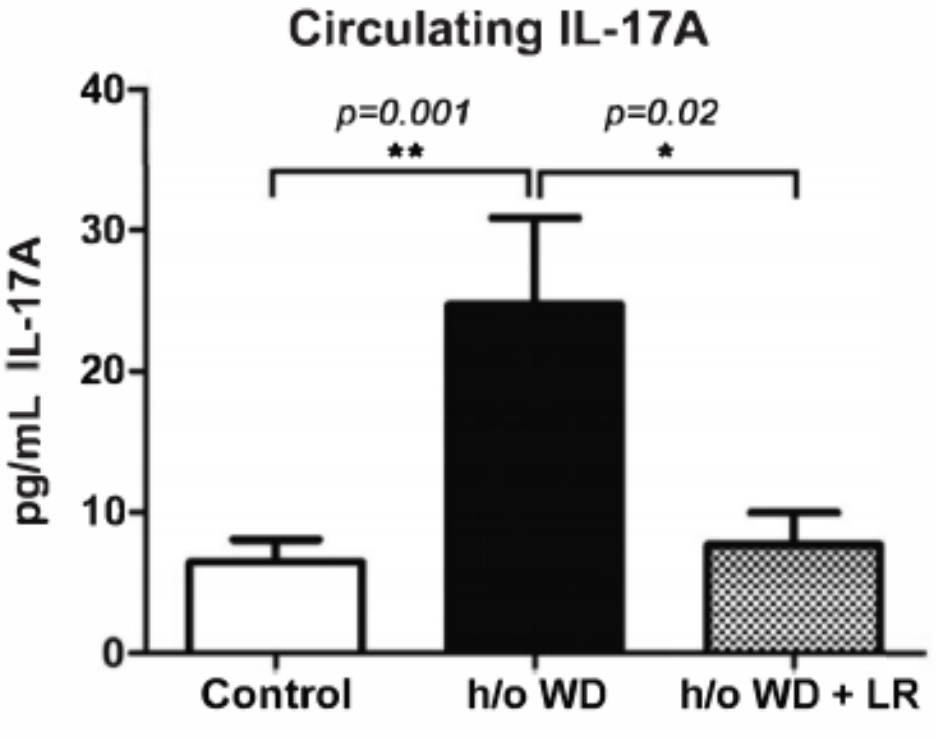
Figure 4. In utero **probiotic *L. reuteri*** effects on FXS-like phenotypes in progeny mice. To test our microbe-driven hypothesis, pregnant mothers with a history of (h/o) WD were randomly subdivided with half receiving probiotic *L. reuteri* in their drinking water ($n = 6$) and half receiving regular drinking water ($n = 6$).

The frequency of FXS-like features was measured in each treatment group. Significant differences (*** $p < 0.001$) were found after in utero dosing with *L. reuteri*, and the benefits of in utero *L. reuteri* were preserved after C-section rederivation.

Results

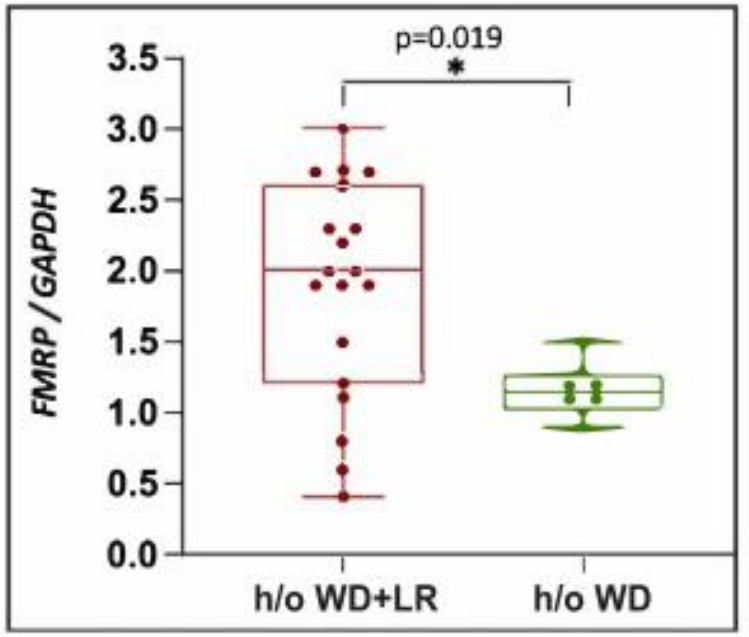
4- Pregnant Mice Supplemented with L. reuteri Exhibit Lower Circulating Levels of Pro-Inflammatory Cytokine Interleukin -17A

Figure 5. To test systemic levels of inflammatory cytokines under different dietary and microbial conditions, we used whole blood from control mothers (*n* = 8), the mothers with a history of (h/o) WD (*n* = 8), and mothers with h/o WD + LR (*n* = 8).To test systemic levels of Il-17A in offspring mice, we used whole blood collected by terminal cardiac puncture and diluted 1:1. Circulating Il-17A levels were determined using ELISA . We found significant differences among treatment groups (* $p < 0.05$ and ** $p < 0.001$).

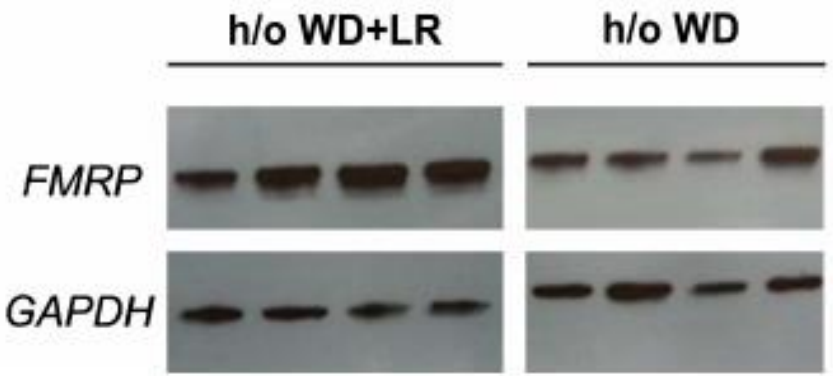


Results

5- In Utero Microbial Rescue with L. reuteri Blunts Methylation of FMRP



- **Figure 6.** Brain expression levels of FMRP in offspring mice. FMRP expression level was measured using Western Blot analysis in male mice with a history of (h/o) WD + LR (*n* = 19) and male mice with h/o WD only (*n* = 8). Significant differences between the groups (* $p < 0.05$) were observed.



Discussion

- Previous studies revealed multigenerational effects of mothers being fed a Westernized diet (WD), which seemingly impacted the gut microbiota and systemic immunity. **A dysregulated microbiome** after a WD has been shown in animal models to lead to neurodevelopmental conditions.

- An association between the **reduction in FMRP** expression and the degree of the severity of neurologic phenotypes, including autism spectrum disorder, schizophrenia, bipolar disorder, and major depressive disorder, has been reported in several studies

- The **reduction in FMRP** likely contributes to the symptoms seen in FXS patients, specifically because FMRP is an **RNA-binding protein** that acts as a **translational regulator of neuronal mRNAs** of many messages that affect synaptic plasticity, connectivity, and memory in the central nervous system.

- The absence of FMRP leads to increased glutamate signaling and downregulation of GABAA pathways, causing an imbalance of excitatory and inhibitory neurotransmitters in the brain that likely contributes to the hyperactivity typically seen in FXS.

- In summary, They present evidence that **in utero effects** of a ‘stress microbiome’ leads to a neurological phenotype in offspring animals, similar to those observed in FXS.

- **Targeted microbe strategy** using probiotic L. reuteri counteracted the development of FXS-like symptoms when introduced during pregnancy, a protective effect that was sustained after C-section rederivation.

- We conclude that administration of L. reuteri during pregnancy with a WD effectively **neutralized the symptoms** of the neurological syndrome after the birth of infants, raising the possibility of similar therapeutic strategies in humans.

Future prospects and recommendations

- Probe possible interactions between probiotics and candidate microbial pathogens during dysbiosis conditions in utero.

- Investigate Specific gut microbiota that triggered pathogenic and potential epigenetic changes

- Examining any potential interaction between the L. reuteri intake and changes in the methylomic profiles correlated with the observed phenotypes.


THANK YOU
FOR YOUR KIND ATTENTION