

# *Development of Synbiotics for Repairing the Microbiota of Children with Acute Malnutrition and Restoring Healthy Growth*

**Jeffrey I. Gordon**

**2021 Balzan Prize for Microbiome in Health and Disease**

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**Jeffrey Gordon** is Dr. Robert J. Glaser Distinguished University Professor and Director of the Edison Family Center for Genome Sciences and Systems Biology at the Washington University School of Medicine in St. Louis, Missouri. He will be Principal Investigator of this three-year project investigating the role of the microbiota in children's health. Michael Barratt, Associate Professor of Pathology and Immunology and Director of the Washington University School of Medicine's Center for Gut Microbiome and Nutrition Research has been named Deputy Supervisor, and Tahmeed Ahmed, Senior Director of the Nutrition and Clinical Services Division and Executive Director of the International Centre for Diarrhoeal Disease Research in Bangladesh (icddr,b) will be Co-Principal Investigator. Administrative responsibility will rest with the Washington University School of Medicine. For nearly ten years, the Washington University-icddr,b collaboration has leveraged the deep clinical and nutritional sciences expertise and long-standing community engagement of Dr. Ahmed and his colleagues at icddr,b, and the experimental and computational approaches (including gnotobiotic animal models) for characterizing the dynamic operations and host effects of the human gut microbiota/microbiome developed in the Gordon lab at Washington University in St. Louis.

## **Background and rationale**

The magnitude of the global health challenge of childhood undernutrition is staggering. In 2020, an estimated 149 million children under five years of age were stunted (reduced length-for-age Z score, or LAZ) while 45 million exhibited wasting (reduced weight-for-length Z score, or WLZ) [1]. Undernutrition and its long-term sequelae (including persistent impairments in linear growth, neurodevelopment, and immune and metabolic functions, all of which have proven to be largely resistant to current therapies) are the leading causes of morbidity and mortality in children five years or younger [2].

Food insecurity is not the sole driver of undernutrition. [3] Gordon's group has provided evidence that disrupted gut microbial community development is a contributing factor to

pathogenesis [4][5][6][7][8][9]. Children with moderate or severe acute malnutrition have impaired ponderal growth (wasting) and microbiota configurations that resemble those of chronologically younger children.

Colonization of gnotobiotic mice with fecal microbiota samples collected from chronologically age-matched healthy children and those with acute malnutrition disclosed that microbial communities from the latter transmitted impaired weight gain phenotypes, altered bone growth, plus immune and metabolic abnormalities [4][6][10]. These results provided preclinical evidence of a causal role for the microbiota in disease pathogenesis.

Gnotobiotic mouse and piglet models were subsequently used to design microbiota-directed complementary food (MDCF) formulations for repairing the microbial communities of children with moderate acute malnutrition (MAM) [8]. In a three-month randomized controlled feeding study of 12-18-month-old Bangladeshi children with MAM, the Washington University-icddr,b team demonstrated clinical proof-of-concept that a lead MDCF formulation (MDCF-2) produced a significant improvement in their rate of weight gain ( $\square$ -WLZ) compared to a control ready-to-use supplementary food (RUSF) that was not designed to alter the gut microbiota. Follow-up mechanistic analyses included quantifying the levels of 5,000 plasma protein mediators and biomarkers of multiple physiologic processes just prior to, one month after initiating, and at the end of the three-month therapeutic intervention [9].

Together, these results provide evidence that the gut microbiota is causally linked to ponderal growth but raise a number of questions. (i) What are the genomic features of bacterial taxa that are positively and negatively associated with WLZ in a significant way, and notably those whose abundances are significantly more affected by MDCF-2 compared to RUSF? (ii) What genes are differentially expressed in response to MDCF-2 in bacterial strains that are positively correlated with WLZ and how does their differential expression relate to the processing of components of MDCF-2? (iii) How are changes in the abundances of these transcriptionally responsive strains related to changes in plasma proteomic biomarkers and mediators of various facets of host ‘systems physiology’? Gaining this information should help enable development of bioequivalent or more efficacious MDCF formulations composed of food ingredients that are culturally acceptable, available, and affordable for different human populations. This information would also serve as a foundation for identifying synbiotic formulations (combinations of pre- and probiotics) for more precise and effective repair of gut microbial communities in these children.

With these findings, questions, and goals in mind, Gordon’s lab aims to characterize treatment-associated responses of key growth-promoting bacterial strains, and how these responsive strains relate to host responses. One notable finding that emerged from their work was the apparent link between certain strains of *Prevotella copri* with distinctive expressed genomic features and host responses, and further research indicated that *P. copri* plays a key role in mediating the effects of MDCF-2.

Thus, the rationale for the current proposal is to develop a next generation of therapeutics targeting *P. copri* for repairing the microbial communities of children with acute malnutrition. These therapeutics are envisioned to consist of advanced MDCF-2 formulations or a synbiotic comprised of *P. copri* and a prebiotic representing a glycan

(or glycans) that increases its fitness and expressed beneficial functions in ways that promote healthy growth.

### **Specific aims of the Balzan Research Project**

This project leverages the long-standing collaboration between Gordon's group at Washington University in St. Louis and their collaborators at the International Centre for Diarrhoeal Disease, Bangladesh (icddr,b). It has four specific aims:

**Aim 1** – Compare the genome sequences of *P. copri* strains that the group cultures from the fecal microbiota of healthy and undernourished Bangladeshi children, and their mothers, living in several demographically distinct areas of Bangladesh.

**Aim 2** – Conduct *in vitro* tests of the capacity of the different cultured *P. copri* strains to utilize different glycan components contained in MDCF-2 ingredients.

**Aim 3** – Advance lead glycans and lead *P. copri* strains from Aim 2 to preclinical studies involving gnotobiotic mouse models of mother-to-child microbial community transmission.

**Aim 4** – Support the creation of a 'Clinical Microbiome Laboratory Medicine Program' at icddr,b through training programs, reciprocal secondments between the two institutions, and regularly scheduled Zoom meetings.

This last aim is particularly significant for the purpose of the second half of the Balzan Prize as stipulated in 2001 in the Articles of the Balzan Foundation, that prizewinners must destine that amount to finance research projects that are carried out by young scientists and researchers.

The project will further the career development of junior icddr,b scientists through secondments at Washington University where they will learn the computational and experimental approaches that underlie their translational medicine pipeline. Based on these experiences, they will be able to implement, at icddr,b, (i) anaerobic microbiological capabilities for culturing members of the human gut microbiota (e.g., additional *P. copri* strains from healthy and undernourished Bangladeshi children and women), (ii) a pipeline for sequencing and annotating the genomes of these isolates (annotation will include *in silico* reconstructions of the metabolic capabilities of these isolated strains) and (iii) methods for characterizing *in vitro* the growth properties of these isolates in different defined nutrient conditions.

Trainees will be junior scientists from icddr,b whose career goals are to eventually play a leadership role in building icddr,b's capacity in clinical microbiome science, and who would thus become a major scientific interface between icddr,b and Washington University, and over time, with other labs around the world. Training in relevant areas of experimental and computational biology during the secondments will be provided by senior staff in the Gordon lab plus others in the Edison Center for Genome Science and Systems Biology. Designated members of the Gordon lab will actively engage in mentoring these junior scientists about developing careers at the interface between microbiome science and human nutrition. This career development program will also

include establishing an online course menu for the junior scientists and icddr,b colleagues so that they can gain expertise in areas relevant to the type of data generation and analyses that this project specifically, and human microbiome science more generally demands.

Reciprocal secondments of Washington University junior scientists will allow them to be exposed to the unique capabilities related to clinical trial design and execution represented within icddr,b, obtain deeper knowledge of issues related to maternal and child health, and become familiar with the societal, educational, and regulatory issues/challenges encountered when developing/deploying new microbiota-directed therapeutics.

During the course of the proposed project, a symposium will be jointly organized by Washington University and icddr,b on the topic *Development of Microbiome-Targeted Therapeutics for Treating Childhood Undernutrition; Scientific, Regulatory, and Policy Considerations*.

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