FORM
Foundations of a Resilient Microbiome

The human gut microbiome functions as a vital organ, playing a central role in physiology and health. Increasingly, we understand how central its role is in nutrition and metabolism[i], endocrine regulation, immune system development[ii], as well as its links to neurological, cognitive, and behavioral outcomes[iii]. There are now well-known biochemical pathways, precursors to neurotransmitters, and immune system signals linking the gut microbiome to development and function in every major physiological system of the human body.

In FORM, we are focused on the role of the maternal and developing infant gut microbiome in healthy infant neurodevelopment. Critically, FORM seeks to identify whether an accumulating set of early-life pressures to the developing gut microbiome is one contributing factor to the rise in one form of neurodevelopmental challenge — namely, autism spectrum disorder (ASD). And further, whether resilience to functional disruption can be measured and supported in key developmental windows as a pathway towards reducing the number of children who could go on to experience severe autism-related difficulties.

Forming a Resilient Microbiome. From birth through to age two, the infant gut microbiome is rapidly forming and reshaping its community and core functions. In this period, everyday environmental exposures and common medical practices impact the formation and function of the infant gut microbiome at different times- some building resilience, others creating stress. For example, birth mode (vaginal or caesarean birth, including whether antibiotics are used) and breastfeeding impact microbiome formation most in the first 6 months; maternal microbiota, solid foods, antibiotics, and family and caregiving environments matter most from 6 months old through to age 2; and maternal factors, such as infection and antibiotic exposure, diet, body mass index, and gestational diabetes impact the maternal microbiome and early infant microbiome throughout the whole period[iv]. Importantly, the maternal microbiome itself shifts markedly in the third trimester[v], affecting microbial transmission at birth and in early life — underscoring the need to see pregnancy and infancy as a connected system for healthy infant microbiome formation. By the end of this multifaceted, formative period, the infant gut microbiome starts to stabilize and approach adult-like maturity.

By age 2, the infant gut microbiome has provided signals critical to immune, metabolic and neurodevelopment. The developmental trajectory and resilience of the infant gut microbiome from birth to age 2 has been shown to predict key aspects of a child's later immune and metabolic health[iv vi]. These developmental trajectories are determined by a combination of environmental exposures and early life challenges acting together that can shape or disrupt function of the microbiome[i]. Increasing evidence also suggests that the formation and resilience of both the maternal and infant gut microbiome are key drivers of a child's brain and cognitive development during this window. Notable work in animal models has demonstrated that:

- Maternal high fat diet during pregnancy and lactation alters the offspring's microbiome, leading to changes in key neural connections in the brain and social behavior in male pups[vii];
- Maternal immune activation and maternal antibiotic exposure during pregnancy can disrupt the maternal microbiome and circulating metabolites, driving differences in fetal brain development and some autism-like behaviors in animal offspring[viii ix];

- Different gut microbiomes from infants in the first year of life can drive functional differences in social, cognitive, and executive-function-related behaviors when these human microbiomes are transferred to germ free mice, with effects mediated by a microbiome-driven metabolic disorder (Unpublished data from the Wellcome Leap First 1000 days program).

Across all of these studies, early targeted intervention with key microbial strains or metabolites indicated that it is possible to restore metabolic balance and minimize severe behavioural challenges in animal models (such as atypical social interactions; repetitive behaviors; anxiety like phenotypes and vocalisations). This suggests the potential for interventions to reduce the severity of neurodevelopmental challenges during critical developmental windows, prior to age 2.

Taken together, these findings indicate that the infant gut microbiome functions much like a vital developing organ, shaping early brain and cognitive development while also guiding immune and metabolic health.

The developing microbiome is under more pressure today than ever before. More pregnancies and early childhoods are shaped by early life exposures known to disrupt the maternal and early infant gut microbiome. In the past twenty-five years, maternal obesity, caesarean births, and antibiotic use in pregnancy have risen by up to 50%[x] [xi] [xii]. Today, over 25% of women in the U.S. enter pregnancy with obesity, 1 in 3 women have a caesarean birth, and almost 40% of women are exposed to antibiotics during pregnancy (with antibiotics accounting for nearly 80% of all medications given in pregnancy)[xiii]. These early-life challenges can also layer and stack up. For example, women that are obese during pregnancy are two to three times more likely to require a caesarean birth[xiv], and caesarean birth always require preventative antibiotics. After birth, despite changes in antibiotic stewardship, antibiotic exposure remains the highest in early childhood, where on average nearly every child under 5 receives at least 1 course of antibiotics each year in the U.S – in some countries, the average is as high as 5 courses[xv]. Some interventions are medically necessary: caesareans save lives, and antibiotics are essential for serious infections. Other interventions and choices may be modifiable. Indeed, studies suggest that more than a quarter of antibiotic prescriptions in children may be unnecessary: given for the wrong infection; not needed at all; or started too long after surgery[xvi].

This suggests several emerging questions:

What if increases in early life pressures are stacking up to disrupt both the maternal and infant gut microbiomes?

What if the result is that the maternal and infant gut microbiome is not able to perform functions critical for healthy neurodevelopment in pregnancy and early infancy?

Could the gut microbiome be a missing link in the rising prevalence of neurodevelopmental challenges?

The rise in neurodevelopmental challenges and autism spectrum disorder. Autism spectrum disorder (ASD) encompasses a range of neurodevelopmental conditions, typically defined by challenges with communication, social interaction, and the presence of restricted or repetitive behaviors. Functional outcomes vary widely. While some children live fairly independently, others with more severe presentations (levels 2 and 3) frequently experience co-occurring cognitive and language challenges, intellectual

disability (ID), and elevated rates of mental health challenges. Approximately 60% of children with ASD experience these more severe presentations[xvii]. Notably, over 70% of this subgroup experience gastrointestinal problems, indicating potential alterations in gut microbiome function[xvii]. These additional challenges often mean that children with more severe presentations need more support to navigate their daily lives. They often miss school, struggle to gain employment or stay employed, and those experiencing ID need, on average, $4.0M in lifetime societal support (in 2025 dollars[1])[xviii xix].

ASD is affecting more families today than ever before. Estimates indicate that worldwide a child is diagnosed with ASD every 22 seconds. In the USA, 1 in 150 children aged 8 years and younger were diagnosed with ASD in the early 2000s. Today it is 1 in 31– one child every 4 minutes. This represents a five-fold increase in the prevalence of ASD in the last twenty-five years in the USA alone. Many have attributed this increase to expanded surveillance, broadening of diagnostic categories (to include milder autism-related difficulties), or increased public awareness. While all are true, the significance of the increase suggests other rising risk factors may also be contributing.

Genes cannot explain it alone. ASD is known to be caused by a complex combination of genetic and environmental factors. Genetic drift happens slowly, across centuries, not decades, and is therefore not consistent with the steep climb in cases seen in the last decades. The question then is whether other exposures – starting even before birth – could help explain why autism has become more common.

Early-life pressures linked to disruption of the microbiome have been associated with increased risk for ASD. The steep rise in ASD diagnoses over the last twenty-five years tracks with the increase in modern pressures on the maternal and early infant gut microbiome. Indeed, a number of these early-life pressures have been robustly linked with ASD risk across multiple studies. For example, during pregnancy, several factors – including infections, gestational diabetes, and maternal obesity – can more than double the risk of a child developing ASD[xx xxi]. And after birth, infection and early antibiotic use in infants, have also been linked to greater ASD risk[xx]. Early studies suggest the timing and type of pressure also matters. Babies exposed to antibiotics in the first 6 months of life may be twice as likely to develop ASD as those exposed later[xxiii] – the same critical window in gut microbiome formation required for healthy neurodevelopment. Some antibiotics appear riskier than others – for example babies exposed to broad spectrum antibiotics that can cause widespread disruption to the gut microbiome (like cephalosporins) may face up to three times the risk of developing ASD versus narrow spectrum antibiotics such as penicillin's[xxii].

What if the developing infant gut microbiome is a missing link in the rising prevalence of ASD?

If the early gut microbiome is a missing link, between increasing early-life pressures and the rising prevalence of neurodevelopmental challenges, it may also present a new path forward to reducing the risk.

The developing gut microbiome can display properties of resilience – meaning it can recover after encountering various, even numerous, early life pressures. And, while both diet and antibiotic use can significantly disrupt the gut microbiome, other factors can offset these challenges and restore balance. For example, studies in infants suggest that breastfeeding can rebalance the gut microbiome after antibiotic exposure in a caesarean

[1] Cost model adapted from Buescher et al 2014 & Cakir et al 2022

birth[xxiii], and work in animal models highlights the role of a high-fibre, low-fat diet in supporting microbiome recovery after antibiotic exposure[xxiv].These examples show that the microbiome is a resilient, actively adapting system in which we can protect, preserve and restore function.

If the gut microbiome does present a new path forward for reducing risk, we would need new objective and scalable methods to assess healthy gut microbiome function and support the resilience needed for healthy neurodevelopment – in pregnancy and in early infancy. Perhaps most importantly, we'll need new methods for preserving and restoring (when disrupted) microbiome function in key developmental windows before age 2.

What's limiting current practice?

Observational cohort studies exploring environmental risk factors and ASD typically consider risks in isolation and rarely consider how early-life challenges can accumulate. Moreover, they rarely consider the biological pathway and potential mechanism of action. While animal models have been invaluable in showing that modern pressures experienced in pregnancy can disrupt the maternal and offspring microbiome, and provide the causal link driving differences in neurodevelopment, these tightly controlled experiments usually test one exposure at a time in artificial conditions. This does do not represent how women and children encounter cumulative pressures in natural environments, nor does it model what would be needed to offset those collective pressures to protect, preserve or restore healthy function.

Additionally, the primary investigational tools used to create a window into the developing infant gut microbiome have focused predominantly on cataloguing *what bacteria* are present and *in what abundance* (taxonomic profiling). This tells us *what is there* but not *whether the system is functioning properly*. Newer tools like shotgun-metagenomic sequencing and metabolomics begin to fill this gap, but the untargeted approach means they are costly, analysis is slow, and they are difficult to scale for clinical use as screening or diagnostic methods. We will need to utilize targeted and more efficient, emerging methods or combinations of methods to assess core gut microbiome functions required for neurodevelopment.

Finally, most gut microbiome interventions that have attempted to minimize autism-related difficulties have been generic in nature and have been focused on children around ages 3–5, after conventional diagnosis. By then, the critical window of microbiome formation – when core functions are required for healthy neurodevelopment – has already passed. Such current approaches rarely account for each child's unique exposures and the timing of exposure, and therefore which microbiome functions may need to be protected, preserved or restored. Timed and targeted protective, preservative and restorative approaches during the critical developmental period before age 2, will likely be needed.

The goal of the FORM program is to develop scalable methods to ensure or restore healthy formation and resilience of the maternal and developing infant gut microbiome. In particular, the key functions needed to support healthy neurodevelopment – in pregnancy and infancy – with the goal of establishing the feasibility of reducing the number of children who could go on to experience severe autism-related difficulties by up to 75%.

We aim to reach these requirements without raising the risk of other microbiome-related childhood conditions, such as obesity or allergies. Our hypothesis focuses on the more severe autism-related difficulties (levels 2 and 3) – where cognitive, language, and GI

challenges are greatest. It is for this subgroup of children, where it seems the gut microbiome could provide the missing link[2].

Why now?

Ensuring healthy formation and resilience of the maternal and developing infant gut microbiome – to support a child's healthy neurodevelopment – is now within reach. Several advances have converged to make this possible.

Advances in data analytics coupled with the availability of rich, longitudinal birth cohorts including repeated gut microbiome samples, make it newly possible to identify which children experience early microbiome dysfunction, which pressures during pregnancy and infancy drive it, and when. This has made it possible to ascertain that: 1) infant gut microbiome development is predictable and follows distinct trajectories; 2) different exposures – caesarean birth, breastfeeding and antibiotics – push trajectories during different developmental windows; and c) trajectories can predict immune or metabolic problems by ages 2-5 years old[iv].

Meanwhile advances in *in vitro* models demonstrate the viability of modelling maternal and infant gut microbiome function, including responses to accumulating early life pressures, and the identification of key factors that promote resilience. Progress has been made in at least two key areas: 1) the ability to mimic nutrients / substrates in the infant intestine in *in vitro* mini bioreactor arrays (MBRAs) has made it possible to recapitulate the infant gut microbiome and identify which bacteria produce or consume key metabolites; and, 2) to test how specific nutrients[xxv] or antibiotics[xxvi] shift function and resilience.

At the same time metabolomic panels coupled with supervised, machine learning approaches are now capable of delineating healthy and unhealthy adults with >80% accuracy based on just a dozen core gut microbiome functions[xxvii]. Meanwhile, machine learning models that can predict an infant's age from metagenomic data show that gut microbial development follows consistent patterns at specific developmental windows – patterns that are strikingly conserved across diverse global populations[xxviii]. This suggests that the gut microbiome provides core functions timed to development. This opens the door for developing scalable functional screens for the maternal and infant gut microbiome.

Digital twins and other *in silico* approaches are also allowing us to model complex, high dimensional metagenomic and metabolomic data and design targeted interventions. For example, *in silico* digital twins of the infant gut microbiome can predict neurodevelopmental challenges in pre-term infants, identify clinical risk factors, and guide early dietary interventions[xxix]. Moreover, advances in optimization algorithms – even quantum inspired – have made it possible to: 1) predict microbiome recovery after antibiotic exposure[xxiv]; 2) design nutrient cocktails that promote recovery[xxiv] ; and 3) propose bacterial consortia to displace drug-resistant pathogens[xxx].

Finally, early, emerging data indicates we may be at the point where we can engineer bacteria to restore missing functions or displace specific bacteria that disrupt these functions. Progress has been made in at least two key areas: engineered probiotics – for

[2] Severe autism with an established genetic origin (about 10–20%) and mild/ moderate ASD (~40%) fall outside the scope of this program.

example, *E. coli* Nissle, has been modified to release specific metabolites that boosted immune cell health in mouse models[xxxi]; and, bacteriophages – the natural viruses that live in the gut – are being used in a targeted way to neutralize antibiotic-resistant bacteria without disturbing the rest of the community and opening opportunities to restore gut microbiome functions[xxxii].

Together, these advances suggest we are at an inflection point. For the first time we can imagine: determining one of the underlying biological and environmental pathways that might cause autism-related difficulties in a subgroup of children with severe difficulties; screen for core gut microbiome functions required for healthy neurodevelopment; and develop targeted strategies to protect, preserve and restore core functions before the brain critical windows close. If we succeed, FORM could offer a path to reducing the number of children who go on to experience severe autism-related difficulties by up to 75%, before age two – when neurodevelopment is most pliable and interventions are most effective for ASD [xxxiii].

To achieve the goals of the program we seek advances in three thrust areas.

To test if the gut microbiome is a missing link and determine if we can meet the requirements of a solution with the potential to reduce the number of children who go on to experience severe autism-related difficulties by 75%, we need advances coordinated across three thrust areas.

- Thrust 1: Identify the proportion of children experiencing ASD attributable to gut microbiome dysfunction, with >90% balanced accuracy, and identify what combination(s) of early life pressures (and when) drive the specific microbiome dysfunction influencing neurodevelopment.

- Thrust 2: Develop objective and scalable methods to: 1) screen the functional contribution of the maternal and infant gut microbiome to infant neurodevelopment to predict cognitive, language, social and executive function challenges, aiming for >70% accuracy; and 2) diagnose a gut microbiome-based functional disorder or disorders predictive of severe autism-related difficulties with >90% accuracy. These performance metrics are comparable to existing early ASD screens like M-CHAT, and sufficient to trigger referral for formal diagnosis, with the clinical grade accuracies expected for a diagnostic test for ASD and other metabolic disorders that result in neurodevelopmental challenges.

- Thrust 3: Pair diagnostic tests with strategies to protect, preserve and restore gut microbiome functions and resilience – in pregnancy and infancy – aiming for >80% effectiveness to support healthy neurodevelopment. Test whether this can meet the requirements of a solution with the potential to reduce the number of new cases of severe autism-related difficulties by 75%.

Advances across thrusts will inform each other to improve and validate screening methods, diagnostic biomarkers, and restoration strategies. It is not necessary to form large consortiums or teams to do accomplish all the thrust goals. Synergies and integrated system demonstrations will be facilitated by Wellcome Leap on an annual basis as we make progress together towards the program goal.

Thrust 1: Identify the proportion of children experiencing severe difficulties associated with ASD that are attributable to gut microbiome dysfunction and identify what combination(s) of early life pressures (and when) drive microbiome dysfunction influencing neurodevelopment.

1A. Determine the proportion of children experiencing severe autism-related difficulties attributable to abnormal gut microbiome function, identify key early-life pressures and windows of exposure.

Autism currently affects about 3.2% of children. To identify what proportion of these cases may be attributable to gut microbiome dysfunction, we will need objective biomarkers that can detect the dysfunction with high accuracy (balanced accuracy >90%). Establishing this will require a large cohort — likely more than 15,000 children — to ensure statistical power. With that sample size, we can reliably estimate whether microbiome dysfunction accounts for as much as 50% of ASD cases (around 1.6% of all children) or as little as 10% (about 0.3%). This sample size will allow us to pin down prevalence estimates within about half a percentage point, enough precision to make strong epidemiological assessments about the role of the gut microbiome in ASD. Experimental designs that could reduce the sample size whilst retaining the same degree of precision in prevalence estimates are welcome.

Develop predictive models that identify functional microbial biomarkers (metabolic, immune, inflammatory, and hormonal) from birth through to age 2 that are: 1) predictive of severe autism-related difficulties with >90% accuracy at age 2 or older; and 2) are predicted by early-life pressures across pregnancy and infancy. These identified biomarkers should provide the foundation for Thrust 2.

Models should integrate data from: gut microbiome metagenomic and metabolomic data from birth through age two; early life pressures that disrupt microbiome formation starting in pregnancy and through to infancy and have been associated with ASD; early life exposures that can promote resilience of the gut microbiome; genetic factors, to account for interactions between inherited and environmental influences; and models should be stratified by sex to test if there are sex differences mediated by interactions between the gut microbiome and endocrine systems. We are interested in models that look at the combined effects of early life pressures on microbial biomarkers. Combined pressures should aim to yield effect sizes >0.8 or odds ratios >4 to support restoration strategies capable of achieving ≥80% effectiveness. Predictive validity of models should be verified against standard ASD screening tools at 18 months or older (e.g. MCHAT, CBCL) and diagnostic tools at 2 years and older (e.g. ADOS-2 or CARS-2).

Of particular interest are *in silico* digital twin computational models that can be used to predict neurodevelopmental outcomes and test the impact of different combinations of early life pressures (e.g. multiple courses of antibiotics or the combined impact of maternal obesity+ caesarean birth with antibiotics + formula feeding) during critical windows (e.g., third trimester or 0-6 months old) of microbiome formation, are of high interest. This will help identify the subgroups of children most at risk, and when that risk peaks. Findings are expected to guide public health strategies to reduce modifiable or avoidable pressures (e.g. diet, antibiotic use, birth practices, breastfeeding support), during critical periods in gestation or infancy when the gut microbiome is forming and thus most vulnerable.

Proposers should draw on large, established birth cohorts with longitudinal and multi-omics data or biological samples (stool, blood, urine) that have already been collected or plan to be collected [3] and can be used to assess microbiome-derived metabolic, immune, inflammatory, and hormone markers. Examples include the HBCD study, C-Gull, HELMI cohort, CHILD, and MAL ED. Cohorts must include at least: one gut microbiome stool sample in the 0–6 month window; one in the 6-12 month window; and one in the 12-24

[3] Funding for new biological sample collection is not within the scope of the program.

month window , with metagenomics data and the potential to perform metabolomics (for those children diagnosed with ASD and an appropriate number of controls). It is desirable for cohorts to also include: maternal stool samples, especially from the third trimester, to study mother–infant microbial transmission and the influence of maternal metabolites on fetal neurodevelopment; and infant neuroimaging or electrophysiology data to identify microbial biomarkers and exposures predictive of neuroendophenotypes (e.g., changes in excitation/inhibition balance or band power) that predict later difficulties associated with severe ASD. Requests to add ASD screening and diagnostic measures[4] onto existing cohorts where children are over 2 years old will be considered (assuming the multi-omics data and biological samples have already been collected from birth through to age 2) as the primary goal is to link early microbial biomarkers and early-life challenges to later ASD diagnoses.

It is expected that multiple cohorts will be synchronised in a multi-site study to achieve the required sample size of ~15,000. This synchronisation will be facilitated by Wellcome Leap. Cohort data and samples should therefore be made accessible to other program participants and to enable testing of screening tools developed in Thrust 2.

1B. Identify the mechanism of action.

Use human-derived model systems to test how early-life pressures alter gut microbiome functions needed for neurodevelopment (e.g., short-chain fatty acids essential for myelination; neuroactive metabolites including GABA, glutamate, and kynurenine; inflammatory cytokines and immune markers, and hormone metabolism, particularly estrogen) in critical developmental windows and test modifiable exposures to identify key resilience factors. In some cases, this could extend to carefully designed randomised clinical trials in infants, but only where microbiome perturbations are safe and ethically justified (e.g., naturally occurring), and feasible to complete within the three-year program.

Of interest is the development of a high-throughput screening platform of *in vitro* mini bioreactor assays (MBRAs) that recapitulate the taxonomic and functional composition of different maternal and infant gut microbiomes in pregnancy and infancy. The MBRAs should be stratified by: different combinations of early life pressures; critical windows of gut microbiome formation (with a particular focus on the third trimester and 0-6 month window in infancy); sex differences; and severe autism-related difficulties at age 2 or later, informed by Thrust 1A and existing literature. Taxonomic and functional fidelity of the MBRAs should be verified via shotgun metagenomic sequencing methods and metabolomics.

Once verified, leverage the *in vitro* models and the *in silico* digital twin models developed in Thrust 1A to: 1) confirm critical functional components of the maternal and infant gut microbiome — including corresponding metagenomes and developmental timepoints- needed for healthy neurodevelopment that can distinguish children with and without severe autism-related difficulties; 2) determine the critical combination(s) of pressures and windows of exposure that discriminate those functional components; and, 3) test modifiable exposures (e.g. antibiotic exposure or dietary environments) to model their impact on gut microbiome function and identify key resilience factors. Advances in Thrust 1B should inform the starting point for diagnostic development in Thrust 2 and restoration strategies in Thrust 3A.

[4] Diagnostic measures should only be added for the subgroup of children identified as at risk for neurodevelopmental challenges

Once functional microbial biomarkers have been identified, mechanism of action needs to be validated by linking microbial biomarkers to neurodevelopmental outcomes. This may include validation *in vitro* 3D brain organoids and assembloids recapitulating critical neural circuits impacted in ASD (e.g. prefrontal-striatal-thalamic-mesencephalic circuits) that include synapses, myelination and microglial activity, as verified by immunohistochemistry, transcriptomic methods and electrophysiology. Alternatively humanized mice may be used, if functional biomarkers of brain development that are conserved across species (e.g., visual evoked potentials, excitation/inhibition balance) are used, which are also predictive of ASD difficulties in children. This will increase the likelihood that findings translate from mice to infants.

Of particular interest are studies that include exploration of possible mechanisms underlying sex differences in ASD. It is well established that a 'mini puberty' takes place between 0-6 months and that the microbiome estrabolome plays an active role in estrogen metabolism. Given estrogen has a neuroprotective effect within specific developmental windows, studies exploring whether specific exposures impact estrabolome activity and may help explain male-female risk gradients are of high interest.

Thrust 2: Develop objective, scalable methods to assess the functional contribution of the maternal and infant gut microbiome to infant neurodevelopment, to predict risk of cognitive, language, social and executive function challenges.

The goal is to deliver a two-tiered approach using objective microbiome derived biomarkers: 1) a universal screening tool (s) to identify infants at risk of cognitive, language, social and executive function challenges aiming for ≥70% accuracy; and, 2) a diagnostic to confirm a gut microbiome functional disorder(s) predictive of severe autism-related difficulties, aiming for ≥90% accuracy (clinical grade accuracy).

Screening should be possible in the third trimester of pregnancy and from as early as 1 month of age in infants through to age 2, so as to be able to integrate into routine maternity and well-child visits. To demonstrate long-term scalability of a universal screening tool for ASD (given a global prevalence range of 0.5-3.2%) tools need to be priced between USD$10-100 and capable of achieving >50% screening coverage in diverse, global populations.

Develop and optimize functional metabolomic panels with supervised machine learning to assess core functions of the maternal and infant gut microbiome in pregnancy and infancy needed for healthy neurodevelopment. We are especially interested in approaches that use accessible samples — stool, urine, or serum — to measure core microbiome functions required for healthy neurodevelopment such as: short-chain fatty acids; neuroactive metabolites including GABA, glutamate, and kynurenine; inflammatory cytokines and immune markers, and hormone metabolism, particularly estrogen.

For the infant gut microbiome, proposals that generate age-specific functional trajectories as well as sex-stratified models are of high interest. These will help determine whether a stable set of microbial functions predicts healthy brain development across the first two years, whether specific functions matter during specific developmental windows, and whether different functions are critical for different sexes. The answer will guide whether screening should be continuous from 1 month through to age 2 or concentrated in "sweet spots" of development; and, whether sex-stratified screens may be needed, shaping how such tools could scale in primary and community healthcare settings globally.

All new screening tools must demonstrate predictive validity for identifying risk of cognitive, language, social, and executive function challenges in infants, benchmarked

against existing measures used in well-child visits (e.g., ASQ, PEDs, M-CHAT, CBCL at 18–24 months). For diagnostic confirmation, methods should be cross-validated against gold-standard ASD instruments such as ADOS-2 or CARS-2 used at ages 2 and older.

Finally, approaches developed in Thrust 2 should be validated across at least two diverse populations (different cultures and geographies) within the birth cohorts used in Thrust 1A.

Thrust 3: Develop strategies to protect, preserve and restore gut microbiome functions needed for healthy neurodevelopment with >80% effectiveness and test if we can meet the requirements of a solution with the potential to reduce the number of children that go on to experience severe autism-related difficulties by 75%.

To demonstrate the potential of reducing the number of new cases of children that go on to experience severe autism-related difficulties by 75%, we need strategies to protect, preserve and restore the gut microbiome functions necessary for healthy neurodevelopment – in pregnancy and in infancy before age 2– that aim for at least 80% effectiveness. These strategies must be fast to deploy, with treatment beginning within seven days of diagnosis, similar to how phenylketonuria (PKU) is managed[5]. This means interventions will be practical for routine maternity and paediatric care in community or primary healthcare settings, without creating added delays or specialist referral bottlenecks, and we can restore core functions before critical brain development windows close.

3A. Develop strategies to protect, preserve and restore maternal and infant gut microbiome function and resilience to support healthy neurodevelopment during critical developmental windows, aiming for ≥80% effectiveness.

Develop computational models – such as flux balance analysis or combinatorial optimization algorithms – that draw on data from Thrusts 1A and 1B (in particular nutrient flow and metabolite utilization data from MBRAs) to design strategies capable of offsetting early-life pressures on the gut microbiome and protecting/ preserving / restoring key functions during the critical developmental period. Strategies of interest include: targeted nutrient-based interventions that guide microbiome formation and development by targeting specific substrates and resources; introducing key microbial communities (living consortia or engineered strains) or microbiome-derived metabolites to restore missing functions; and next-generation therapies to displace particular bacterial strains disrupting function, such as phage-based therapies. Once designed, strategies should be tested in the MBRA system used in Thrust 1B to evaluate whether they are capable of offsetting the effects of causal early-life pressures and restoring microbiome functions needed for healthy neurodevelopment with >80% effectiveness.

Particular value lies in models that can: a) test whether combining strategies (e.g., diet plus key bacterial communities) can achieve >80% effectiveness in restoring function and resilience; b) match women and children to the right strategy at the right time, based on both the early life pressures they were exposed to (and when); and, c) if different strategies are needed for different sexes. For example, a child with disrupted microbial function in the first six months of life may require different interventions from one whose gut microbiome collapses at 12 months following multiple rounds of antibiotics. Models capable of making this distinction are of high priority.

[5] A metabolic disorder that causes neurodevelopmental delay.

3B. Develop an integrated model to test whether microbial biomarkers coupled with effective restoration strategies can meet the requirements of a future solution with the potential to reduce the number of new cases of severe autism-related difficulties by 75%.

Perform experimental validation in animal models to verify whether functional microbial biomarkers can predict severe autism-like behaviors with >90% accuracy and restoration strategies can achieve >80% effectiveness for restoring key microbial function and resilience needed for healthy neurodevelopment during critical developmental windows. Humanized mice are of particular interest to: a) verify the full causal chain from early life pressures and gut microbiome disruption → microbial biomarkers → severe ASD-like behaviors in offspring; and b) test whether interventions applied during critical gestational or infant windows can recover gut microbiome function and reduce the number of offspring encountering severe autism-like behaviors in the preclinical model by up to 75%. Validation must use cross-species conserved biomarkers of brain development (as outlined in Thrust 1B) alongside established ASD-like behaviors such as sociability.

Interventions must prioritize safety and stability. We aim to optimize gut microbiome function without introducing pathogenic strains, antimicrobial resistance genes, disrupting essential immune and metabolic functions or triggering an adverse immune or inflammatory reaction. Proposers are therefore encouraged to: a) perform deep sequencing of candidate bacterial strains to screen for antimicrobial resistance genes and virulence factors; b) assess microbiome stability, taxonomic composition, and functional outputs before and after intervention (using MBRAs or humanized mice), to confirm that no harmful shifts are introduced that could elevate risks for obesity or allergy, as verified by biomarkers known within the literature; and, c) assess immune and inflammatory markers.

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