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Autism Gene Variants Disrupt Enteric Neuron Migration and Cause Gastrointestinal Dysmotility

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1. Abstract

The biological basis of the well-established comorbidity between severe gastrointestinal symptoms and autism spectrum disorders is yet unknown. By examining these genes within the framework of the gastrointestinal system, it is possible to uncover convergent, underlying biology that may be responsible for the identification of high-confidence large-effect autism risk genes. Here, we demonstrate that the expression of these genes is enriched in human prenatal gut neurons and their migrating progenitors, implying that pathogenic mutations linked to autism may interfere with the formation and/or function of these neurons, resulting in gastrointestinal dysfunction. Here, we report the frequency of gastrointestinal problems in individuals who have large-effect variations in sixteen of these genes. Dysmotility is highlighted, which is consistent with possible failure of the enteric nervous system. We specifically target five of these using the highthroughput diploid frog Xenopus tropicalis

2. INTRODUCTION

The neurodevelopmental diseases known as autism spectrum disorders (ASD) are characterized by atypical social relationships, recurring acts, and constrained interests1. Gastrointestinal (GI) distress is one of the most common and debilitating comorbidities of Autism Spectrum Disorder (ASD). It typically manifests as symptoms of gastric motility such as constipation, diarrhea, or abdominal pain2-4. Even though this comorbidity is well-established, the underlying molecular pathways are still unclear. The identification of genes associated with high-confidence (hc), large-effect autism has made it possible to investigate these genes in vivo and determine the biological causes of this co-occurrence. The development of neural progenitor cells in the central nervous system has been demonstrated to be regulated by hcASD risk genes 6–13, supporting the theory that these genes may also play a role in the development of the enteric nervous system (ENS), which produces the neurons that regulate the gastrointestinal system. In fact, the function of CHD8, one of the first large-effect autism genes discovered, has been clarified in zebrafish, where chd8 disruption resulted in decreased enteric neuron colonization into the stomach as well as gut dysmotility14. It has repeatedly been demonstrated that the expression of autism genes is enriched in adult human enteric neurons, which regulate stomach motility. Taken together, these data suggest that aberrant ENS development could be the cause of the observed comorbidity.

3. Results

The expression of the hcASD gene is more abundant in enteric neurons and their precursors. Previous research has demonstrated an enrichment of hcASD gene expression in mature human enteric neurons15. We used scRNA sequencing data from various points in the human fetal gut development process to evaluate the expression of the hcASD gene in this tissue during development26. In the data set, all 252 hcASD genes with an FDR < 0.15 were expressed. The average expression of these genes and their relative expression enrichment across cell types were determined using Module Score Analysis (AddModuleScore, Seurat R Package) in comparison to a comparably expressed random control geneset (Fig. S1A-B). When compared to all other cell types, enteric neurons and their progenitors, enteric neural crest-derived cells (ENCCs), had a much greater hcASD gene expression enrichment.

4. DISCUSSION

Although the co-occurrence of GI problems and autism is well-established2, the molecular processes underlying this comorbidity are still unknown. Here we are record how often GI problems are in people with hcASD gene variations, emphasizing constipation as a sign of GI dysmotility. Several hcASD genes have been demonstrated by our lab and others to regulate neuronal development in the central nervous

system6–13. This has led us to wonder if these genes also play a role in the development of the ENS. We consistently see that during human embryonic development, enteric neurons and their migratory progenitor cells have higher levels of hcASD gene expression. Thus, we deploy a vertebrate in vivo model to individually disrupt five hcASD genes with different functional annotations (neurotransmission, chromatin control, kinase) in order to explore this notion.action). For each, we find aberrant ENCC migration, which raises the possibility that GI dysmotility in ASD is caused by atypical ENCC migration. As we look more closely at Dyrk1a's function in gut motility, we find that when Dyrk1a is disrupted, excretion decreases but can be increased again when acutely exposed to serotonergic signaling agonists. This study contributes to the expanding body of research indicating that variations in ASD genes disrupt the development of the ENS3,50. This is in line with studies on CHD814 in zebrafish and FOXP151 and PTEN52 in mice. Although the gut neuron number 53 was not significantly altered in a fish investigation of SHANK3, the researchers did uncover impaired serotoninrgic transmission in the gut, which is consistent with our findings that regulating serotonin signaling may be a viable therapeutic approach. In fact, Since serotonin signaling plays a well-established function in gut motility44–47, addressing GI problems in the context of ASD may benefit from a wider exploration of this pathway. Further, important concerns include how variations in the ASD gene impact the quantity, differentiation, structure, appearance, and activity of enteric neurons in mature animals. Given that we have shown a significant loss of function for these genes and that individuals carrying pathogenic mutations in these genes are heterozygous, the impact of gene dosage on these parameters will undoubtedly play a significant role in the future.

5. MATERIALS AND METHODS

Analysis of Ciitizen® Patient Data and Simons Searchlight

The UCSF IRB office, IRB#23-39-079, decided that research using data from the Simons Foundation Autism Research Initiative (SFARI), the Simons Collection (Searchlight), and Ciitizen® medical records was not considered research involving human participants. GI data from afflicted individuals and unaffected families was accessible via the Simons Searchlight Single Gene Dataset v8.0. Every family with any genetic variation in a hcASD gene—defined by Fu et al. 20225 with an FDR < 0.1—was included in the analysis. Additionally, patient GI data was retrieved from Citizen®. Individuals who had a genetic variation categorized as "pathogenic," "likely pathogenic," or "variant of uncertain significance" on their genetic report were included in the study. People having "benign" mutations in one of the hcASD genes were not included. People that possess a Both a "pathogenic" mutation in a non-ASD gene and a "variant of uncertain significance" in a hcASD gene were both ruled out. Using Prism (v.10.1.1), data analysis and visualization were carried out. scRNA-sequencing study and enrichment scoring in the human prenatal gut first, Cell Ranger (GRCh38) was used to process the scRNA-seq FASTQ data of the human fetal gut (ArrayExpress: E-MTAB-948926). We read the gene-barcode matrix outputs of all samples using the normal Seurat analysis pipeline. We then removed low-quality cells with >10% mitochondrial counts and unique feature counts over 2,500 or less than 200, leaving 39,095 cells. The gene-barcode matrix was then normalized using the LogNormalize technique, which multiplied by the default scale factor (10,000) and normalized each cell by total expression. 2,000

Each sample's highly variable genes were found using the FindVariableFeatures function, and all samples were then integrated using the IntegrateData method to create a single Seurat object for further analysis. The integrated object was subsequently scaled, and dimensions reduction was achieved using Principal Component Analysis. The object's cell clusters were computed using FindNeighbors and FindClusters. In order to complete cell annotations, gene markers from Elmentaite et al. 202057 were used to identify cell clusters in addition to ENCC indicators (SOX10, FOXD3, PHOX2B) and enteric neuron markers (TUBB3, ELAVL4, RET). The 252 hcASD genes from Fu et al. 2022 were subjected to the AddModuleScore function in order to evaluate the enrichment of these genes. The results were compared with a control geneset that had equal average expression in all cells. Every cell was given a module score and was then categorized using the previously mentioned cell annotation method. When a cell expressed the control geneset higher than the hcASD genes, it scored less than 0, but when a cell expressed the hcASD genes higher than the control geneset, it scored more than 0. The cells were then categorized using the aforementioned clustering into three groups: enteric neurons, ENCCs, and Non-ENS cells. Next, we used a Kruskal-Wallis test, Wilcoxon rank-sum testing, and Benjamini-Hochberg adjustment for multiple comparisons to determine if ENCCs and enteric neurons had hcASD gene expression enrichment values that were substantially greater than those of any other cell. Using VlnPlot, the enrichment of these genes in each cluster or group was shown.

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