HHS Public Access

Author manuscript

Am J Med Genet B Neuropsychiatr Genet. Author manuscript; available in PMC 2025 January 01.

Published in final edited form as:

Am J Med Genet B Neuropsychiatr Genet. 2024 January ; 195(1): e32952. doi:10.1002/ajmg.b.32952.

Genetic liability for gastrointestinal inflammation disorders and association with gastrointestinal symptoms in children with and without autism

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AUTHOR CONTRIBUTIONS

This study was conceived and designed by Christine Ladd-Acosta with input from Kelly Benke, M. Daniele Fallin, and Valerie Morrill. Polygenic score derivation and statistical analyses were completed by Valerie Morrill under the mentorship of Christine Ladd-Acosta and Kelly Benke with additional input from M. Daniele Fallin. John Brinton performed reproducibility analyses. Ann M. Reynolds, Victoria Fields, Gnakub N. Soke, and Calliope Holingue provided input on measures of GI symptoms and data variables in SEED and input on their analyses. Funding and participant recruitment, sample collection, and genotype measurements was led by M. Daniele Fallin, Craig J. Newschaffer, and Laura A. Schieve. The paper was written by Valerie Morrill, Christine Ladd-Acosta, and Kelly Benke. All authors contributed to interpretation of results and edited and reviewed the manuscript.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

CONFLICT OF INTEREST STATEMENT

Dr. Christine Ladd-Acosta reports receiving consulting fees from the University of Iowa for providing expertise on epigenetics outside of this work. The other authors have no conflicts of interest to disclose.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Abstract

Children with autism spectrum disorder (ASD) have a greater prevalence of gastrointestinal (GI) symptoms than children without ASD. We tested whether polygenic scores for each of three GI disorders (ulcerative colitis, inflammatory bowel disease, and Crohn's disease) were related to GI symptoms in children with and without ASD. Using genotyping data (564 ASD cases and 715 controls) and external genome-wide association study summary statistics, we computed GI polygenic scores for ulcerative colitis (UC-PGS), inflammatory bowel disease (IDB-PGS), and Crohn's disease (CD-PGS). Multivariable logistic regression models, adjusted for genetic ancestry, were used to estimate associations between each GI-PGS and (1) ASD case–control status, and (2) specific GI symptoms in neurotypical children and separately in ASD children. In children without ASD, polygenic scores for ulcerative colitis were significantly associated with experiencing any GI symptom (adjusted odds ratio (aOR) = 1.36, 95% confidence interval (CI) = 1.03–1.81, p = 0.03) and diarrhea specifically (aOR = 5.35, 95% CI = 1.77–26.20, p = 0.01). Among children without ASD, IBD-PGS, and Crohn's PGS were significantly associated with diarrhea (aOR = 3.55, 95% CI = 1.25–12.34, p = 0.02) and loose stools alternating with constipation (aOR = 2.57, 95% CI = 1.13–6.55, p = 0.03), respectively. However, the three PGS were not associated with GI symptoms in the ASD case group. Furthermore, polygenic scores for ulcerative colitis significantly interacted with ASD status on presentation of any GI symptom within a European ancestry subset (aOR = 0.42, 95% CI = 0.19–0.88, p = 0.02). Genetic risk factors for some GI symptoms differ between children with and without ASD. Furthermore, our finding that increased genetic risks for GI inflammatory disorders are associated with GI symptoms in children without ASD informs future work on the early detection of GI disorders.

Keywords

autism spectrum disorder; co-occurring condition; gastrointestinal; genetic; polygenic score

1 | INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition defined as having challenges in social communication and interactions as well as restricted or repetitive behaviors (American Psychiatric Association, 2013). The most recent prevalence estimates from 2018 show 1 out of every 44 children from 11 communities in the United States has ASD (Maenner et al., 2021). Studies have shown a significantly higher prevalence of gastrointestinal (GI) symptoms among children with ASD compared to those without ASD (Bresnahan et al., 2015; Chaidez et al., 2014; Galli-Carminati et al., 2006; McElhanon et al., 2014).

While there is no consensus among the scientific community about why children with ASD experience more GI symptoms, at least two primary explanations have emerged. The first postulates that GI symptoms are a result of sensory differences or increased restrictive and repetitive eating behaviors among children with ASD. Recent research supporting this theory focused on problem eating behaviors (Bandini et al., 2010; Fields et al., 2021; Johnson et al., 2008), sensory-processing difficulties (Ahearn et al., 2021), and family factors (Geraghty et al., 2010). Children with ASD exhibit more food refusal than typically

developing children (Bandini et al., 2010), and have more mealtime behavioral differences (Johnson et al., 2008). These behavioral differences, however, have not been linked to specific GI symptoms experienced among ASD cases.

The second mechanistic theory posits that GI symptoms could be due to inherent biologic factors that are part of a causal pathway leading to both GI symptoms and ASD. Supporting this theory, publications have shown a role of inappropriate immune functioning (Careaga et al., 2010; Onore et al., 2012), alterations to the microbiome (Finegold, 2008; Finegold et al., 2010), the gut-brain axis (Hediger et al., 2008), and impaired intestinal barrier (de Magistris et al., 2010; d'Eufemia et al., 1996) in ASD etiology or treatment. Children with ASD have widespread changes in their immune systems and ongoing inflammation (Careaga et al., 2010; Onore et al., 2012), alterations in microbiome (Finegold et al., 2010), an altered intestinal permeability (d'Eufemia et al., 1996), and differences in their micro-transcriptome (Beversdorf et al., 2022) compared to those without ASD. Although these biological differences have been observed in ASD, there have been no data examining associations between these biological differences and ASD GI symptoms, and no clear evidence-based treatment guidelines for physicians exist to reduce GI symptoms among individuals with ASD (Furuta et al., 2012).

In this study, we derive three GI disorder polygenic scores (PGS), proxy measures of genetic susceptibility to GI symptoms, and investigate differences in GI genetic susceptibility between ASD and typically developing children enrolled in the Study to Explore Early Development (SEED). GI disorder PGS values can approximate genetic risk for symptoms and can be used to compare whether GI genetic liability is associated with GI symptoms in children with ASD compared to children without ASD. Our goal is to inform our understanding of the role of genetic mechanisms in influencing the co-occurrence of GI symptoms in ASD and inform strategies for treating these symptoms to improve daily functioning for people living with ASD.

2 | METHODS

2.1 | The Study to Explore Early Development (SEED): overall study design

In this study, we examined a subset of 1279 participants enrolled in SEED Phase I (SEED 1) with genome-wide genotyping and an adjudicated ASD or typical control outcome classification. SEED is a multisite case–control study based in the United States that was designed to investigate risk factors for ASD, including environmental and genetic factors (Schendel et al., 2012). SEED 1 enrolled children aged 2–5 years, approximately balanced between ASD cases, population-based controls (Peterson et al.), and children with non-ASD developmental delays, and their parents. All children were recruited from the same geographic areas and birth date range (Schendel et al., 2012). SEED ascertained rigorous clinical phenotyping, information on GI symptoms and conditions, as well as biospecimens. A detailed description of SEED recruitment, inclusion/exclusion criteria, clinical phenotyping, and data collection procedures can be found in Schendel et al. (2012). All families provided written consent for participation and institutional review boards at local study sites and the Centers for Disease Control and Prevention (CDC) approved the study.

2.2 | ASD case definition

Detailed information on SEED 1 clinical evaluations and ASD diagnostic criteria are previously published (Wiggins et al., 2015). Briefly, SEED participants were recruited from one of three ascertainment groups: (1) the general population (Peterson et al., 2019); (2) children with a broad array of developmental delays or disorders (DD); and (3) children with ASD. Only ASD cases and POP controls were included in this report. Children were screened with the Social Communication Questionnaire (SCQ) (Rutter et al., 2007). Children who scored 11 or greater, or who had previously been diagnosed with ASD (regardless of SCQ score), completed a full ASD developmental evaluation that included both the Autism Diagnostic Observation Schedule (ADOS) (Anttila et al., 2018) (Gotham et al., 2007) and Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994). All children (including those with SCQ < 11) completed the Mullen Scales of Early Learning (Mullen, 1995) developmental evaluation. Children with a mental age of 24 months or greater were classified as an ASD case if they met ASD diagnostic criteria on the revised ADOS and met the ADI-R diagnostic criteria or any one of three ADI-R relaxed criteria, as approved by the instruments' authors. Children who had a mental age less than 24 months were classified as ASD if they met the above criteria and a clinician was certain they had ASD via the adapted Ohio State University Autism Rating Scale. Children recruited as POP controls who scored 11 or greater on the SCQ but did not otherwise meet the criteria for ASD classification were retained as POP controls (Schendel et al., 2012) (Wiggins et al., 2015).

2.3 | Gastrointestinal symptom ascertainment

GI symptoms were ascertained in SEED using a GI questionnaire designed for the SEED study (Reynolds et al., 2021). Parents reported whether their child had any current "gastrointestinal (bowel) problems on a regular basis", that is, greater than two times per month. They also reported nine more specific symptoms: vomiting, diarrhea, loose stools, constipation, loose stools alternating with constipation, abdominal pain with meals, abdominal pain relieved by defecation, pain on stooling, and gas.

2.4 | Genome-wide genotyping data measures and quality control

Genomic DNA was isolated from individuals through blood or buccal samples at the SEED biorepository. Samples were sent to the Genetic Resources Core Facility at Johns Hopkins University for processing on the Illumina Omni1M-Quad array, or to the University of California—San Francisco for processing on the Affymetrix Axiom KP array. Rigorous quality control (QC) metrics were applied to the genotype measurements to ensure data integrity by removing poorly performing samples and genomic markers. Samples with marker call rate <98%, a sex discrepancy between genotype and self-report, evidence of unexpected relatedness (Pi-hat >0.2), or excess hetero- or homozygosity were removed. Single nucleotide polymorphisms (SNPs) that had a call rate <96%, were monomorphic, had minor allele frequency <5%, or that deviated significantly ($p < 0.0001$) from Hardy–Weinberg equilibrium were also removed. Eigensoft (Price et al., 2006) was used to compute principal components that represent the genetic ancestry of each sample, which were used as covariates in our downstream analyses. Phasing was performed using SHAPEIT (Delaneau

et al., 2013) and imputation was performed using IMPUTE2 (Howie et al., 2009), with 1000 Genome Project, version 3, as the reference panel (Howie et al., 2011).

A total of 1279 unrelated individuals passed QC, completed imputation, had information on GI symptoms, and had a final classification definition as POP or ASD. Three hundred and forty-five of these samples were genotyped on the Axiom array, and 934 were genotyped on the omni array. This included 564 ASD cases, and 715 POP controls.

2.5 | Polygenic score derivation

To create a proxy for genetic susceptibility to GI symptoms, we chose inflammatory bowel disease, including Crohn's disease and ulcerative colitis as they have a well-documented genetic basis, and similar symptoms as those more frequently observed in ASD cases, such as diarrhea, loose stool, and abdominal pain (Baumgart & Sandborn, 2012; Danese & Fiocchi, 2011). Crohn's disease and ulcerative colitis are the two types of inflammatory bowel disease (Liu et al., 2015). Large-scale genome-wide association studies have identified genetic variants associated with inflammatory bowel disease (Alonso et al., 2015; Duerr et al., 2006; Festen et al., 2011; Liu et al., 2015), and estimated heritabilities of 0.67 for ulcerative colitis and 0.75 for Crohn's disease (Chen et al., 2014). Polygenic scores take variation in multiple genes into account and can serve as a measure of underlying aggregate genomic susceptibility (Wray et al., 2007).

We calculated PGSs for Crohn's disease (CD-PGS), ulcerative colitis (UC-PGS), and inflammatory bowel disease (IBD-PGS) in our SEED analytic sample based on summary statistics from a large genome-wide association study (GWAS) conducted by the International Inflammatory Bowel Disease Genetics Consortium (Liu et al., 2015). After downloading the summary statistics, we applied several filters for QC purposes. We removed SNPs that had minor allele frequency >5%, an imputation information score >0.6, and a p-value for heterogeneity in direction of effect between cohorts >0.05. We excluded duplicate SNPs, insertions, deletions, and palindromic SNPs. In order to account for linkage disequilibrium among SNPs, a two-step clumping procedure was performed in PLINK 1.9 (Chang et al., 2015). We first carried out short rage clumping of SNPs by specifying an r^2 value > 0.5 within a 250 kb window, and then carried out a longer-range clumping step on the remining SNPs by specifying an r^2 value > 0.2 within a 5000 kb window. Discovery GWAS sample sizes before and after filtering are listed in Table S1.

Ideally, we would evaluate the associations of our PGSs with each GI disorder in our sample to evaluate an optimal p-value threshold for inclusion of SNPs in a GI-PGS calculation. Our analytic sample of young children has only one UC and no CD cases, as expected in a childhood sample, so we could not set a p-value threshold for PGS prediction of these disorders in our sample empirically. Instead, we included SNPs in the three PGSs whose discovery findings fell below $p < 0.001$, as suggested by a previous simulation study (Zhang et al., 2018) to maximize prediction. The total number of SNPs included in the PGS calculated for ulcerative colitis was 615, for Crohn's disease was 740, and for inflammatory bowel disease was 826. Genetic scoring was carried out in PLINK 1.9 using the—score command, which computes a score for each individual by summing across the number of risk alleles for each SNP, weighted by the discovery effect size. We standardized each PGS

by centering to the mean and rescaling so a 1-unit increase is equal to 1 *SD* to aid in the interpretation of the association effect sizes.

2.6 | Statistical analyses

Density plots for each PGS by ASD status were visually inspected to assess normality and identify outliers. To formally test the associations of each GI-PGS with ASD case status, we employed logistic regression. All modeling included the first 10 principal components for ancestry. In addition, we performed logistic regression stratifying by European ancestry status to reduce population stratification. Each regression coefficient represents the change in log odds of ASD for every one standard deviation unit increase in the PGS. *p*-Values <0.05 were considered statistically significant.

Using logistic regression, we assessed the strength of association between the three GI-PGS and each of nine GI symptoms in POP controls and ASD cases separately. The results among POP control children could help determine which symptoms could be explained by the variants captured by the GI-PGS, uninfluenced by the potential behavioral causes of GI symptoms in ASD cases. In *Model 1*, each of nine GI symptoms served as the dependent variable, and were regressed onto each of the three GI-PGS, for a total of 27 models which were restricted to controls:

$$\text{Model 1}: \text{E}(\text{GI symptom among controls}) = \alpha + \beta 1\text{GI} - \text{PGS} + C(\textit{ancestry principal components}\ 1-10) + \varepsilon .$$

In *Model 2*, we performed the same analysis in ASD cases to give insight on whether the PGS has a different effect across ASD cases and controls and to inform the modeling below:

$$\text{Model 2}: \text{E}(\text{GI symptom among cases}) = \alpha + \beta 1\text{GI} - \text{PGS} + C(\textit{ancestry principal components}\ 1-10) + \varepsilon .$$

In *Model 3*, we include a cross-product term to evaluate the statistical interaction between ASD status and GI-PGS to formally test for effect modification of the PGS effect on GI symptoms by ASD case and control status. Outcomes for Model 3 included any GI symptom, and specific GI symptoms that were significantly predicted by the GI-PGS at a multiple testing threshold of $p < 0.016$: In addition, we model the GI-PGS as a binary covariate, with a “high GI risk” group that had GI-PGS in the top tertile, and a “low GI risk” group that had GI-PGS in the bottom two tertiles.

$$\text{Model 3}: \text{E}(\text{GI symptom among both cases and controls}) = \alpha + \beta_1\text{ASD} + \beta 2\text{GI} - \text{PGS} + \beta_3\text{ASD*GI} - \text{PGS} + C(\textit{ancestry principal components}\ 1-10) + \varepsilon .$$

3 | RESULTS

3.1 | Descriptive statistics for GI symptoms, GI-PGS and demographics in SEED

Children with ASD were significantly more likely to have any GI symptoms, and all nine specific GI symptoms, compared to POP controls (Table 1). A larger percentage of ASD

cases were males and European ancestry was significantly higher among controls (70.0%) than ASD cases (53.7%) (Table 1).

The three GI disorder PGSs appeared normally distributed within both the ASD cases and the control samples, and we did not identify any outliers (Figure S1). Unadjusted mean differences between ASD cases and POP controls were observed for each PGS (Table 1). For example, the CD-PGS was significantly ($p = 0.019$) lower in the ASD group (mean = −0.08) compared to the control group (mean = 0.05). However, these associations were not statistically significant after adjusting for genetic principal components or after stratifying by European ancestry (Table 2).

3.2 | Gastrointestinal polygenic score associations with GI symptoms in POP controls and ASD cases

Among POP control children, each GI-PGS was associated with increased odds of having any GI symptom, adjusting for ancestry, but this only reached statistical significance for the UC-PGS ($p = 0.05$) (Figure 1a). When considering nine specific GI symptoms, control children with an increased GI-PGS showed higher odds of constipation, diarrhea, gas, loose stools, and loose stools alternating with constipation, with loose stools alternating with constipation reaching statistical significance for CD-PGS and diarrhea reaching statistical significance for IBD-PGS and UC-PGS (Figure 1b).

Among controls, Diarrhea was significantly predicted by the UC-PGS ($p = 0.015$) at a multiple testing threshold of $p < 0.016$ (Figure 1b), suggesting that this PGS captured variation in diarrhea in our POP control sample. No other GI symptoms were significantly associated with other PGS at the multiple testing threshold.

Among ASD cases, none of the GI-PGS were significantly associated with increased odds of having any GI symptom (Figure 2a) nor with any specific symptoms (Figure 2b).

3.3 | GI effect modification for UC polygenic risk group by ASD case status

Given the differences in ASD outcome-stratified results for the UC-PGS, we tested for GI effect modification for UC-PGS risk group by ASD status. Children were included in the "high GI risk" group if their UC-PGS was in the top tertile of all children included in the analysis. We assessed effect modification on any GI symptom and on diarrhea because these traits were well captured by the UC-PGS in our POP control sample. We find a difference in the effect of UC-PGS on any GI symptom risk by ASD status (cross-product term for interaction aOR = 0.68, 95% CI = 0.37–1.27, $p = 0.22$). This relationship reached statistical significance within a European ancestry subset (aOR = 0.42, 95%CI = 0.19–0.88, $p = 0.02$). Similarly, we also found UC-PGS effect modification on diarrhea risk by ASD among a European ancestry subset (cross-product term for interaction aOR = 0.68, 95% CI = 0.11–3.52, $p = 0.66$).

4 | DISCUSSION

In a national sample of preschool-aged children, we showed that polygenic scores for GI disorders and presentation of GI symptoms differs among those with and without ASD. We

identified polygenic scores associated with the presentation of GI symptoms, specifically diarrhea, among typically developing young children. However, this relationship was not observed among children with ASD. We also found a significant interaction between GI PGS risk group and ASD status on presentation of any GI symptom within a European ancestry subset. This supports the hypothesis that GI symptom presentation in ASD children may have other biologic or behavioral mechanisms.

Beyond relevance to ASD, our results showing increased UC-PGS associated with diarrhea in neurotypical children provides important insights into potential early diagnosis and monitoring of UC. A UC-PGS could be used to identify children at high risk of developing diarrhea prior to diagnoses, although such recommendations would require replication of our findings in larger childhood samples and further input from pediatric gastroenterologists.

Our results provide important insights into potential mechanisms for an increased prevalence of GI symptoms in ASD, but there are several limitations that need to be considered. First, clinician diagnosed GI disorders/symptoms were not available, so we used parent-reported symptoms, which may be subject to recall bias due to collection after ASD diagnoses. GI measurements that include symptoms such as abdominal pain may be further biased in individuals with ASD, especially in young children, and those with language impairment, intellectual disability, or impairments in interoception (Holingue et al., 2018; Holingue et al., 2021). Future studies might benefit from a more objective GI symptom ascertainment, such as clinical diagnosis or incorporating the Bristol stool scale. Second, the GWAS summary statistics that we used to create the PGS were derived from GWAS discovery analyses that only included individuals of European ancestry, but our SEED pediatric sample is much more ethnically diverse. We addressed this concern by adjusting for genetic ancestry in our analyses. Efforts are underway to include more diverse samples in future GWAS (Mahajan et al., 2022; Peterson et al., 2019). Third, we focused on genetic risk variants for IBD including CD, and UC, but it is possible that there are other genetic variants that we did not examine that are associated with both GI symptoms and ASD, similar to the *CD38* and *OXTR* variants identified by Schindler et al (Shindler et al., 2020), or the *MET* variants identified by Campbell et al (Campbell et al., 2009). Thus, our findings do not eliminate the potential role of genetic susceptibility for GI symptoms to account for the increased observation of GI symptoms in ASD cases. Future studies could expand on our results to include PGS for other GI symptoms or conditions (e.g., celiac disease, disorders of gut-brain interaction such as irritable bowel syndrome), as well as other biologic or environmental factors (e.g., microbiome, impaired intestinal barrier function, RNA profile). Other studies could explore the association between behavioral factors (e.g. repetitive eating behaviors, sensory differences) and GI symptoms, including assessing interactions with cofactors such as immunity, sleep, and stress reactivity.

Our findings are consistent with two existing theories about why children with ASD experience more GI symptoms. The first theory supports a behavioral component to the association between GI symptoms and ASD. Mealtime behavioral differences between children with ASD and typically developing children may lead to an increase in GI symptoms (Johnson et al., 2008). These behavioral differences may include food selectivity (Bandini et al., 2010), insistence on nonfunctional mealtime routines, or refusal to try new

foods (Johnson et al., 2008). The second theory supports a unique biological etiology for the co-occurrence of ASD and GI symptoms. Shared biological processes may lead to both GI symptoms and ASD (Johnson et al., 2008). These biological processes may include immune dysfunction (Careaga et al., 2010), abnormal intestinal permeability (d'Eufemia et al., 1996), or abnormal microbiota (Finegold et al., 2010).

Future studies to examine the contribution of behavioral traits and biological mechanisms of this co-occurrence are warranted. Our findings also highlight the potential value of PGS in capturing GI problems related to UC well before the typical age of diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank other SEED collaborators and the SEED Data Coordinating Center team at the Clinical and Translational Sciences Institute of Michigan State University for their support throughout this study. This research was supported by 12 cooperative agreements from the Centers for Disease Control and Prevention: Cooperative Agreement Number U10DD000180, Colorado Department of Public Health; Cooperative Agreement Number U10DD000181, Kaiser Foundation Research Institute (CA); Cooperative Agreement Number U10DD000182, University of Pennsylvania (PA); Cooperative Agreement Number U10DD000183, Johns Hopkins University (MD); Cooperative Agreement Number U10DD000184, University of North Carolina at Chapel Hill (NC); Cooperative Agreement Number U10DD000498, Michigan State University (DCC); Cooperative Agreement Number U01000750, University of Colorado Denver; Cooperative Agreement Number U01000748, Kaiser Foundation Research Institute (CA); Cooperative Agreement Number U01000752, University of Pennsylvania; Cooperative Agreement Number U01000746, Johns Hopkins University; Cooperative Agreement Number U01000749, University of North Carolina at Chapel Hill; and Cooperative Agreement Number U10DD000901, Michigan State University.

Funding information

Centers for Disease Control and Prevention; European Commission; National Institute of Environmental Health Sciences

DATA AVAILABILITY STATEMENT

The SEED 1 data analyzed for this study are not publicly available due to lack of explicit consent for such sharing in the written informed consents for SEED sites, according to the CDC IRB that governs the SEED network. The SEED network is currently working on a protocol to recontact participants and will reconsent for this type of genomic data sharing as part of the protocol. Therefore, it is possible that in the future these genomic data can be deposited in a data sharing repository such as National Database for Autism Research (NDAR; ndar.nih.gov) for the participants that consent to such sharing. We have made the code used to perform statistical analyses publicly available on github at the following location: https://github.com/vmorrill/GI_PRS_in_ASD.

Abbreviations:

ADI-R	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
aOR	adjusted odds ratio

ASD	autism spectrum disorder
CD-PGS	polygenic score for Crohn's disease
CI	confidence interval
GI	gastrointestinal
GWAS	genome-wide association study
IBD-PGS	polygenic score for inflammatory bowel disease
PGS	polygenic score
QC	quality control
SEED	Study to Explore Early Development
SCQ	Social Communication Questionnaire
SNP	single nucleotide polymorphism
UC-PGS	polygenic score for ulcerative colitis

REFERENCES

Ahearn WH, Castine T, Nault K, & Green G (2021). An assessment of food acceptance in children with autism or pervasive developmental disorder-not otherwise specified. Journal of Autism and Developmental Disorders, 31(5), 505–511. 10.1023/A:1012221026124

Alonso A, Domènech E, Julià A, Panés J, García-Sánchez V, Mateu PN, Gutiérrez A, Gomollón F, Mendoza JL, Garcia-Planella E, Barreiro-de Acosta M, Muñoz F, Vera M, Saro C, Esteve M, Andreu M, Chaparro M, Manyé J, Cabré E, … Gisbert JP (2015). Identification of risk loci for Crohn's disease phenotypes using a genome-wide association study. Gastroenterology, 148(4), 794–805. [PubMed: 25557950]

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.).APA.

Anttila V, Bulik-Sullivan B, Finucane HK, Walters RK, Bras J, Duncan L, Escott-Price V, Falcone GJ, Gormley P, Malik R, Patsopoulos NA, Ripke S, Wei Z, Yu D, Lee PH, Turley P, Grenier-Boley B, Chouraki V, Kamatani Y, … Neale BM (2018). Analysis of shared heritability in common disorders of the brain. Science, 360(6395), eaap8757. 10.1126/science.aap8757 [PubMed: 29930110]

Bandini LG, Anderson SE, Curtin C, Cermak S, Evans EW, Scampini R, Maslin M, & Must A (2010). Food selectivity in children with autism spectrum disorders and typically developing children. The Journal of Pediatrics, 157(2), 259–264. [PubMed: 20362301]

Baumgart DC, & Sandborn WJ (2012). Crohn's disease. The Lancet, 380(9853), 1590–1605.

Beversdorf DQ, Sohl K, Levitskiy D, Tennant P, Goin-Kochel RP, Shaffer RC, Confair A, Middleton FA, & Hicks SD (2022). Saliva RNA biomarkers of gastrointestinal dysfunction in children with autism and neurodevelopmental disorders: Potential implications for precision medicine. Frontiers in Psychiatry, 12, 2606.

Bresnahan M, Hornig M, Schultz AF, Gunnes N, Hirtz D, Lie KK, Magnus P, Reichborn-Kjennerud T, Roth C, Schjølberg S, Stoltenberg C, Surén P, Susser E, & Lipkin WI (2015). Association of maternal report of infant and toddler gastrointestinal symptoms with autism: Evidence from a prospective birth cohort. JAMA Psychiatry, 72(5), 466–474. [PubMed: 25806498]

Campbell DB, Buie TM, Winter H, Bauman M, Sutcliffe JS, Perrin JM, & Levitt P (2009). Distinct genetic risk based on association of MET in families with co-occurring autism and gastrointestinal conditions. Pediatrics, 123(3), 1018–1024. [PubMed: 19255034]

Careaga M, Van de Water J, & Ashwood P (2010). Immune dysfunction in autism: A pathway to treatment. Neurotherapeutics, 7(3), 283–292. [PubMed: 20643381]

Chaidez V, Hansen RL, & Hertz-Picciotto I (2014). Gastrointestinal problems in children with autism, developmental delays or typical development. Journal of Autism and Developmental Disorders, 44(5), 1117–1127. [PubMed: 24193577]

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, & Lee JJ (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. Gigascience, 4(1), 7. 10.1186/s13742-015-0047-8 [PubMed: 25722852]

Chen G-B, Lee SH, Brion M-JA, Montgomery GW, Wray NR, Radford-Smith GL, Visscher PM, & The International IBD Genetics Consortium. (2014). Estimation and partitioning of (co) heritability of inflammatory bowel disease from GWAS and immunochip data. Human Molecular Genetics, 23(17), 4710–4720. [PubMed: 24728037]

Danese S, & Fiocchi C (2011). Ulcerative colitis. New England Journal of Medicine, 365, 1713–1725. 10.1056/NEJMra1102942 [PubMed: 22047562]

de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, … Bravaccio C (2010). Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. Journal of Pediatric Gastroenterology and Nutrition, 51(4), 418–424. [PubMed: 20683204]

Delaneau O, Howie B, Cox AJ, Zagury J-F, & Marchini J (2013). Haplotype estimation using sequencing reads. The American Journal of Human Genetics, 93(4), 687–696. [PubMed: 24094745]

D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, … Giardini O (1996). Abnormal intestinal permeability in children with autism. Acta Paediatrica, 85(9), 1076–1079. [PubMed: 8888921]

Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, … Cho JH (2006). A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science, 314(5804), 1461–1463. [PubMed: 17068223]

Festen EA, Goyette P, Green T, Boucher G, Beauchamp C, Trynka G, Dubois PC, Lagacé C, Stokkers PCF, Hommes DW, Barisani D, Palmieri O, Annese V, van Heel DA, Weersma RK, Daly MJ, Wijmenga C, & Rioux JD (2011). A meta-analysis of genome-wide association scans identifies IL18RAP, PTPN2, TAGAP, and PUS10 as shared risk loci for Crohn's disease and celiac disease. PLoS Genetics, 7(1), e1001283. [PubMed: 21298027]

Fields VL, Soke GN, Reynolds A, Tian LH, Wiggins L, Maenner M, DiGuiseppi C, Kral TVE, Hightshoe K, Ladd-Acosta C, & Schieve LA (2021). Association between pica and gastrointestinal symptoms in preschoolers with and without autism spectrum disorder: Study to explore early development. Disability and Health Journal, 14(3), 101052. [PubMed: 33358227]

Finegold SM (2008). Therapy and epidemiology of autism–clostridial spores as key elements. Medical Hypotheses, 70(3), 508–511. [PubMed: 17904761]

Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, Liu M, Molitoris DR, & Green JA III. (2010). Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe, 16(4), 444–453. [PubMed: 20603222]

Furuta GT, Williams K, Kooros K, Kaul A, Panzer R, Coury DL, & Fuchs G (2012). Management of constipation in children and adolescents with autism spectrum disorders. Pediatrics, 130(Supplement 2), S98–S105. [PubMed: 23118260]

Galli-Carminati G, Chauvet I, & Deriaz N (2006). Prevalence of gastrointestinal disorders in adult clients with pervasive developmental disorders. Journal of Intellectual Disability Research, 50(10), 711–718. [PubMed: 16961700]

Geraghty ME, Depasquale GM, & Lane AE (2010). Nutritional intake and therapies in autism: A spectrum of what we know: Part 1. ICAN: Infant, Child, & Adolescent Nutrition, 2(1), 62–69.

Gotham K, Risi S, Pickles A, & Lord C (2007). The autism diagnostic observation schedule: Revised algorithms for improved diagnostic validity. Journal of Autism and Developmental Disorders, 37(4), 613–627. [PubMed: 17180459]

Hediger ML, England LJ, Molloy CA, Kai FY, Manning-Courtney P, & Mills JL (2008). Reduced bone cortical thickness in boys with autism or autism spectrum disorder. Journal of Autism and Developmental Disorders, 38(5), 848–856. [PubMed: 17879151]

Holingue C, Newill C, Lee LC, Pasricha PJ, & Daniele Fallin M (2018). Gastrointestinal symptoms in autism spectrum disorder: A review of the literature on ascertainment and prevalence. Autism Research, 11(1), 24–36. [PubMed: 28856868]

Holingue C, Poku O, Pfeiffer D, Murray S, & Fallin MD (2021). Gastrointestinal concerns in children with autism spectrum disorder: A qualitative study of family experiences. Autism, 26(7), 1698–1711. 10.1177/13623613211062667 [PubMed: 34903078]

Howie B, Marchini J, & Stephens M (2011). Genotype imputation with thousands of genomes. G3: Genesj Genomesj Genetics, 1(6), 457–470.

Howie BN, Donnelly P, & Marchini J (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genetics, 5(6), e1000529. [PubMed: 19543373]

Johnson CR, Handen BL, Mayer-Costa M, & Sacco K (2008). Eating habits and dietary status in young children with autism. Journal of Developmental and Physical Disabilities, 20(5), 437–448.

Liu JZ, van Sommeren S, Huang H, Ng SC, Alberts R, Takahashi A, Ripke S, Lee JC, Jostins L, Shah T, Abedian S, Cheon JH, Cho J, Daryani NE, Franke L, Fuyuno Y, Hart A, Juyal RC, Juyal G, … Weersma RK (2015). Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nature Genetics, 47(9), 979–986. [PubMed: 26192919]

Lord C, Rutter M, & Le Couteur A (1994). Autism diagnostic interview-revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. Journal of Autism and Developmental Disorders, 24(5), 659–685. [PubMed: 7814313]

Maenner MJ, Shaw KA, Bakian AV, Bilder DA, Durkin MS, Esler A, Furnier SM, Hallas L, Hall-Lande J, Hudson A, Hughes MM, Patrick M, Pierce K, Poynter JN, Salinas A, Shenouda J, Vehorn A, Warren Z, Constantino JN, … Cogswell ME (2021). Prevalence and characteristics of autism spectrum disorder among children aged 8 years—Autism and developmental disabilities monitoring network, 11 sites, United States, 2018. MMWR Surveillance Summaries, 70(11), 1–16.

Mahajan A, Spracklen CN, Zhang W, Ng MC, Petty LE, Kitajima H, … Morris AP (2022). Multi-ancestry genetic study of type 2 diabetes highlights the power of diverse populations for discovery and translation. Nature Genetics, 54(5), 560–572. [PubMed: 35551307]

McElhanon BO, McCracken C, Karpen S, & Sharp WG (2014). Gastrointestinal symptoms in autism spectrum disorder: A meta-analysis. Pediatrics, 133(5), 872–883. [PubMed: 24777214]

Mullen EM (1995). Mullen scales of early learning AGS Circle Pines.

Onore C, Careaga M, & Ashwood P (2012). The role of immune dysfunction in the pathophysiology of autism. Brain, Behavior, and Immunity, 26(3), 383–392. [PubMed: 21906670]

Peterson RE, Kuchenbaecker K, Walters RK, Chen C-Y, Popejoy AB, Periyasamy S, Lam M, Iyegbe C, Strawbridge RJ, Brick L, Carey CE, Martin AR, Meyers JL, Su J, Chen J, Edwards AC, Kalungi A, Koen N, Majara L, … Duncan LE (2019). Genome-wide association studies in ancestrally diverse populations: Opportunities, methods, pitfalls, and recommendations. Cell, 179(3), 589–603. [PubMed: 31607513]

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, & Reich D (2006). Principal components analysis corrects for stratification in genome-wide association studies. Nature Genetics, 38(8), 904–909. [PubMed: 16862161]

R-CoreTeam. (2021). R: A language and environment for statistical computing Retrieved from https://www.R-project.org/

Reynolds AM, Soke GN, Sabourin KR, Croen LA, Daniels JL, Fallin MD, … Levy SE (2021). Gastrointestinal symptoms in 2-to 5-year-old children in the study to explore early development. Journal of Autism and Developmental Disorders, 51, 3806–3817. [PubMed: 33394243]

Rutter M, Bailey A, Lord C, Cianchetti C, & Fancello GS (2007). SCQ: Social Communication Questionnaire: Manuale Giunti OS.

Schendel DE, DiGuiseppi C, Croen LA, Fallin MD, Reed PL, Schieve LA, … Yeargin-Allsopp M (2012). The study to explore early development (SEED): A multisite epidemiologic study

of autism by the centers for autism and developmental disabilities research and epidemiology (CADDRE) network. Journal of Autism and Developmental Disorders, 42(10), 2121–2140. [PubMed: 22350336]

Shindler A, Hill-Yardin E, Petrovski S, Bishop N, & Franks A (2020). Towards identifying genetic biomarkers for gastrointestinal dysfunction in autism. Journal of Autism and Developmental Disorders, 50, 76–86. [PubMed: 31535339]

Wiggins LD, Reynolds A, Rice CE, Moody EJ, Bernal P, Blaskey L, Rosenberg SA, Lee LC, & Levy SE (2015). Using standardized diagnostic instruments to classify children with autism in the study to explore early development. Journal of Autism and Developmental Disorders, 45(5), 1271–1280. [PubMed: 25348175]

Wray NR, Goddard ME, & Visscher PM (2007). Prediction of individual genetic risk to disease from genome-wide association studies. Genome Research, 17(10), 1520–1528. [PubMed: 17785532]

Zhang Y, Qi G, Park J-H, & Chatterjee N (2018). Estimation of complex effect-size distributions using summary-level statistics from genome-wide association studies across 32 complex traits. Nature Genetics, 50(9), 1318–1326. [PubMed: 30104760]

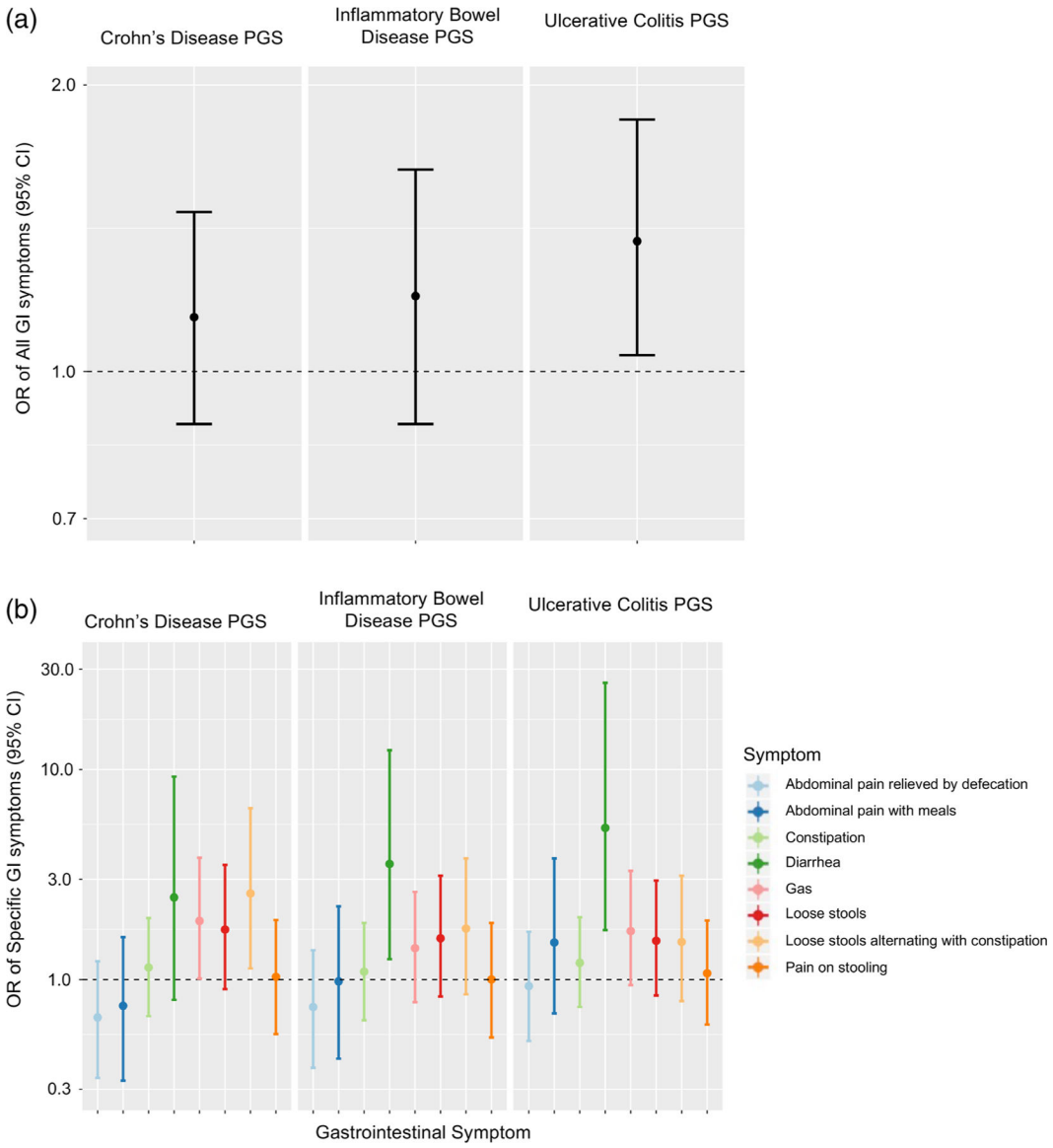

FIGURE 1.

Forest plot among population controls showing the association between 1 *SD* (Beversdorf et al., 2022) increase in each of the three gastrointestinal-polygenic score (GI-PGS) and (a) all GI symptoms ($n = 715$), and (b) specific GI symptoms ($n = 81$). Odds ratio (OR) and 95% confidence interval (CI) are adjusted for 10 genetic principal components.

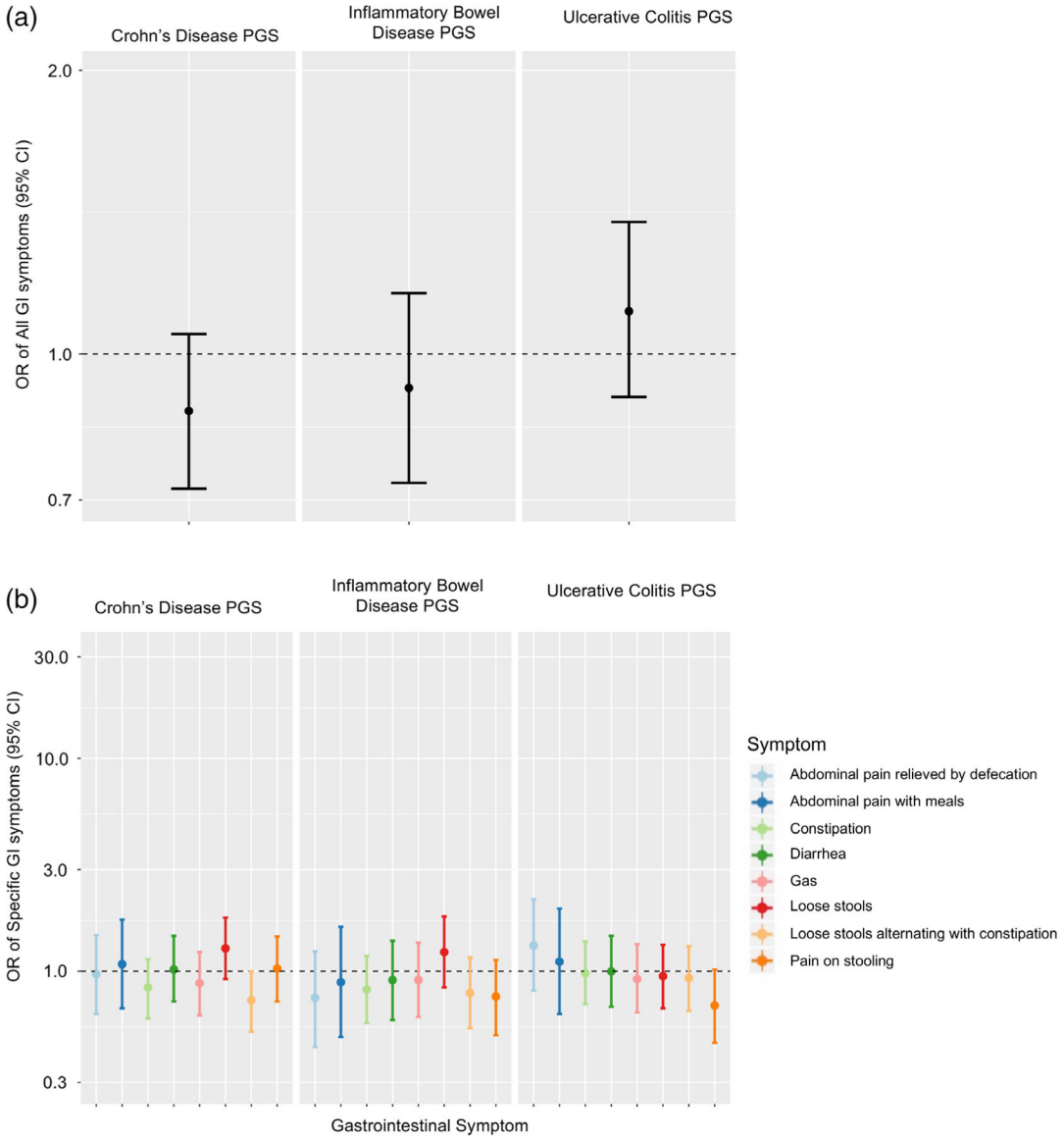

FIGURE 2.

Forest plot among autism spectrum disorder (ASD) cases showing the association between 1 *SD* (Beversdorf et al., 2022) increase in each of the three gastrointestinal-polygenic score (GI-PGS) and (a) all GI symptoms ($n = 564$), and (b) specific GI symptoms ($n = 187$). Odds ratio (OR) and 95% confidence interval (CI) are adjusted for 10 principal components.

TABLE 1

Descriptive statistics of gastrointestinal symptoms and polygenic scores across autism spectrum disorder cases and population controls, Study to Explore Early Development.

	ASD (*n* = 564)	POP (*n* = 715)	*p*-value*
Any GI symptoms (*n* (%))	187 (33.2%)	81 (11.3%)	<0.00001
Specific GI symptoms (*n* (%))			
Vomiting	33 (5.9%)	6 (0.8%)	<0.00001
Diarrhea	50 (8.9%)	12 (1.7%)	<0.00001
Loose stools	76 (13.5%)	24 (3.4%)	<0.00001
Constipation	132 (23.4%)	54 (7.6%)	<0.00001
Loose stools alternating with constipation	71 (12.6%)	17 (2.4%)	<0.00001
Abdominal pain with meals	30 (5.3%)	13 (1.8%)	0.0006
Abdominal pain relieved by defecation	42 (7.4%)	29 (4.1%)	0.0085
Pain on stooling	72 (12.8%)	25 (3.5%)	<0.00001
Gas	86 (15.2%)	25 (3.5%)	<0.00001
Male (*n* (%))	453 (80.3%)	391 (54.7%)	<0.00001
European ancestry (*n* [%])	303 (53.7%)	501 (70.0%)	<0.00001
Crohn's disease PGS[a] (mean [*SD*])	−0.08 [1.04]	0.05 [0.97]	0.019
Ulcerative colitis PGS[a] (mean [*SD*])	−0.07 [1.04]	0.078 [0.98]	0.0093
Inflammatory bowel disease PGS[a] (mean [*SD*])[a]	−0.08 [1.05]	0.07 [0.95]	0.0045

Abbreviations: ASD, autism spectrum disorder; GI, gastrointestinal; PGS, polygenic score; POP, population control.

[a]Standardized PGS.

*_p_-Value reflect univariate, unadjusted associations between GI symptom and ASD status from 2 sample z-test.

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TABLE 2

Comparison of gastrointestinal polygenic scores between children with autism spectrum disorder and population controls, Study to Explore Early Development.

	Crude OR (95% CI)[a] of ASD	Adjusted OR (95% CI)[b] of ASD
Crohn's disease PGS		
All	0.87 (0.78–0.97)	0.97 (0.86–1.09)
European ancestry	0.91 (0.79–1.06)	0.91 (0.78–1.05)
Non-European ancestry	1.00 (0.82–1.21)	1.13 (0.90–1.41)
Ulcerative colitis PGS		
All	0.86 (0.77–0.96)	1.07 (0.93–1.22)
European ancestry	1.08 (0.92–1.28)	1.08 (0.91–1.27)
Non-European ancestry	0.88 (0.74–1.06)	1.02 (0.82–1.28)
Inflammatory bowel disease PGS		
All	0.86 (0.76–0.95)	1.06 (0.92–1.23)
European ancestry	1.05 (0.88–1.25)	1.03 (0.86–1.23)
Non-European ancestry	0.91 (0.76–1.08)	1.13 (0.86–1.48)

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; GI, gastrointestinal; OR, odds ratio; PGS, polygenic score.

[a]Odds of ASD for each 1 SD unit increase in GI-PGS.

[b]Odds of ASD for each 1 SD unit increase in GI-PGS, adjusted for 10 genetic ancestry principal components.