



# De novo duplication on Chromosome 19 observed in nuclear family displaying neurodevelopmental disorders

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**Abstract** Pleiotropy and variable expressivity have been cited to explain the seemingly distinct neurodevelopmental disorders due to a common genetic etiology within the same family. Here we present a family with a de novo 1-Mb duplication involving 18 genes on Chromosome 19. Within the family there are multiple cases of neurodevelopmental disorders including autism spectrum disorder, attention deficit/hyperactivity disorder, intellectual disability, and psychiatric disease in individuals carrying this copy-number variant (CNV). Quantitative polymerase chain reaction (PCR) confirmed the CNV was de novo in the mother and inherited by both sons. Whole-exome sequencing did not uncover further genetic risk factors segregating within the family. Transcriptome analysis of peripheral blood demonstrated a ~1.5-fold increase in RNA transcript abundance in 12 of the 15 detected genes within the CNV region for individuals carrying the CNV compared with their noncarrier relatives. Examination of transcript abundance across the rest of the transcriptome identified 407 differentially expressed genes ( $P$ -value  $< 0.05$ ; adjusted  $P$ -value  $< 0.1$ ) mapping to immune response, response to endoplasmic reticulum stress, and regulation of epithelial cell proliferation pathways. 16S microbiome profiling demonstrated compositional difference in the gut bacteria between the half-brothers. These results raise the possibility that the observed CNV may contribute to the varied phenotypic characteristics in family members through alterations in gene expression and/or dysbiosis of the gut microbiome. More broadly, there is growing evidence that different neurodevelopmental and psychiatric disorders can share the same genetic variant, which lays a framework for later neurodevelopmental and psychiatric manifestations.

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**Ontology terms:** autism;  
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immune dysregulation;  
intellectual disability, severe;  
microretrognathia

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## INTRODUCTION

Genomic variation is an important contributor to observable phenotypic differences in human traits ranging from morphology, physiology, and behaviors to disease predisposition (Albert and Kruglyak 2015). Individuals differ by thousands to millions of DNA sequence variants, which include single-nucleotide polymorphisms (SNPs), small insertions and deletions (indels), and large structural variants such as block substitutions, inversions, and copy-number variants (CNVs) (Scherer et al. 2007; Frazer et al. 2009; Albert and Kruglyak 2015). Although SNPs are the most common genetic variant, comprising 78% of all variants,

structural variants account for 74% of all variant nucleotides (Frazer et al. 2009; Gamazon and Stranger 2015). Recent efforts have made tremendous strides in identification, categorization, and mapping common and rare variants including the Single Nucleotide Polymorphism Database (dbSNP) (Sachidanandam et al. 2001), haplotype map (The International HapMap Consortium 2005; Frazer et al. 2007), 1000 Genomes Project (Abecasis et al. 2010), DECIPHER (Firth et al. 2009), Exome Aggregation Consortium (ExAC) (Lek et al. 2016), gnomAD (Karczewski et al. 2019), and the Database of Genomic Variants (DGV) (MacDonald et al. 2014). Although identification of variants has progressed rapidly over the past few years, the fundamental challenge to understand the effect of variants has remained (Zarrei et al. 2015).

Although some adaptive CNVs have been described (e.g., the higher copy number of salivary amylase gene (*AMY1*) in populations with a high-starch diet [Perry et al. 2007]), most CNVs are maladaptive and implicated in human disease (Coe et al. 2014; Sudmant et al. 2015; Gambin et al. 2017). CNVs have an established role in autism spectrum disorder (ASD), in which inherited CNVs contribute to an estimated 5%–15% of cases of ASD (Sudmant et al. 2015; Uddin et al. 2015). Likewise, novel deletions and duplications are observed in 15% of cases with schizophrenia (Walsh et al. 2008). CNVs have also been linked with hemiplegic cerebral palsy (Oskoui et al. 2015; Zarrei et al. 2018), developmental delay and intellectual disability (ID) (Cooper et al. 2011; Rees et al. 2016), epilepsy (Helbig et al. 2009), bipolar disorder (Green et al. 2016), and many other disorders (Fanciulli et al. 2010; Sudmant et al. 2015; Uddin et al. 2015; Zarrei et al. 2015; Gambin et al. 2017).

There are a variety of factors that complicate our understanding of the phenotypic consequence of carrying a CNV. First, CNVs in neurodevelopmental disorders are not consistently penetrant with individuals carrying an identical mutation displaying a large degree of phenotypic variability (Woodbury-Smith and Scherer 2018). Penetrance estimates for ASD vary from 5% for mutations in the dystrophin (*DMD*) and neurofibromin (*NF1*) genes to 80% for mutations involving the synaptic scaffold gene (*SHANK3*) and the calcium ion channel gene (*CACNA1C*) (Torre-Ubieta et al. 2016). Second, CNVs can have variation in expressivity resulting in different disease outcomes for the same CNV (Cooper et al. 2011). Stefansson et al. (2014) compared schizophrenia and control patients carrying the same CNV against the general population with a battery of cognitive tests. They showed that schizophrenia patients had the greatest impairment in attention, spatial working memory, executive function, cognitive flexibility, language, and processing speed, and that CNV control patients also showed impaired cognitive abilities in comparison to the general population. Third, the pleiotropic effect may result in multiple different clinical presentations. For example, the 1q21.1 duplication had been described in individuals with ASD, ID, epilepsy, schizophrenia, and other disorders (Bernier et al. 2016). Fourth, ASDs and other neurodevelopmental disorders have high rates of comorbidity: 40%–50% of individuals with an ASD also present symptoms of attention deficit/hyperactivity disorder (ADHD) and 70% present symptoms of ID (Matson and Nebel-Schwalm 2007). Finally, many neurodevelopmental disorders are clinically and genetically heterogeneous, with a complex etiology involving potential risk factors in hundreds of different genes (Damiano et al. 2014; De Rubeis and Buxbaum 2015).

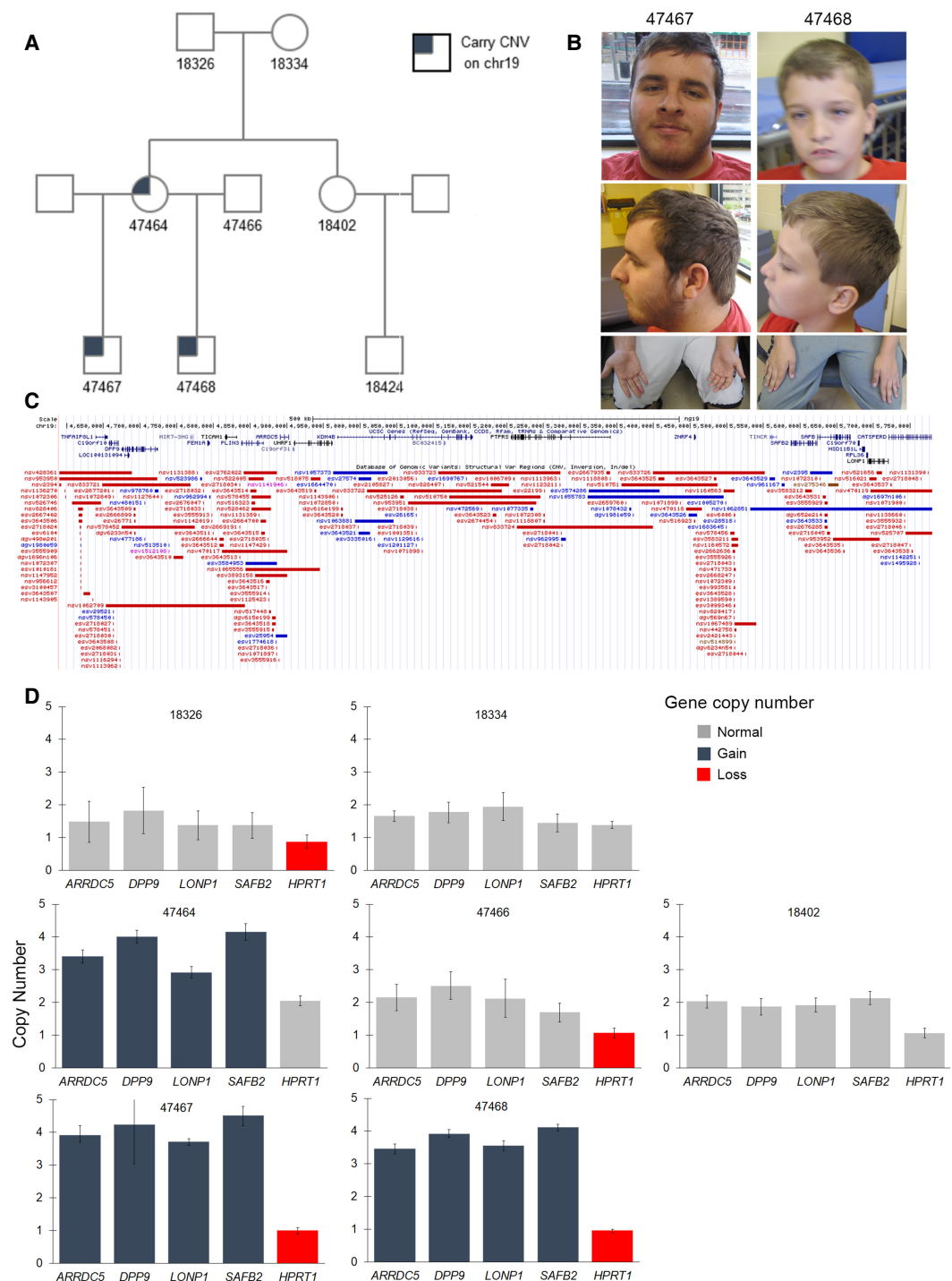
As the number of studies describing novel de novo and inherited CNVs increase, genetic and phenotypic overlap between seemingly distinct neurodevelopmental disorders may begin to illustrate the phenotypic consequence of carrying a particular CNV. In this report we present a family with a 1.089-Mb duplication involving 18 genes on Chromosome 19. Within the family are multiple cases of neurodevelopmental disorders including ASD, ADHD, ID, and psychiatric disease. Quantitative polymerase chain reaction (PCR) confirmed that the CNV is de novo in the mother and inherited by both of her sons. Transcriptome data demonstrates a dosage effect of genes within the CNV and

differential expression of 407 genes throughout the genome. Finally, we use gastrointestinal microbiome data to speculate on the phenotypic differences observed between the boys carrying the CNV.

## RESULTS

Family 23538 (extended pedigree shown in Fig. 1A) has two maternal half-brothers diagnosed with neurodevelopmental disorders. The elder half-brother (47467) has diagnoses of autism, ID, and ADHD; his clinical history indicates he has been followed by pediatricians since the age of 2 yr old for “significant global developmental impairment” and “pervasive and incredible hyperactivity and overflow aggressive behaviors.” The younger half-brother (47468) has diagnoses of ADHD, ID, and suspected ASD; the Autism Diagnostic Observation Scale–2 (ADOS-2) showed that he met cutoff in the moderate severity range. The Wechsler Abbreviated Scale of Intelligence–Second Edition (WASI-II) showed both half-brothers’ IQ scores in the extremely low range (60 for 47467; 69 for 47468) and the mother’s IQ score as 89. Both boys were taking stimulant medication to treat their ADHD (Adderall and quetiapine for 47467; Concerta and clonidine for 47468). Both boys had normal growth parameters, with the exception of truncal obesity in the older brother. Both boys had simple ears, tubular nose, high arched palate, and microretrognathia with prominent front teeth (Fig. 1B). On examination they both had bilateral fetal pads. Their mother (47464) has physical and psychiatric diagnoses that include borderline personality disorder, depression, anxiety, polycystic ovarian syndrome (PCOS), type I diabetes, speech delay, thyroid condition, and irritable bowel syndrome (IBS). The mother has self-reported ASD-like behaviors during childhood, which were confirmed by the maternal grandmother including rocking behaviors, difficulty interacting with her peers, and a lack of eye contact with others. The mother was recently assessed using the Diagnostic Interview for Social and Communication Disorders (DISCO) (Wing et al. 2002) and the International Personality Disorder Examination (Loranger et al. 1994). She fulfilled DSM-5 criteria for borderline personality disorder. She did not meet the criteria for ASD. In the extended family there are diagnoses of ADHD in the mother’s half-brother and in maternal half-cousins. Some of the mother’s conditions are shared by other family members, including PCOS, IBS, type I diabetes, and thyroid conditions. Furthermore, there is a history of mood disorders in the maternal family.

A clinical microarray report provided by the family identified a 1.089-Mb duplication in both half-brothers (47467 and 47468). The CNV was located on Chromosome 19 spanning locations 4,660,013–5,749,143 (hg19 coordinates) and impacts 18 genes *ARRDC5*, *MYDGF*, *C19orf70*, *DPP9-AS1*, *DPP9*, *FEM1A*, *HSD11B1L*, *KDM4B*, *LONP1*, *PLIN3*, *PTPRS*, *RPL36*, *SAFB*, *SAFB2*, *TICAM1*, *CATSPERD*, *UHRF1*, and *ZNRF4* (Fig. 1C). Because both half-brothers carried the CNV, it seemed to be inherited from the mother, although she was not formally tested. We used quantitative PCR (qPCR) to measure copy number of four genes within the CNV (*ARRDC5*, *DPP9*, *LONP1*, *SAFB2*), two genes outside the CNV as a control for copy number (*SDHA*, *DYRK1A*), and a gene on the sex chromosome (*HPRT1*) to validate that the method was effective at detecting copy number. We validated that the CNV was present in the mother and both half-brothers but absent in all other family members and unrelated controls. Figure 1D shows that the maternal grandfather (18326) and father (47466) carry two copies (gray) of the genes in the CNV region and one copy (red) of the gene on the X Chromosome and the maternal grandmother (18334) and maternal aunt (18402) carry two copies of the CNV genes and *HPRT1*, whereas the mother (47464) and both half-brothers carry three copies (blue) of the CNV genes and the expected copy number of the *HPRT1* gene for their sex. Further genetic testing by whole-exome sequencing (WES) for both boys and their mother identified 10 rare, heterozygous variants that were predicted



**Figure 1.** Individuals in Family 23538 carry a 1.089-Mb duplication on Chromosome 19. (A) Pedigree showing two half-brothers and their mother that carry a copy-number variant (CNV) and extended family members that do not carry the CNV. All family members shown with a sample number were evaluated for the CNV. (B) Face, profile, and hand pictures of both boys. (C) View of the CNV, which spans 18 genes, in genome browser, as well as previously reported CNVs (Database of Genomic Variants [DGV]). (D) Quantitative polymerase chain reaction (qPCR) identifies copy number of genes within the CNV region (*ARRDC5*, *DPP9*, *LONP1*, *SAFB2*) and copy number of the X Chromosome (*HPRT1*).

by in silico models to be damaging to the function of that gene (Table 1). The four variants shared by at least both boys were also observed in the neurotypical, maternal aunt (Table 1).

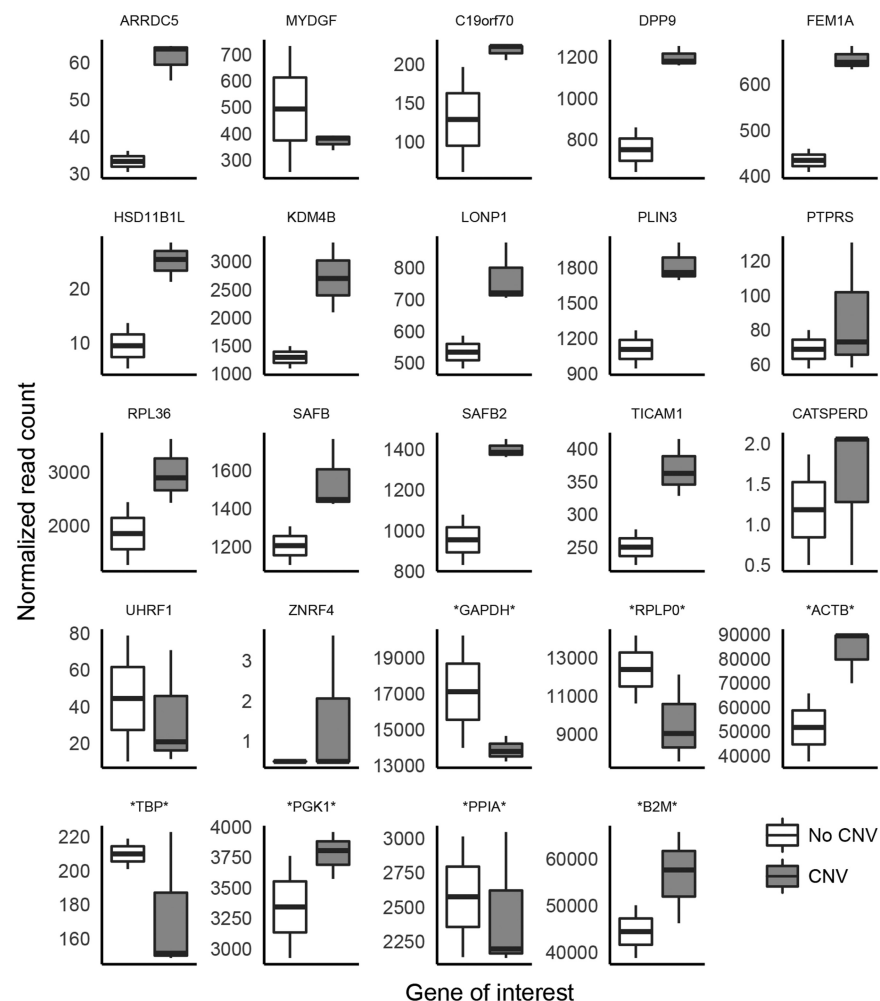
Whole-transcriptome data was analyzed for the three individuals with the CNV and two individuals without the CNV; the maternal aunt (18402), who is an approximate age and gender match for the mother, and a maternal first cousin (18424), who is an approximate age and gender match for the two half-brothers. We detected reads for 15 of the 18 genes located within the CNV region (Fig. 2); reads of the pseudogene *DPP9-AS1* were not detected, and the number of reads aligned to *CATSPERD* and *ZNRF4* were almost negligible. Of the remaining 15 genes in the CNV, 12 were expressed ~1.5-fold higher in the individuals with the CNV when compared to the individuals without the CNV. When considering the whole transcriptome, principal components analysis clustered the individuals carrying the CNV together (Fig. 3A), and differential gene expression identified 407 genes that were differentially expressed in the individuals carrying the CNV versus those without the CNV (Fig. 3B:  $-1.5 \leq FC \leq 1.5$ ;  $P\text{-value} < 0.05$ ;  $P\text{-adj} \leq 0.1$ ). Mapping the 407 genes to gene ontology pathways using WebGestalt identified three overrepresented biological processes including immune response, response to endoplasmic reticulum stress, and regulation of epithelial cell proliferation (Fig. 3C).

Finally, we collected environmental and dietary information relevant to bacterial composition of the gastrointestinal tract and performed microbiota profiling for the two half-brothers using 16S rDNA sequencing. The boys were raised together and are cohabiting, so they share many environmental factors and dietary habits. Several differences reported in their current dietary habits include higher consumption of fish and lower consumption of dairy, artificial sweeteners, and fruit/vegetables by the elder half-brother (47467) compared with the younger half-brother (47268). 16S rDNA sequencing generated 75658 and 83038 mapped reads for individuals 47467 and 47468, respectively. Alpha diversity was similar between the half-brothers with 26 families of bacteria in the stool of individual 47467 and 29 families of bacteria in 47468. Comparing the relative abundance of bacteria at the phylum taxonomic level (Fig. 4A) demonstrates the composition within participant 47467 includes predominantly Firmicutes (85.2%) but also Bacteroidetes (9.9%), Proteobacteria (4.14%), and Actinobacteria (0.75%); whereas 47468 has approximately equal abundance of Firmicutes (41.5%) and Bacteroidetes (54.8%) but also Proteobacteria (2.83%), Tenericutes (0.67%), and Actinobacteria (0.28%). Notable observations at the family taxonomic level include the presence of *Christensenellaceae* in 47468 (Fig. 4B), increased abundance of

**Table 1.** Variant discovery by whole-exome sequencing (WES) in Family 23538

Gene	Chr	HGVS DNA reference	HGVS protein reference	Variant type	dbSNP	Genotype	pLI score	Carriers <sup>a</sup>
<i>RAB3GAP1</i>	2	c.C2011T	p.R671X	Stop-gain	rs137853052	Het	0.00	O
<i>ITPR1</i>	3	c.C4218G	p.H1406Q	Nonsynonymous	rs61757110	Het	1.00	MOYA
<i>FLNB</i>	3	c.G2935A	p.V979M	Nonsynonymous	rs376511120	Het	0.00	Y
<i>HTR1B</i>	6	c.T212C	p.I71T	Nonsynonymous	rs199727324	Het	0.65	MOYA
<i>EPHB6</i>	7	c.G364A	p.G122S	Nonsynonymous	rs8177173	Het	0.25	MOAC
<i>DLGAP2</i>	8	c.C2039T	p.T680M	Nonsynonymous	rs759725513	Het	0.95	MOYA
<i>LAMC3</i>	9	c.C2282T	p.S761L	Nonsynonymous	rs376240028	Het	0.00	MO
<i>AAAS</i>	12	c.T1145C	p.M382T	Nonsynonymous	rs200871966	Het	0.00	Y
<i>SCN4A</i>	17	c.G3313A	p.V1105M	Nonsynonymous	N/A	Het	0.01	MYAC
<i>PTPRT</i>	20	c.G541A	p.E181K	Nonsynonymous	rs868295248	Het	1.00	MOYAC

<sup>a</sup>Indicates family members that carry the variant: Mother-47464; Older sibling-47467; Younger sibling-47468; maternal Aunt-18402, maternal Cousin-18424.

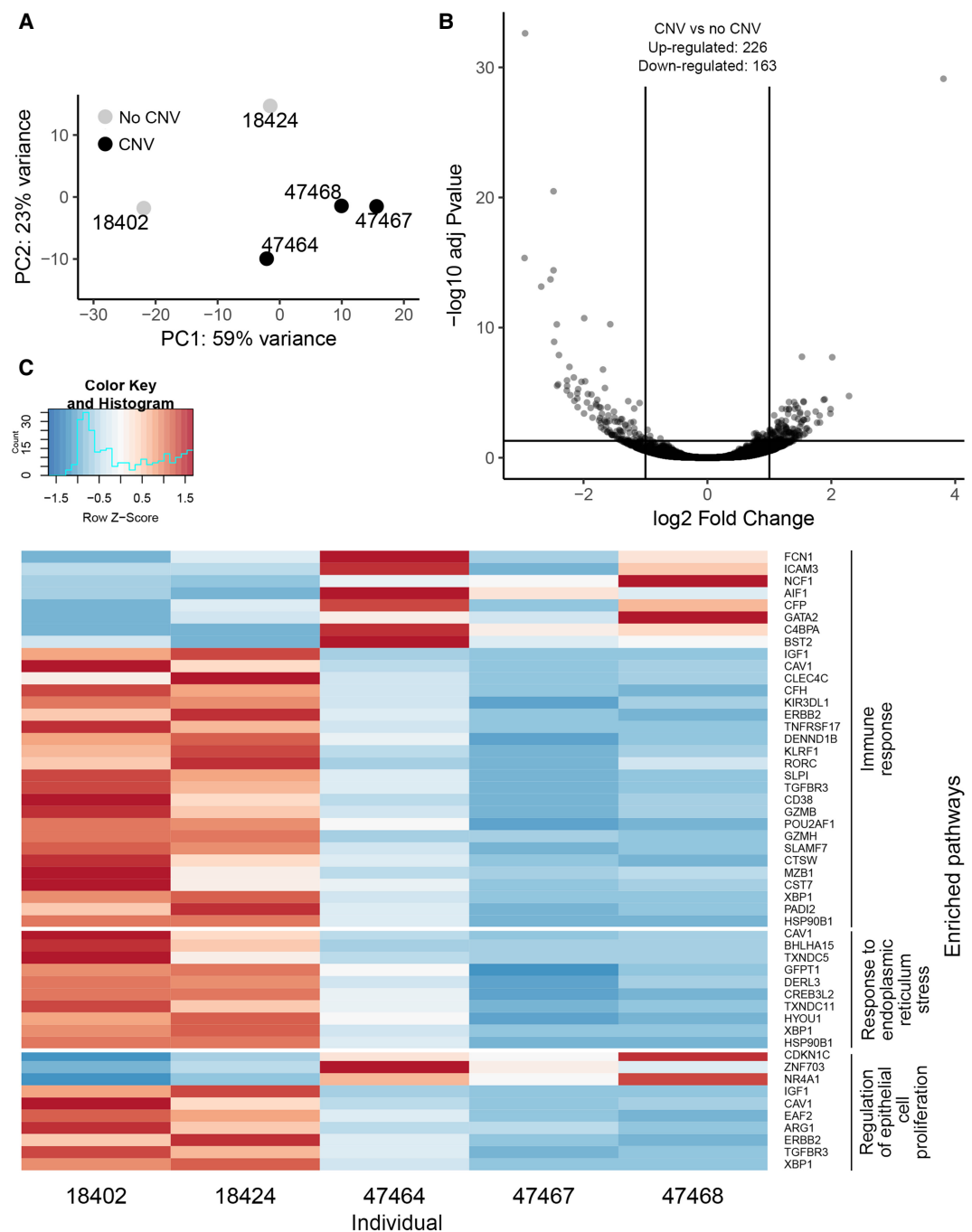


**Figure 2.** Normalized read count of 17 genes within the CNV and seven housekeeping genes (denoted by \*) in family members carrying the CNV (47464, 47467, 47468) and their relatives without the CNV (18402, 18424). Individuals carrying the CNV show an ~1.5-fold increase in transcript abundance in 12 of the genes contained within the CNV region when compared to their relatives not carrying the CNV. *DPP9-AS1* is not shown on this figure because no reads mapping to this gene were observed in the transcriptome data set.

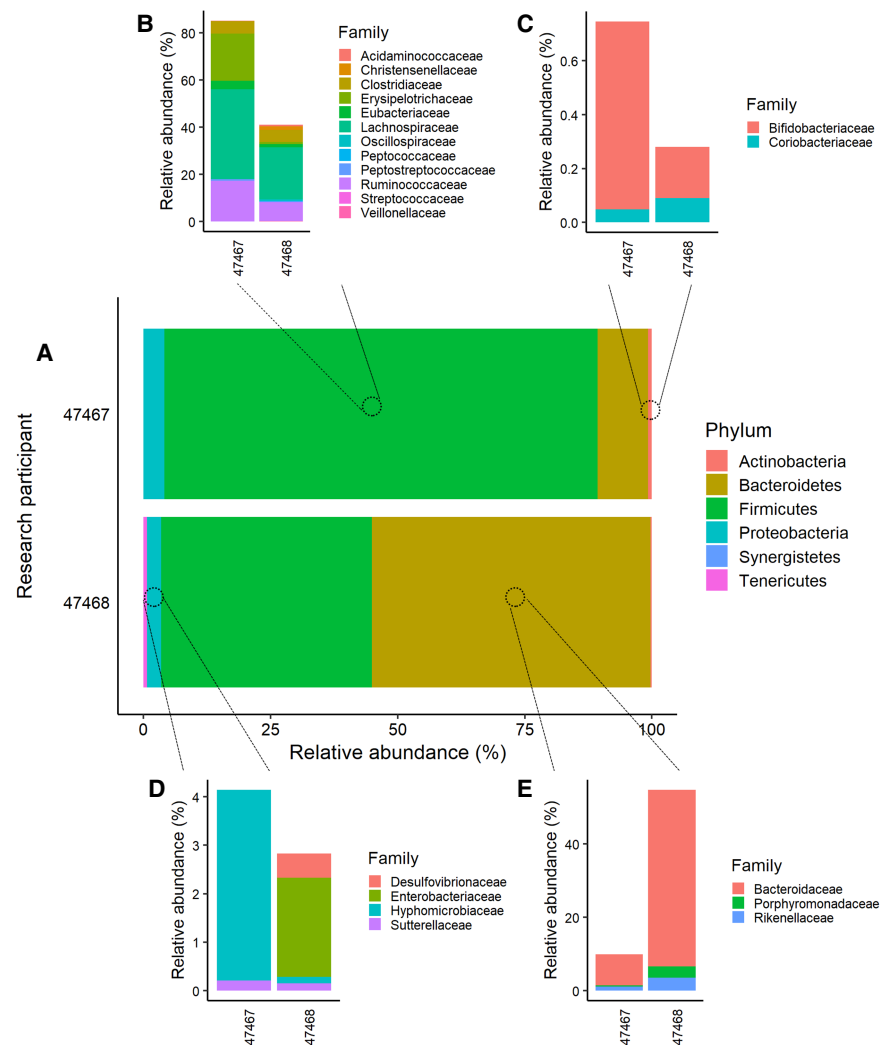
*Bifidobacteriaceae* in 47467 (Fig. 4C), and presence of *Hyphomicrobiaceae* in 47467 but *Desulfovibrionaceae* and *Enterobacteriaceae* in 47468.

## DISCUSSION

Family 23538 enrolled in our research registry because both children had a diagnosis of a neurodevelopment disorder. The family provided clinical reports demonstrating that both children had a 1.089-Mb duplication on Chromosome 19 detected by microarray. Using qPCR, we validated that the CNV was carried by both boys and their mother. This CNV was absent in the father, who provided a sample, and the rest of the extended maternal family indicating that the CNV occurred de novo in the mother and was inherited by both sons. To identify other potential contributing genetic factors, we used WES to identify 10 rare,



**Figure 3.** Whole-transcriptome and pathway analysis of genes differentially expressed in the peripheral blood of family members carrying the CNV. (A) Principal components analysis (PCA) for the first two axes explain 82% of the variability in the data set. (B) Volcano plot shows 245 up-regulated and 162 down-regulated genes in the family members carrying the CNV compared with noncarrier family members. (C) Gene Ontology pathway analysis using WebGestalt identified three overrepresented biological processes including immune response, response to endoplasmic reticulum stress, and regulation of epithelial cell proliferation.



**Figure 4.** Bacterial composition of the gastrointestinal tract of the two half-brothers carrying the CNV as determined by 16S rRNA sequencing. (A) Relative abundance of bacteria at the phylum taxonomic level. (B–D) Relative abundance of the bacteria at the family taxonomic level within the (B) Firmicutes, (C) Actinobacteria, (D) Proteobacteria, and (E) Bacteroidetes phylum.

damaging variants in the family. A nonsynonymous variant in the *DLGAP2* gene was confirmed by Sanger sequencing in all three family members. *DLGAP2* encodes a membrane-associated protein that has a role in synaptic organization and neuronal signaling and has evidence as an ASD candidate gene (Marshall et al. 2008; Pinto et al. 2010; Chien et al. 2013). However, the variant in *DLGAP2*, and five other variants in the *ITPR1*, *HTR1B*, *EPHB6*, *PRPRT*, and *SCN4A* genes, are also carried by the neurotypical aunt, suggesting that they are not responsible for the phenotypes observed in Family 23538. A stop-gain variant in *RAB3GAP1* was observed only in the older brother, indicating that this variant may be de novo or inherited from his father. *RAB3GAP1* encodes a subunit of RAB GTPase activating protein and is thought to be involved in the neurotransmitter exocytosis which is important during eye and brain development (Aligianis et al. 2005). Inactivation of *RAB3GAP* via homozygous mutations in *RAB3GAP* has been implicated in Warburg Micro

syndrome (Aligianis et al. 2005), a developmental disorder that often includes severe intellectual disability (Bokhoven 2011; Rump et al. 2016). The *RAB3GAP1* variant reported in participant 47467 is a heterozygous variant, and the pLI score of *RAB3GAP1* is 0.00, indicating that this heterozygous variant is likely not responsible for the deficiencies observed in this family. Therefore, the 1.089-Mb duplication on Chromosome 19 was the only detected genetic risk factor that segregates with disease in the family, raising the possibility that the duplication contributes to the observed phenotypic deficiencies and neurodevelopmental disability diagnoses observed in this nuclear family.

### The Female Protective Model

Levy et al. (2011) reported that females with ASDs have a higher frequency of de novo events than males, but males are more likely to be referred for genetic testing (Jacquemont et al. 2014). The female protective model (or male susceptibility model) suggests that females have a protective mechanism that shields them from the effects of genetic variation (Jacquemont et al. 2014), and a mother can pass dominant mutations that have reduced penetrance in females to multiple affected male children (Torre-Ubieta et al. 2016). This model is supported by the 4:1 male to female gender disparity in ASD prevalence (Newschaffer et al. 2007). The 1.089-Mb CNV reported in this family supports the female protective mechanism. The mother displays less severe phenotypic characteristics, such as an IQ that is in the low average range and gene expression more similar to family members without the CNV, as seen in both the PCA plot (Fig. 3A) and heatmap (Fig. 3C).

A recent study demonstrated no significant difference in the Adult Autism-Spectrum Quotient score between individuals diagnosed with borderline personality disorder and ASD (Dudas et al. 2017), suggesting that there may be overlap between the two disorders or that individuals (especially females) with borderline personality disorder may be misdiagnosed (their ASD was overlooked), and a proportion may have both conditions. The mother presented in this report was diagnosed with borderline personality disorder and has self-reported ASD-like behaviors during childhood. Further testing demonstrated that the mother does not have ASD supporting the presence of distinct but possibly etiologically related disorders.

### Gene Dosage Effect

The 1.089-Mb duplication on Chromosome 19 is much larger than previously reported CNVs in this region. The DGV contains 331 CNVs in this region of Chromosome 19 (Fig. 1C) including 280 deletions and 44 insertions ranging in size from single-nucleotide indels to a 362,050-bp insertion (Coe et al. 2014), which overlaps the genes at the 3' end of this CNV. The gnomAD-SV browser (Collins et al. 2019) contains 145 CNVs in this region, including 77 deletions and 62 duplications/insertions ranging from 51 bp to 80,417 bp. CNVs in this region of Chromosome 19 have been reported in CNV maps for individuals with ASD, although they did not maintain statistical significance following Benjamini–Hochberg correction (Cooper et al. 2011). Although dysfunction of each gene may not result in psychiatric disability, the cumulative involvement of multiple genes within this large CNV may reflect an accumulated risk for presentation of several psychiatric disorders. We measured the expression levels of the 18 genes in the peripheral blood for the three individuals carrying the CNV as well as controls from the extended maternal family that were approximate age and gender matched and found that 15 genes were expressed in the peripheral blood. Of the 15 genes whose expression was detectable in the blood, 12 genes were expressed ~1.5-fold higher in individuals carrying the CNV than their relatives, indicating the CNV may have a dosage effect on the expression of the genes contained within the CNV. The Clinical Genome Resource (ClinGen) consortium has developed a community resource

designed to improve our understanding of genomic variation and its use in clinical care (Rehm et al. 2015) and are currently curating genes and regions of the genome to assess whether there is evidence to support that these genes/regions are dosage-sensitive. However, the genes in this reported CNV have not yet been curated by ClinGen. Although there is not an obvious connection between the genes differentially expressed in the peripheral blood and neurodevelopmental disability, the CNV dosage effect may also affect other tissues and other periods of development that may impede typical neurological development. For example, *PTPRS* is a member of the protein tyrosine phosphatase (PTP) family, which is involved in cell–cell interaction, primary axonogenesis, axon guidance during embryogenesis (Shen et al. 2009), and molecular control of adult nerve repair (Lang et al. 2015). Several rodent studies have shown that 60% of *Ptp* double-knockouts are lethal, whereas the surviving mice show severe developmental delays, craniofacial defects, and neurologic defects (Wallace et al. 1999; Uetani et al. 2009). Although overexpression of *PTPRS* in the human brain has not been reported to date, regulated expression of *PTPRS* appears to be very important for typical embryonic and brain development.

When considering the whole transcriptome, the family members carrying the CNV demonstrated differential expression of 407 genes, 245 overexpressed and 162 underexpressed, compared with their typical relatives. Pathway analysis maps the genes to three overrepresented pathways including immune response, response to endoplasmic reticulum stress, and regulation of epithelial cell proliferation. There is extensive evidence of immune system implications in neurological processes, and research supporting immune system dysfunction in ASDs (Onore et al. 2012). Dysfunctional immune activity has been observed in ASD spanning both the innate and adaptive immune systems, which can impact neurodevelopment, cognitive function, and behavior (Onore et al. 2012). *CD38* has an important role in social recognition through its role as a transmembrane protein engaged in oxytocin secretion (Munesue et al. 2010; Sauer et al. 2012). The three individuals carrying the CNV demonstrated a reduction in peripheral blood expression levels of *CD38* (Fig. 3C). Reduced *CD38* expression has been reported previously in immortalized lymphocytes derived from an individual with ASD (Lerer et al. 2010). Furthermore, two *CD38* SNPs have been associated with high functioning cases of ASD (Munesue et al. 2010), in which the genotype of one of these *CD38* SNPs (rs3796863) modulates oxytocin activation of the fusiform gyrus (Sauer et al. 2012). Recently, Martucci et al. (2019) reported that *CD38* knockout male mice show an abnormal cortex development, a shifted excitation–inhibition balance, and impaired synaptic plasticity in the prefrontal cortex such as those observed in various mouse models of ASD.

The transcriptome data presented has two key limitations: the use of peripheral blood to understand the biological consequence of carrying a mutation and the small sample size. Though gene expression in the peripheral blood may indicate some effects of the CNV, the observed gene expression profile reported here may not represent gene expression in the brain or during development. Comparing the transcriptomes of three individuals carrying a CNV with two individuals without the CNV does not allow for a proper statistical power analysis, so these results only have exploratory value (Conesa et al. 2016).

### The Role of the Gut Microbiome

A female protective model may account for the phenotypic differences observed between the mother and her two sons (discussed above), but it does not account for the differences between the two boys. Although some of these differences may be accounted for by their different fathers, there may be other factors to consider. Emerging studies have demonstrated the gut microbiome is an integral component of human health by contributing to the host's digestion, nutrition, and immune system where dysbiosis of the gut microbiome has been implicated in a wide range of diseases including ASD (DeGruttola et al. 2016).

Several of these dysbiosis trends are observed in the elder half-brother (47467) who presents with a more severe phenotype when compared with the younger half-brother (47468), including lower Bacteroidetes/Firmicutes ratio (Strati et al. 2017) and increased *Clostridium* (Eshraghi et al. 2018) and *Sutterella* (Williams et al. 2012; Wang et al. 2013b). Although both half-brothers display neurodevelopmental disorders, potentially as a result of the CNV, we propose that the observed differences in phenotype may be, in part, a result of differences in the composition of the gut microbiome. The microbiome data has the same sample size limitation as the transcriptome data set (described above) and should be viewed as exploratory. Future studies correlating gut microbiota and blood metabolites may provide a better view of which bacterial compounds make it into circulation and may impact host metabolism and health (Wilmanski et al. 2019).

In conclusion, we present a family in which the mother carries a de novo 1.089-Mb duplication on Chromosome 19 and passed the CNV to both of her sons. All three individuals display neurodevelopmental disorders including ASD, ADHD, ID, and psychiatric disorders. WES sequencing did not identify any additional variants that segregate within the family, which may account for the observed phenotype. As extended, neurotypical family members carry the same damaging variants but not the CNV, we propose that the CNV may contribute to the etiology underlying these disorders. Exploratory analysis comparing the transcriptome of individuals carrying the CNV with relatives without the CNV demonstrated a dosage effect on the expression of the 18 genes within the CNV region and altered transcriptome in the peripheral blood including dysregulation of many genes involved in immune response. Finally, microbiome differences between the half-brothers may contribute to the observed phenotypic differences. These results raise the possibility that the observed CNV may contribute to varied phenotypic characteristics in different family members through altered gene expression or through altered gut microbiota composition, and more broadly that different psychiatric disorders can share genetic variants that lay a framework for later psychiatric dysfunction.

## METHODS

### Sample Collection

Family members carrying the CNV (47464, 47467, 47468) and two members not carrying the CNV, a maternal aunt (18402) and a maternal cousin (18424), supplied whole blood in EDTA tubes for DNA extraction, and whole blood in Biometrika's RNAgard tubes for RNA extraction. One of the fathers (47466), the maternal grandparents (18326, 18334), and other members of the extended family supplied saliva for DNA extraction. The other father was unavailable to provide a sample. Additionally, both half-brothers provided stool samples to represent the gastrointestinal microbiome community. DNA was isolated from saliva following QIAGEN's FlexiGene DNA Handbook and from whole blood in EDTA tubes using Affymetrix's PrepEase Genomic DNA Isolation Kit. Total RNA was isolated from whole blood collected in RNAgard tubes following Biometrika's RNAgard Blood RNA Purification Kit User Manual. Bacterial DNA was isolated from 200 mg of stool using QIAGEN's Allprep PowerFecal Kit. DNA quantity was determined using the Qubit Fluorometer 2.0 dsDNA Broad Range Assay Kit (ThermoFisher Scientific) and quality via gel electrophoresis. RNA quantity was determined using the Qubit 2.0 RNA Assay Kit and quality on an RNA 6000 Pico Kit on the Agilent Technologies Bioanalyzer 2100.

### Confirmation of CNV

Forward and reverse primers were designed using NCBI PrimerBlast for four genes in the duplicated region (*ARRDC5*, *DPP9*, *LONP1*, and *SAFB2*), two autosomal genes for copy-

**Table 2.** Sequence and location of primers used for quantitative polymerase chain reaction (qPCR) validation of the copy-number variant (CNV) on Chromosome 19 in members of family 23538

Gene name	Primer sequence (5'–3')	Chromosome:location
<i>DPP9</i>	Forward: ATGTGGCCCTTAGCCTCTC Reverse: CTGACTCGTGCCCTGGACTA	Chr 19:4,689,228–4,689,312
<i>LONP1</i>	Forward: TTCCAGCACGGGATTGTG Reverse: GAGGTTGGGGGAAAATGCG	Chr 19:5,707,215–5,707,325
<i>SAFB2</i>	Forward: TTACCGGAATCAACCACCTAATG Reverse: TGTGCTTTTACCATCCCATAG	Chr 19:5,610,429–5,610,541
<i>ARRDC5</i>	Forward: AGTGCTGCCCCGAGGATAGAA Reverse: TCCACCTTCACTATGGGGTC	Chr 19:4,902,710–4,902,811
<i>SDHA</i>	Forward: CAATATACACGTCTTCCCTTC Reverse: CTCAGATACGAAATGAAAAAGGCAC	Chr 5:223,240–223,326
<i>DYRK1A</i>	Forward: CTGGGCCTTGTTTTACCCTC Reverse: TGAACCTGTGCCCTCATCAAG	Chr 21:38,794,783–38,794,888
<i>HPRT1</i>	Forward: TACGGGCCAACCTGACAA Reverse: GGTGTGTGCTGTCTTCAGTC	Chr X:133,604,318–133,604,403

number controls (*SDHA* and *DYRK1A*), and a sex chromosome gene (*HPRT1*) (Table 2). qPCR was performed on Applied Biosystems Viia7 Real-Time PCR machine using ThermoFisher Scientific's 1X Power SYBR Green Master Mix, 100 nM of each primer, and 3 ng of gDNA with the following cycling conditions: initial 10 min denaturation at 95°C, 40 cycles of 15 sec at 95°C, 1 min at 60°C with data collection, and melt curve at 0.5°C/sec increments from 60°C to 95°C. The qPCR data was analyzed using qbase+ v3.1 ([www.qbaseplus.com](http://www.qbaseplus.com)).

### Whole-Exome Sequencing

Exome sequencing and variant discovery were performed for both half-brothers (47467 and 47468) and their mother (47464) as we have described previously (Sjaarda et al. 2019), with the exception that we used an Ion 550 Chip on the Ion GeneStudio S5 Plus Semiconductor Sequencer. WES generated an average of 36,858,736 reads and base coverage depth of 94.36 (Table 3). The 10 reported variants were validated in the three family members and the maternal aunt and cousin by Sanger sequencing as previously described (Sjaarda et al. 2019).

### Whole-Transcriptome Sequencing

mRNA was isolated from total RNA using ThermoFisher Scientific's Dynabeads mRNA DIRECT Micro Kit recommended protocol for low total RNA input. Libraries were constructed using ThermoFisher Scientific's Ion Total RNA-seq Kit v2 for Whole-Transcriptome Libraries using IonXpress RNA 3' Barcode Primers to allow for multiplex sequencing. Library yield and size distribution were assessed with the Bioanalyzer 2100 system with

**Table 3.** Exome sequencing coverage

Sample	Mapped reads	Average base coverage depth	Uniformity of base coverage
47464	34,050,284	86.2	92.71%
47467	40,538,962	106.9	94.38%
47468	35,986,961	90.0	94.43%

the High Sensitivity DNA Kit. ThermoFisher Scientific's Ion OneTouch 2 System was used for templating following Ion PI Hi-Q OT2 200 Kit protocol. Whole-transcriptome sequencing runs were performed with the Ion Proton System following Ion PI Hi-Q Sequencing 200 Kit protocol on an Ion PI Chip v3 (ThermoFisher Scientific).

### Transcriptome Data Analysis

Raw sequencing reads were exported from the Ion Torrent Suite and analyzed using Partek Flow software, version 7.0.18.0401 (Partek Inc.). Reads were aligned to the hg19 reference genome using Torrent Mapping Alignment Program (TMAP) and gene counts were quantified to the RefSeq annotation model. Differential gene expression was determined using DESeq2 release 3.7 in R version 3.5.0. Significant differential expression was determined based on  $P$ -value  $< 0.05$ , adjusted  $P$ -value  $< 0.1$ , and fold change of  $< -1.5$  or  $> 1.5$ . The on-line tool WebGestalt (WEB-based Gene SeT AnaLysis Toolkit) (Zhang et al. 2005; Wang et al. 2013a) was used to identify overrepresented biological processes and gene ontology pathways.

### Microbiome Sequencing

Using 3 ng of DNA as input, 16S hypervariable regions were amplified using both primer sets and reagents in the Ion 16S Metagenomics Kit (ThermoFisher Scientific) following manufacturer's recommendations and 18 PCR cycles on an Applied Biosystems GeneAmp PCR System 9700. PCR products were ligated with Ion Xpress Barcode Adapters 1-16 Kit (ThermoFisher Scientific) to allow for multiplexing, and libraries were quantified using the Ion Universal Library Quantitation Kit (ThermoFisher Scientific) on a Vii7 Real-Time PCR machine (ThermoFisher Scientific). Using 10 pM of library as input, template preparation was performed using the Ion OneTouch 2 System and the Ion PGM Hi-Q OT2 Kit, before loading on an Ion 318 chip v2 and sequencing on the Ion PGM System using the Ion PGM Hi-Q Sequencing Kit (ThermoFisher Scientific). Raw sequence data files were uploaded to ThermoFisher's Ion Reporter and analyzed using the Metagenomics 16S 21.1 version 5.10 workflow. The consensus file for each sample was imported into R version 3.5.2 and figures generated using ggplot2 version 3.1.0. In addition, both boys completed a survey collecting information regarding birth delivery method, early childhood health and diet information, current dietary habits, and gastrointestinal history.

## ADDITIONAL INFORMATION

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### Data Deposition and Access

Whole-exome, RNA-seq, and microbiota data are available under the NCBI Sequence Read Archive (SRA) study accession numbers PRJNA625607 and PRJNA626020.

### Ethics Statement

Informed written consent was obtained from all participants or their legal caregivers prior to the study. All experimental protocols were approved by and conducted in accordance with the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (PSIY-074-99 and PSIY-121-01).

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**Competing Interest Statement**

The authors have declared no competing interest.

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**Author Contributions**

C.P.S. and X.L. designed the study; C.P.S., B.K., A.J.M.M., and M.L.H. recruited the participants and collected samples; A.G., M.L.H., B.K., and M.A. collected the phenotype data; C.P.S. performed next-generation sequencing; C.P.S., B.K., L.H.-L., and K.L. analyzed data and performed validation; C.P.S. drafted the manuscript; and all authors reviewed the manuscript.

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## De novo duplication on Chromosome 19 observed in nuclear family displaying neurodevelopmental disorders

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