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Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders

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ABSTRACT

Recent studies have determined that the microbiome has direct effects on behavior, and may be dysregulated in neurodevelopmental conditions. Considering that neurodevelopmental conditions, such as autism, have a strong genetic etiology, it is necessary to understand if genes associated with neurodevelopmental disorders, such as Shank3, can influence the gut microbiome, and if probiotics can be a therapeutic tool. In this study, we have identified dysregulation of several genera and species of bacteria in the gut and colon of both male and female Shank3 KO mice. *L. reuteri*, a species with decreased relative abundance in the Shank3 KO mice, positively correlated with the expression of gamma-Aminobutyric acid (GABA) receptor subunits in the brain. Treatment of Shank3 KO mice with *L. reuteri* induced an attenuation of unsocial behavior specifically in male Shank3 mice, and a decrease in repetitive behaviors in both male and female Shank3 KO mice. In addition, *L. reuteri* treatment affected GABA receptor gene expression and protein levels in multiple brain regions. This study identifies bacterial species that are sensitive to an autism-related mutation, and further suggests a therapeutic potential for probiotic treatment.

1. Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by a lack of social communication and the presence of repetitive or stereotypical behaviors (American Psychiatric Association, 2013). The etiology of ASD includes a very strong genetic component, where heritability has been estimated to be from 60% (Gaugler et al., 2014; Huguet et al., 2013) to as much as 83% (Sandin et al., 2017). However, the genetic etiology of ASD is very heterogeneous, and hundreds of genetic aberrations have been associated with ASD, which include chromosome copy number variations, common variants, and de novo rare mutations (Chaste and Leboyer, 2012; Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2013). There are a few genes whose association to ASD have been well characterized (Durand et al., 2007; Griswold et al., 2012) and mouse models have been produced to study their role in the development of ASD (Hulbert and Jiang, 2016; Peça et al., 2011). Some of these genes encode proteins that are related to synaptic transmission (Bourgeron, 2015), while others are related to epigenetic regulation (Loke et al., 2015). A few examples of such genes are *SHANK3* (Durand et al., 2007; Guilmatre et al., 2014; Leblond et al., 2014; Peça et al., 2011), *CNTNAP2* (Jonsson et al., 2014; Poot et al., 2010), and more recently, *CHD8* (Wilkinson et al., 2015). In particular, both de novo mutations and deletions in the *SHANK3 gene* have been identified in individuals with autism, and deletions in 22q13, the genetic region containing *SHANK3* and other genes, causes Phelan-McDermid syndrome, which includes autistic behavior (Durand et al., 2007; Gauthier et al., 2009; Phelan and McDermid, 2012).

Recent evidence suggests that the microbiome may play a role in neurodevelopmental disorders. Each person has a unique fingerprint of microbial diversity that live in symbiosis with us. The gut microbiome is influenced by many factors, including diet, life experiences,

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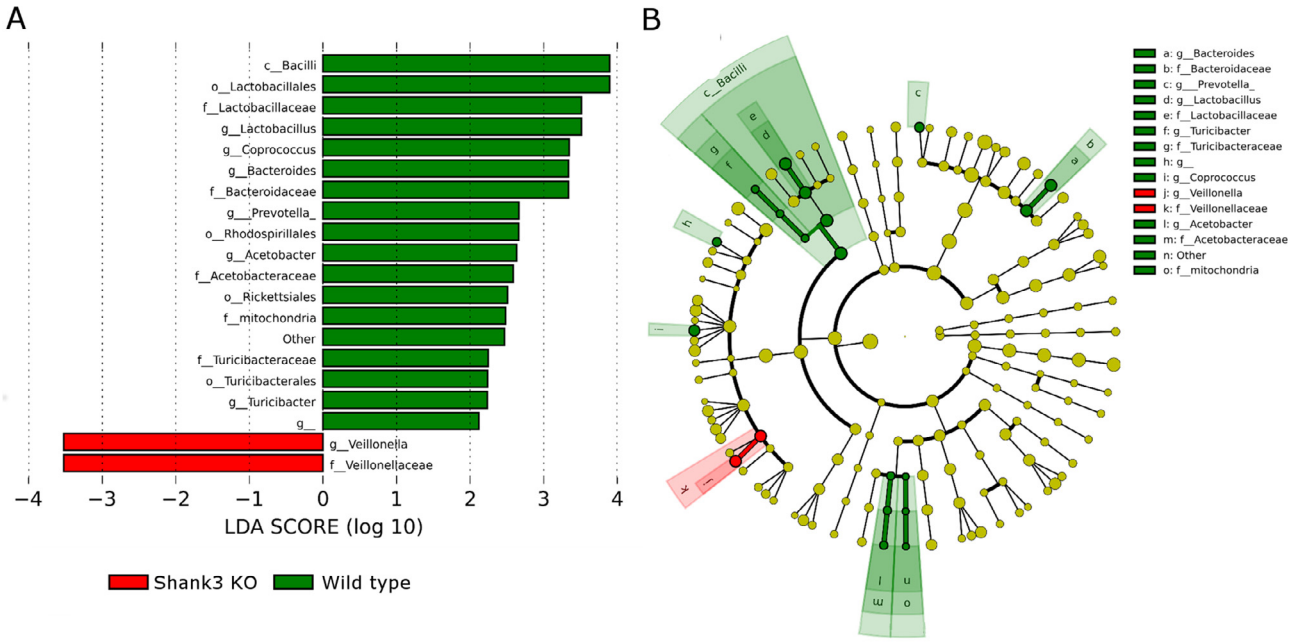

Fig. 1. 16S high-throughput sequencing to determine bacterial taxa that are differentially represented in Shank3 KO mice A.) List of bacterial taxa that are found overrepresented (red) or underrepresented (green) in Shank3 KO mice, according to LefSe package analysis of 16S high-throughput sequencing data B.) Cladogram of the LefSe output. n = 27 wild type mice, 31 Shank3 KO mice.

environment, and genetics. Their genes are 150 times more numerous than human genes and it is estimated that more than 1000 species of microorganisms exist in the gut (Blottière et al., 2013; Consortium, 2013). Within the past decade, several studies determined that the gut microbiome may have a direct effect on behavior (Cryan and Dinan, 2012; Heijtz et al., 2011), neurodevelopment (Borre et al., 2014; Cryan and Dinan, 2015), and brain disorders (Borre et al., 2014; Felice and O'Mahony, 2017). While the mechanisms of these effects are still poorly understood, some evidence has suggested that the gut microbiome can affect brain function through the excretion of metabolites (De Vadder et al., 2014; Parashar and Udayabanu, 2016), host immune response regulation (Fung et al., 2017; Shamriz et al., 2016), and possibly through the excretion of active neuropeptides and neurotransmitters (Buffington et al., 2016; Mazzoli and Pessione, 2016).

Several high-throughput studies have been performed to determine changes in the microbiome among individuals diagnosed with ASD (De Angelis et al., 2013; Finegold et al., 2010; Kang et al., 2018; Strati et al., 2017). While most of these studies have identified dysregulation of the microbiome in individuals diagnosed with ASD, there is little consensus regarding the specific bacterial species that are commonly dysregulated. It is worth noting that multiple studies have identified differential abundance of the phylum *Bacteroidetes* in individuals with ASD, compared to controls. However, while some studies have identified an increase of *Bacteroidetes* in ASD (De Angelis et al., 2013; Finegold et al., 2010), a recent study reported a decrease (Strati et al., 2017). Therefore, further studies of larger populations and well defined populations are necessary to gain a firm consensus regarding specific changes to the microbiome in ASD. A recent preliminary study has also determined positive effects of fecal transplant therapy in children diagnosed with ASD (Kang et al., 2017). Therefore, modulators of the microbiota–gut–brain axis, such as probiotics, might serve as a promising strategy for ASD treatment (Navarro et al., 2016).

Furthermore, animal studies have suggested that specific environmental factors which may be involved in the etiology of autism can induce a dysregulation of the microbiome, and that probiotic treatment of mice can attenuate autism-like behaviors. For example, Maternal Immune Activation (MIA) in pregnant female mice induces deficient intestinal barrier integrity and autism-like symptoms in the offspring, which correlate with dysbiosis of the microbiome (Hsiao et al., 2013). Treatment with *Bacteriodetes fragilis*attenuated repetitive behaviors among the offspring. A separate study found that maternal high fat diet induces deficits in social behavior among the offspring and dysbiosis of the microbiome. Treatment with *Lactobacillus* attenuated social deficits (Bravo et al., 2011; Buffington et al., 2016). Therefore, the microbiome has been implicated as a mediator between the environment and the development of autism-related behaviors, including social deficits and repetitive behaviors.

Despite the more established role of environment in the regulation of the microbiome, the relationship between host genetics and the microbiome is poorly understood. Nevertheless, a few studies have established such a relationship. For example, Goodrich *et al.* demonstrated that host genetics can influence the presence of particular bacterial taxa, including the family *Christensenellaceae* (Goodrich et al., 2014). Of particular interest, members of this family can influence body mass index (BMI), demonstrating a direct relationship between host genomics, microbiome, and body weight. In the case of autism, there is no knowledge of whether autism-associated genes may influence the microbiome. Considering the current interest in the role of the microbiome in autism, this knowledge is necessary to decipher how the microbiome may be involved in the development of ASD. Therefore, in the current study, we have tested the relationship between an ASD-associated gene and dysregulation of the microbiome, utilizing a well-established genetic model, the Shank3 KO mice. This study reveals a clear dysbiosis of the microbiome in this animal model. Moreover, a probiotic treatment regulated the behavior and several molecular mechanisms associated with ASD.

2. Results

To determine if the microbiota is dysregulated in the Shank3 KO mice, microbial DNA was extracted from stool samples of Shank3 KO (n = 31) and wild type (WT) mice (n = 27), followed by 16S rRNA gene sequencing. Bacterial richness (Alpha diversity) was decreased in the Shank3 KO mice (Supplementary Fig. 1A). Alpha diversity was particularly affected in Shank3 KO females (Supplementary Fig. 1B). Examination of between sample diversity (Beta diversity) revealed that