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Model-based hypothesis of gut microbe populations and gut/brain barrier permeabilities in the development of regressive autism

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ABSTRACT

Regressive autism is a devastating disorder affecting children between the ages of 15–30 months. The disorder is characterized by the loss of social interaction and communication ability following otherwise healthy development. In spite of rising autism prevalence, current detection methods and treatment options for this disease are lacking. Therefore, this study introduces a systems-level model, which suggests that gut microbes and intestinal inflammation influence the onset of regressive autism through increasing gut permeability. This computational model provides a framework for quantitative understanding of how imbalances in populations of gut microbes alters the whole-body and brain distributions of neurotoxins produced by GI tract bacteria. Our results indicate that increased levels of the bacteria *Bacteroides vulgatus* lead to increased brain levels of propionic acid, a neurotoxin which has been known to cause symptoms characteristic of autism when injected into the brain of rats. Our results further indicate that immune response to virulence factors produced by bacteria in the gut leads to increased systemic levels of inflammatory cytokines, such as IL-1β, which significantly alter the permeability of the gut epithelial layer and the blood–brain barrier. Due to the large size of cytokines, however, we predict the time required for concentrations in the brain to stabilize to be on the order of years. This suggests that treatments preventing autism development could be administered after identifying microbial biomarkers of disease but before debilitating brain inflammation leads to regressive autism progression. Future research extending this work could provide new treatment options and diagnostic techniques to help combat regressive autism.

Introduction

Autism Spectrum Disorders (ASDs) are neurodevelopmental disorders characterized by behavioral symptoms including communication deficits, dependency on routine, sensitivity to environmental changes, and intense focus on inappropriate items [1]. Regressive autism is a subset of this disease generally characterized by normal development, with minor deficiencies in some cases, followed by the emergence of autistic symptoms at approximately 18 months of age [2]. Despite different characteristics that generally combine to form a conclusive autism diagnosis, there is no definitive cause and therefore no quantitative, biological measurement for diagnosis [3]. By determining underlying mechanisms contributing to the development of autism, more effective means of diagnosis and targeted therapeutics can be designed.

To this end, it is generally accepted that both genetic vulnerabilities and environmental factors contribute to the occurrence of autism [3,4]. One potential environmental factor contributing to autism development may be composition of the gut microbiome. It has recently been discovered that there is a significant difference in the populations of gut bacteria of autistic individuals compared to normally-developed individuals [5]. This difference in the gut microbial composition indicates that certain microbes resident in the gut may contribute to autism development through various mechanisms [4]. Further understanding of this microbial difference in the gastrointestinal tract and the mechanisms by which it affects the brain could lead to potential non-behavior based diagnostic methods and eventually to therapeutics for autism.

Several recent studies have investigated the gut microbiome in an effort to understand the potential relationship between gut bacteria and autism [5–7]. Finegold published findings that showed increased levels of *Desulfovibrio* and *Bacteroides vulgatus* in autistic children when compared to control subjects [6]. *Desulfovibrio* is an anaerobic bacterium that produces virulence factors such as lipopolysaccharides (LPS) and hydrogen sulfide (H_2S) and is resilient to unfavorable environmental conditions [6]. *B. vulgatus* is a known producer of propionic acid (PA), which has been shown to cause symptoms characteristic of autism when injected into the brains

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of rats [7]. An additional study showed that *Bacteroides* that produce PA have been shown to cause "biologic, chemical, and pathologic changes that are characteristic of autism" [5]. Additionally, Finegold et al. found that *Bifidobacterium* levels were decreased in autistic individuals compared to controls. *Bifidobacterium* was shown to have an inhibitory relationship with both *B. vulgatus* and *Desulfovibrio* [5,8]. Similarly, *Desulfovibrio* growth was found to have an inhibitory relationship with some *Bifidobacterium* species [9].

In addition to their implication in autism development, *Desulfovibrio* and *B. vulgatus* have also each been implicated in inflammatory colonic diseases, which can inflame the epithelial layer of the gut, increasing its permeability to antigens, such as cytokines [5,8]. Cytokines are produced by the immune response to virulence factors in the gut and can induce systemic inflammation of other body regions including the blood–brain barrier (BBB) and the brain [10]. Once inflammatory cytokines cross the BBB, they can induce neuroinflammation by affecting neuroglial cells [10]. Clinically, this presents itself as increased levels of the inflammatory cytokines TNF-α, IL-6, IL-1β, GM-CSF, INF-γ, and IL-8 found in the blood and brains of autistic patients [11,12].

Because of the systemic nature of the impact of gut bacterial populations on autism development, we propose a theoretical model describing a mechanism by which changes in the gut microbiome can lead to the etiology of regressive autism by affecting gut epithelial and BBB permeability via inflammatory cytokines. In this model, bacteria in the gut produce virulence factors implicated in autism, which lead to an elevated immune response producing inflammatory cytokines localized within the gut. These cytokines lead to an increase in gut epithelial permeability allowing cytokines and bacteria-derived virulence factors to circulate in the blood and potentially increase BBB permeability. Under healthy gut microbe conditions, with normal levels of cytokines throughout the body, the epithelial and BBB permeability are small enough to significantly reduce propionic acid produced in the gut from entering the blood stream and brain. Under autistic gut microbe conditions, however, there is an overproduction of inflammatory cytokines, which lead to increased gut and BBB permeability. We hypothesize that this weakened barrier causes increased levels of PA and inflammatory cytokines in the brain, which leads to brain inflammation contributing to the etiology of symptoms characteristic of regressive autism. A flow diagram representing this mechanism is presented in Fig. 1.

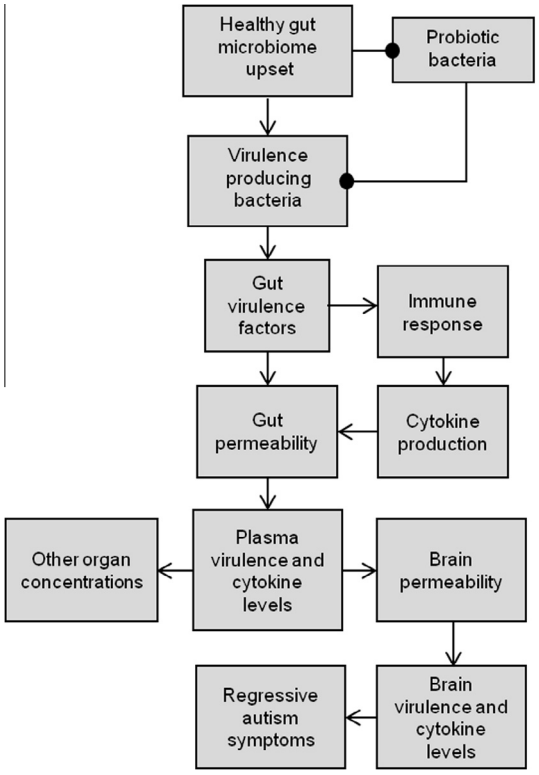

Fig. 1. Model flow diagram of gut influence on autism development. Arrows indicate promotion, dots indicate inhibition. Increasing levels of virulence-producing bacteria lead to a localized immune response. If this immune response becomes chronic, however, gut epithelial lining may become more permeable, leading to cytokine release into the blood, systemic inflammation, and potential damage to the blood–brain barrier.

A computational model is used to characterize this system because of the complexity of the interactions involved in the gut microbiome's effect on the body. To our knowledge, there has been no attempt in the literature to develop a quantitative connection between these relationships and the etiology of autism. Therefore, with the data currently available, we propose the following predictive model to provide insight into this highly complex disease and suggest directions for future work.

The model

Gut microbe populations

The model simulations were computed assuming constant microbial populations over time, representative of the conditions found within the gut of autistic individuals compared to healthy individuals [5]. This allowed for the determination of the overall effect that different bacteria population sizes would have on inflammation and virulence concentration profiles.

Virulence production by gut microbes

Several different model equations were used to capture the time dependent behavior of the virulence factors. In the model, each factor was formed by the appropriate bacterium following the equation:

$$\frac{dC_i}{dt} = k_i x_j - q_i C_i \tag{1}$$

where C_i is the concentration of the virulence factor i, x_j is the population of bacterium j, k_i is the production rate constant, and q_i is the clearance rate of the toxin from the gut.

The cytokine response was assumed to be proportional to the sum of the virulence factors using Michaelis–Menten kinetics according to the following equation:

$$C_{cyto} = \frac{C_{\max} \sum C_i}{K_C + \sum C_i} \tag{2}$$

here $C_{\max}$ represents the maximal concentration of cytokines produced in the body based on measurements of regressive autistic children, and K_C describes the rapidity of the immune response. The values chosen for $C_{\max}$ and K_C were based on literature values for IL-1β plasma concentrations; however, the model can easily be expanded to include more cytokines [12].

BBB/gut epithelial permeability

Under normal conditions, the epithelial cells lining the gut lumen and BBB act as a physical barrier between the gut contents and tissue [13]. They are spaced such that they are highly selective to molecular transport and the radius of the pore spacings, called tight junctions, determines their permeability [13,14].