TITLE: Parental inflammatory bowel disease and autism in the offspring: Triangulating the evidence using four complementary study designs.

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ABSTRACT

Evidence linking parental diagnoses of inflammatory bowel disease (IBD) with offspring autism is inconclusive. We conducted four complementary studies to investigate associations between parental diagnoses of IBD and offspring autism and elucidate their underlying aetiology. (1) Nationwide population-based cohort study using Swedish registers to examine associations between parental IBD diagnoses and autism diagnoses in offspring, (2) Linkage disequilibrium (LD)-score regression to estimate the genetic correlation between the phenotypes. (3) Polygenic risk score (PRS) analyses in the Avon Longitudinal Study of Parents and Children (ALSPAC) to investigate associations between maternal genetic liability to IBD and autism factor mean score in offspring. (4) Two-sample Mendelian randomization (MR) to assess bidirectional causal links between genetic liability to IBD and autism. We found evidence of an association between parental IBD diagnoses and offspring autism (maternal: OR= 1.32; 95% CI: 1.25 to 1.40; p<0.001; paternal: OR= 1.09; 95% CI: 1.02 to 1.17; p=0.012; n= 2 324 227, 52.3% male). PRS analyses in ALSPAC indicated associations between maternal PRS for IBD subtypes and a measure of broad autism phenotype, autism factor mean score, in the offspring (UC: β_{PRS}= 0.02; 95%CI: 0.003 to 0.05; p= 0.02; R^2=0.06; Crohn's: β_{PRS}= 0.03; 95%CI: 0.01 to 0.05; p= 0.004; R^2= 0.06; n= 7348, 50.3% male). MR analyses provided evidence of a potential causal effect of genetic liability for IBD, especially ulcerative colitis, on autism (OR_{MR}= 1.03; 95%CI: 1.01 to 1.06). Triangulating evidence from a nationwide register-based cohort study, genetic correlation, polygenic risk score analyses and MR, we found evidence of a potentially causal link between parental, particularly maternal, diagnoses and genetic liability to IBD and offspring autism. Perinatal immune system dysregulation, micronutrient malabsorption and anaemia may be implicated.

INTRODUCTION

Autism spectrum disorder (autism) is a chronic neurodevelopmental condition with a highly variable clinical manifestation[1]. Beyond the core phenotypic expressions of autism (social communication difficulties and restricted interests/repetitive behaviours), emerging evidence suggests that almost half of autistic individuals present with gastrointestinal symptoms (median prevalence 47% in a review of studies published between 1980 and 2017[2]). In addition, a recent study of 48,762 autistic children and 243,810 controls in the United States (US), suggested that children with autism were approximately 47% more likely to be diagnosed with Crohn's disease (Crohn's) and 94% more likely to be diagnosed with ulcerative colitis (UC) compared to controls[3].

Crohn's and UC are the major subtypes of Inflammatory bowel disease (IBD), a chronic condition associated with immune system dysregulation, intestinal microbiome alterations, micronutrient malabsorption and anaemia[4–6]. There is evidence suggesting that these characteristics of IBD might be perinatal factors associated with autism[7–11]. On this basis, a potential link between parental IBD and offspring autism could be hypothesised. Evidence so far is inconclusive, with only one out of the four registry-based studies in the field[12–15] indicating an association between maternal UC and offspring autism[15]. Moreover, the underlying aetiology of any associations is unclear.

We conducted four complementary studies (Figure 1 and Table 1) to investigate: (1) associations between parental diagnoses of IBD and offspring autism in a nationwide cohort in Sweden; (2) genetic correlation between IBD and autism using genome-wide association study (GWAS) summary statistics; (3) polygenic associations between maternal genetic liability to IBD and offspring autistic traits in a large UK birth cohort; and (4) potential causal effects of genetic liability to IBD on autism and the possibility of reverse causation using bidirectional two-sample Mendelian randomization (MR).

RESULTS

Study 1: Associations between parental IBD diagnoses and offspring autism

In a sample of 2,324,227 offspring born to 1,282,494 mothers and 1,285,719 fathers from "Psychiatry Sweden", a comprehensive national register linkage, we assessed the associations between parental IBD diagnosis and offspring autism (Online Methods, Supplementary Figure S1, Supplementary Figure S2, Supplementary Tables S1 & S2). Using logistic regression, we assessed the associations between parental IBD diagnoses and offspring autism. We ran crude models (Model 1), as well as models adjusted for covariates that have been previously identified to be associated with autism in the Swedish registers, including parental age at delivery[16], migrant status[17], education level, family income quintile at birth[18], parents' history of psychiatric diagnosis[19] prior to the birth of the child and offspring sex, birth year and birth order (Model 2). In order to avoid potential bias from assortative mating in Model 2, we additionally mutually adjusted for maternal and paternal IBD diagnoses (Model 3)[20]. Maternal IBD diagnosis was associated with offspring autism in crude and adjusted models (Any IBD diagnosis: OR_{MODEL3}= 1.32; 95% CIs: 1.25 to 1.40; Table 2). Similar results were observed in analyses of maternal UC and Crohn's diagnoses and offspring autism (Table 2), and in analyses restricted to maternal IBD diagnoses prior to the index person's birth (Any IBD diagnosis: OR_{MODEL3}= 1.20; 95% CIs: 1.09 to 1.32; Supplementary Table S3). The paternal IBD associations with autism were weaker (OR_{MODEL3}= 1.09; 95% CIs 1.02 to 1.17) than the maternal associations (Table 2). Point estimates for associations of parental IBD diagnoses to autism without intellectual disabilities (ID) were higher than those for autism with ID, although confidence intervals overlapped (Supplementary Table S4).

Study 2: Genetic correlation between IBD and autism

Using the latest GWAS summary statistics on IBD (N_{cases}= 25,042; $N_{controls}$= 34,915)[21], Crohn's (N_{cases}= 12,194; $N_{controls}$= 28,072)[21], UC (N_{cases}= 12,366; $N_{controls}$= 33,609)[21] and autism (N_{cases}= 18,381; $N_{controls}$= 27,969)[22], we performed Linkage disequilibrium score regression (LDSC)[23]. We found no evidence of a genetic correlation between genetic liability to autism and IBD, UC, or Crohn's (Table 3). Heritability

scores (z-scores: 8.34-11.75), chi-squares (1.20-1.53) and intercepts (1.01-1.12) satisfied the conditions to provide reliable LD-score regression estimates (Supplementary Table S5)..

Study 3: Associations between polygenic risk for IBD, UC, Crohn's and broad autism phenotype in ALSPAC

In 7,348 mothers and 7,503 children of the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort we calculated polygenic risk scores (PRS) for IBD, Crohn's and UC, using the latest available GWAS summary data[21], and assessed associations with an available measure of broad autism phenotype, autism mean factor score[24] (Online Methods, Supplementary Figure S3). Maternal polygenic risk for IBD, UC, Crohn's and offspring broad autism phenotype

Maternal polygenic risk for UC and Crohn's was associated with a higher autism factor mean score in the offspring (UC: β_{PRS}= 0.02; 95%CIs: 0.003 to 0.05; p= 0.03; Crohn's: β_{PRS}= 0.03; 95%CIs: 0.01 to 0.05; p= 0.004). Similar results were found across other p-value thresholds (0.5- 0.05). The effect size of the association between maternal polygenic risk for IBD and autism factor mean score, was comparable to that of UC and Crohn's, although confidence intervals crossed the null (β_{PRS}= 0.02; 95%CIs: -0.004 to 0.04; p= 0.1; R^2= 0.06; Table 4, Supplementary Figure S4, Supplementary Table S6).

Child's polygenic risk for IBD, UC, Crohn's and broad autism phenotype

There was no evidence of associations between child's PRS for IBD, UC, Crohn's and autism mean factor score in children (IBD: β_{PRS}= 0.003; 95%CIs: -0.02 to 0.02; p= 0.79; R^2= 0.05; UC: β_{PRS}= 0.001; 95%CIs: -0.02 to 0.02; p= 0.89; R^2= 0.05; Crohn's: β_{PRS}= 0.007; 95%CIs: -0.01 to 0.03; p= 0.49; R^2= 0.05; Table 4, Supplementary Figure S5, Supplementary Table S7).

Study 4: Causal effects of genetic liability to IBD on risk of autism

Within a two-sample Mendelian randomization (MR)[25] framework, we extracted common genetic variants robustly associated (p≤5e-08) with IBD, Crohn's and UC using the latest available GWAS summary data[21] and assessed their causal effects on 18,381 autism cases and 27,969 controls of the PGC and the iPSYCH consortia[22](Online Methods, Supplementary Figure S6, Supplementary Table S8).

MR analyses were additionally performed using a subsample of the iPSYCH excluding all ID cases (N_{cases}= 11,203; $N_{controls}$= 22,555; Online Methods, Supplementary Figure S7, Supplementary Table S9).

The mean F statistics of the IBD, UC and Crohn's instruments were 67, 68 and 70, respectively, suggesting adequate strength[26]. There was evidence of a causal effect of genetic liability to UC on risk of autism ($_{IVW}$OR= 1.04; 95% CIs: 1.01 to 1.07; p= 0.006). Evidence for the effect of genetic liability to IBD and Crohn's on autism risk was weaker, although the magnitude and direction of the effect estimates was comparable to the UC results (Table 5).

The magnitude and direction of causal effect estimates was consistent across all sensitivity analyses, and there was no evidence to suggest the influence of horizontal pleiotropy (Supplementary Table S10). Results of analyses with instruments extracted from the autism GWAS excluding ID cases were comparable to our primary effect estimates (Supplementary Table S11).

Causal effects of genetic liability to autism on risk of IBD

We assessed the possibility of reverse causation by performing bidirectional two-sample MR. We extracted common genetic variants associated (p≤5e-07) with autism as well as autism without ID[22] and assessed their potential causal effects on IBD (N_{cases}= 25,042; $N_{controls}$= 34,915), UC (N_{cases}= 12,366; $N_{controls}$= 33,609) and Crohn's (N_{cases}= 12,194; $N_{controls}$= 28,072)[21] (Online Methods, Supplementary Figures S5& S6, Supplementary Tables S8 & S9). The mean F statistic of the autism instruments was 28, suggesting adequate strength. There was no evidence of a causal effect of genetic liability to autism on risk of IBD, UC or Crohn's (Table 5). The estimates were consistent across sensitivity analyses, with overlapping confidence intervals, and were unlikely to be influenced by horizontal pleiotropy (Supplementary Table S12). Repeating our analyses with instruments extracted from the autism GWAS excluding all ID cases yielded similar results (Supplementary Table S13).

DISCUSSION

We used four complementary approaches to investigate the associations between parental diagnoses and genetic liability to IBD and offspring autism. Conducting a nationwide register-based cohort study

in Sweden we found evidence of associations between parental diagnoses of IBD and offspring autism. Importantly, the maternal effect sizes were larger than paternal, without overlapping confidence intervals. PRS analyses in the ALSPAC birth cohort suggested associations between maternal genetic liability to IBD and offspring autism, while two-sample MR studies provided evidence of a potentially causal effect of genetic liability to IBD on autism risk. There was no evidence to suggest a genetic correlation between autism and IBD, as indicated by LD-score regression analyses.

A number of studies have investigated the potential associations between parental autoimmune conditions and autism. Several parental autoimmune conditions have been previously identified to be linked to offspring autism, including rheumatoid arthritis[27] and psoriasis[28]. In the case of IBD, evidence from previous studies is inconclusive. In contrast to studies to date, the use of four distinct study designs is a notable strength of our approach. Using study designs with different strengths and sources of bias (Table 1) allowed the triangulation of our findings, rather than relying on arbitrary p-value thresholds[6,7]. Using study designs with different strengths and sources of bias (Table 1) allowed the triangulation of our findings, rather than relying on arbitrary p-value thresholds[29,30]**.** The Swedish nationwide register-based cohort study of over 2 million parent-child pairs is the largest to date on parental IBD-offspring autism. In addition, the present study benefited from the longest to date follow-up period (1987-2016), as well as exposure and outcome ascertainment from both inpatient and outpatient specialist care.

The ALSPAC cohort containing genotype data for over 7,000 mothers and children as well as broad autism phenotype measures for over 13,000 children, is one of the richest resources for the investigation of the potential polygenic associations between maternal polygenic risk for IBD and offspring autism.. Finally, in the MR analyses we used the largest GWAS data available for all conditions and conducted several sensitivity analyses to test the robustness of our findings.

Considering study limitations, in the Swedish registers the possibility of measurement error in IBD diagnoses cannot be excluded. However, this is likely to be non-differential in relation to our study

outcome and would therefore bias our findings towards the null. Secondly, while PRSs were based on large GWAS samples, it was not possible for us to investigate the variance explained by the PRSs in our target sample. However, based on previous studies[31,32], it could be expected that our PRS potentially explain little variance in the phenotype (≈3-1.5%), a limitation which could be overcome with future larger GWAS. Thirdly, the autism mean factor score used in the present analyses, was derived from individual measures that were not primarily intended to assess autism. However, the score has been found to be predictive of a clinical autism diagnosis (measured independent of the variables contributing to the derivation of the mean factor score) and presents associations with autism PRS in ALSPAC, as suggested by previous studies[24,33].Fourth, in two-sample MR analyses investigating the effects of genetic liability to autism on risk of IBD, we used a relaxed instrument inclusion p-value threshold, this could potentially result in including weak instruments and therefore bias the causal effect estimates. The F statistic of the autism instruments in our analyses suggested that weak instrument bias is unlikely. Fifth, although we performed a series of sensitivity analyses to assess the robustness of the causal effect estimates, the possibility of horizontal pleiotropy influencing the present findings cannot entirely be ruled out, especially considering emerging evidence on the genetic architecture of IBD, implicating immune and endocrine-related genes[34]**.** Sixth, using GWAS data we could only investigate the possible contribution of common variants acting under an additive model and not any contribution from rare variation which has been found to be implicated in autism[35,36]. Finally, an important consideration is that the present study has been conducted using samples and GWAS data of predominantly European ancestry individuals. Although a proportion of index children in the registry-based study had at least one parent of non-European descent (10%), the use of European ancestry summary and individual-level genetic data in LDSC, PRS and MR analyses, was unavoidable considering that the largest available GWAS on autism and IBD have been conducted in European ancestry samples. The increasing representation of ethnically diverse populations in biobanks and health registers will allow future studies to build on the present findings.

Overall, our findings suggesting larger maternal effect sizes than paternal in the registry-based study, in combination with the identified associations between maternal, but not child's, PRS for IBD and offspring autism factor mean score, could potentially indicate in utero effects. This could be further supported considering that we did not find evidence of a genetic correlation between autism and IBD. Specifically, based on liability-threshold models of inheritance[37–40] (and assuming that liability to IBD is normally distributed in the population), it could be hypothesised that liability to IBD will be expressed after a threshold has been exceeded, depending on a synergy of genetic variation, environmental factors and chance. Mothers below but close to the threshold, could be expected to express sub-phenotypic manifestations of IBD such as immunological alterations, micronutrient deficiencies, or anaemia. These sub-phenotypic manifestations could influence fetal development. In fact, several immune pathways have been implicated in both Crohn's and UC (which are strongly genetically correlated: r_g= 0.5; p= $2*10^{-13\ 23}$), including T-helper 1 (TH1), T-helper 2 (TH2) and T-helper 17 (TH17) cytokines[41], which are increasingly identified to be linked to perinatal complications[42–44] as well as autism[45–47]. Similarly, micronutrient malabsorption and anaemia during pregnancy have been found to be associated with offspring autism[10,11]. The availability of genotype and biospecimen data in autism family cohorts such as the Simons Simplex Collection (SSC) and the Simons Foundation Powering Autism Research (SPARK)[48,49], is expected to allow the integration of genomic, immune, and gut microbiome profiling approaches to elucidate the potential aetiology and biological pathways underlying the identified associations.

In conclusion, triangulating evidence from a nationwide register-based cohort study, genetic correlation, polygenic risk score analyses and MR, we found evidence suggesting associations between parental, particularly maternal, diagnoses of IBD and offspring autism. Links between maternal genetic liability to IBD and offspring autism may reflect the influence of the maternal genotype on the prenatal/intrauterine environment. Investigating the mechanisms behind these findings may provide valuable insights into the origins of autism.

ONLINE METHODS

Study 1: Investigating associations between parental diagnoses of IBD and offspring autism- Swedish cohort study.

We used individual-level data from 'Psychiatry Sweden', a comprehensive national register linkage, to investigate whether parental IBD diagnosis is associated with offspring autism diagnosis.

All children born in Sweden from 1-January-1987 to 31-December-2010 (n= 2,837,045) were eligible index persons, with follow-up to 31-December-2016. Exclusion criteria were: children born outside Sweden (n=292,023), not registered in the Medical Birth Register (n=74,240), resident in Sweden for <5 years (n=23,495), multiple pregnancy (n=67,309), adopted (n=2,425), known genetic/metabolic causes of neurodevelopmental conditions (e.g. trisomies) (n=7,873) or incomplete parental records (n=45,453)[50]. The study population included 2,324,227 offspring born to 1,282,494 mothers and 1,285,719 fathers (Supplementary Figure S1). The Stockholm Regional Ethical Review Committee (DNR 2010/1185-31/5) approved the study.

Offspring autism was identified in the National Patient Register (NPR) using ICD-9 and ICD-10 codes (Supplementary Methods S1). Lifetime history of parental IBD, Crohn's disease (Crohn's) and ulcerative colitis (UC) were identified using ICD-9 and ICD-10 codes in the NPR (Supplementary Methods S1).

Using STATA/MP15, we estimated the odds ratios and 95% confidence intervals of the association of mother's and father's diagnosis of IBD (any IBD, Crohn's, or UC) with offspring autism using generalised estimating logistic models with robust standard errors accounting for clustering of multiple children born to the same parents.

Model 1 was unadjusted. Model 2 was adjusted for parental age at delivery[16], migrant status[17], education level, family income quintile at birth[18], parents' history of psychiatric diagnosis prior to the birth of the child and offspring sex, birth year and birth order (Supplementary Table S14 for collinearity diagnostics of covariates included in the models). Model 3 was additionally mutually

263 adjusted for maternal and paternal IBD diagnoses to avoid bias from assortative mating[20]. As a
264 sensitivity analysis, we restricted parental IBD diagnoses to those recorded prior to the birth of the
265 index person and investigated associations with offspring autism. Additionally, we investigated
266 associations between any parental IBD diagnoses and offspring autism with and without intellectual
267 disabilities (ID) separately, since these groups may have distinct genetic and environmental risk
268 factors[19,51–53] and outcomes[54,55]. Due to the number of analyses run in the study we applied a
269 Bonferroni correction to account for multiple testing (0.05/42= 0.0012).

270 ## Study 2: Investigating genetic correlations- LD-Score regression

271 We used LD-score regression to estimate the genetic correlation between genetic liability to autism
272 and IBD, Crohn's and UC.

273 LD-score regression allows the estimation of the genetic correlation between polygenic traits using
274 GWAS summary statistics by capitalising on patterns of linkage disequilibrium among common genetic
275 variants[23]. We used the latest available GWAS summary data on autism (N_{cases}= 18,381; $N_{controls}$=
276 27,969)[22], IBD (N_{cases}= 25,042; $N_{controls}$= 34,915)[21], Crohn's (N_{cases}= 12,194; $N_{controls}$= 28,072)[21] and UC
277 (N_{cases}= 12,366; $N_{controls}$= 33,609)[21]. Detailed information on study samples and case definition can be
278 found in the original publications.

279 We followed the suggested protocol for LD-score regression analyses
280 (https://github.com/bulik/ldsc/wiki). Using the LDSC (LD Score) v1.0.1 software in Python, we
281 estimated genetic correlations using pre-computed LD scores from the 1000 Genomes project
282 European data[56] (from: https://data.broadinstitute.org/alkesgroup/LDSCORE/eur_w_ld_chr.tar.bz) with
283 an unconstrained intercept term to account for any sample overlap, and population stratification.

284

Study 3: Investigating associations between genetic liability to IBD and childhood broad autism phenotype- Polygenic Risk Score analysis in mothers and children of the ALSPAC cohort

Discovery Sample

Common genetic variants, corresponding alleles, effect sizes and p-values were extracted in order to calculate polygenic risk scores (PRSs), from the GWAS summary data of IBD[21], UC[21] and Crohn's[21] described above.

Target Sample

ALSPAC is a UK prospective birth cohort study based in Bristol and surrounding areas, which recruited pregnant women with expected delivery dates from 1 April 1991 to 31 December 1992; 14,541 women were initially enrolled, with 14,062 children born, and 13,988 children alive at 1 year of age. Detailed information on the cohort is available elsewhere[57,58]. A fully searchable study data dictionary is available at : http://www.bristol.ac.uk/alspac/researchers/our-data/. Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Genetic data

10,015 ALSPAC mothers were genotyped on the Illumina Human660W-quad genome-wide single nucleotide polymorphism (SNP) genotyping platform, and 9,912 ALSPAC children were genotyped on the Illumina HumanHap550-quad. After standard quality control (Supplementary Methods S2) and excluding participants who had withdrawn consent, genetic data were available for 7,921 mothers and 7,977 children of European ancestry. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004).

Broad autism phenotype- autism factor mean score

We used a measure of the broad autism phenotype previously estimated in ALSPAC as the mean score of 7 factors derived from a factor analysis of 93 measures related to autism in ALSPAC[24]. The measure was available in 13,103 children and strongly predictive of the autism diagnosis measured independently via school records, record linkage and parental reports[24]. Other autism trait measures

or diagnoses were not used as there were fewer genotyped mothers and children with these measures.

306 *Calculation of Polygenic Risk Scores in ALSPAC and statistical analysis*

307 PRS were calculated using PLINK version 1.9, applying the method described by the Psychiatric
308 Genomics Consortium (PGC)[59]. SNPs with mismatching alleles between the discovery and target
309 dataset were removed. The MHC region was removed (25 Mb – 34 Mb), except for one SNP
310 representing the strongest signal within the region. Using ALSPAC data as reference panel, SNPs were
311 clumped with an r^2 of 0.25 and a physical distance threshold of 500 kB. The optimal p-value threshold
312 for PRS is dependent on discovery and target sample sizes, as well as SNP inclusion parameters (e.g.,
313 r^2)[60,61]. For this reason, we calculated PRS for each participant across 13 p-value thresholds (p<5e-8 to
314 p<0.5), standardised by subtracting the mean and dividing by the standard deviation. We defined PRS
315 corresponding to p-value threshold 0.05 as our primary exposure, based on a previous ALSPAC
316 study[62]. This threshold has been found to have sufficient predictive ability for IBD and its subtypes[32].
317 We could not directly assess the predictive power and optimal p-value threshold of our PRSs in our
318 target sample as there were few UC (n=12) and Crohn's cases (n=16).

319 After constructing PRS for IBD, UC and Crohn's in mothers and children, we performed linear
320 regressions using STATA/MP 15 to examine associations with the standardised autism factor mean
321 score in childhood. Analyses were adjusted for child's sex and the first 10 principal components of the
322 ALSPAC genotype data to avoid population stratification bias[60].

323 Study 4: Investigating bidirectional causal links- Two-sample Mendelian randomisation

324 We performed two-sample Mendelian randomisation (MR) to assess bidirectional causal links
325 between genetic liability to autism and IBD and its subtypes, and vice versa.

326 MR can be implemented as an instrumental variable approach, utilising common genetic variants as
327 instruments for exposures of interest, allowing assessment of causal effects and their direction on
328 outcomes. MR relies on the following assumptions : (i) there must be a robust association between

the common genetic variants and the exposure, (i.e., no horizontal pleiotropy, the phenomenon in which the genetic variant influences multiple phenotypes through biologically distinct pathways), (ii) the variants should operate on the outcome entirely via the exposure, (iii) the variants should not be associated with any confounders of the associations between the exposure and the outcome[63]. In this study, we applied two-sample MR, in which the effect sizes and standard errors of the instruments for the exposure and the outcome were extracted from separate GWASs conducted in independent samples from the same underlying population[25].

Genetic Instruments

Genetic instruments were extracted from the overlapping set of SNPs between the autism[22], IBD[21], UC[21], and Crohn's[21] GWASs. This ensured that all selected genetic instruments would be present in the outcome GWAS.

GWAS summary data were restricted to a common set of SNPs and then clumped in PLINK 1.90 using the 1000Genomes[56] phase 3 European ancestry reference panel, and an r2= 0.01, within a 10,000 kb window. Among the independent variants, instruments were defined using a genome-wide significance threshold of $p \leq 5 \times 10^{-08}$. The only exception was autism, as only two independent and genome-wide significant variants were identified. We therefore relaxed the p-value threshold to 5×10^{-07} to improve statistical power, as used previously [64]. Supplementary Figure S6 illustrates the process of instrument definition, and supplementary table S8 contains information on the genetic instruments used.

Harmonisation

We harmonised the alleles of the outcome on the exposure, to ensure SNP-exposure and SNP-outcome effects correspond to the same allele. Variants identified as palindromic were removed, as the effect allele frequencies in the IBD, UC, and Crohn's GWASs were not provided. Supplementary tables S15 and S16 contain details of the harmonised datasets.

Inverse Variance Weighted MR

The primary MR analysis was the Inverse Variance Weighted (IVW) method which provides an overall causal effect estimate of the exposure on the outcome, estimated as a meta-analysis of the ratios of the SNP-outcome effect to the SNP-exposure effect weighted by each SNP's relative precision[65].

Sensitivity Analyses to test robustness of causal effect estimates

We assessed the strength of the instruments by estimating the mean F statistic. As a rule of thumb, the IVW is unlikely to suffer from weak instrument bias if mean F>10[26].

We assessed the consistency of the IVW causal effect estimates using sensitivity analyses, including: MR Egger regression[65], Weighted Median[66] and Weighted Mode[67] (Supplementary Methods S3).

Sensitivity Analyses to test the consistency of the causal effect estimates in autism without intellectual disabilities (ID)

The autism GWAS used in our primary analyses included a proportion of autism cases with ID[22]. We tested the consistency of the causal effect estimates using GWAS summary data on a sub-sample of the iPSYCH cohort[68] excluding all intellectual disability cases (N_{cases}= 11,203; $N_{controls}$= 22,555). Supplementary figure S7 visualises the process of instrument definition, and supplementary tables S9, S17 and S18 contain details on the instruments used and the harmonised datasets.

Two-sample MR analyses were performed using the TwoSampleMR R package[69] in R version 3.5.1.

DATA AVAILABILITY

Swedish registry data: Individual-level data from 'Psychiatry Sweden' were used and under ethics approval from the Stockholm regional ethical review committee (DNR 2010/1185-31/5). Data are not publicly available. Data must remain in the countries, according to national laws and registry regulations, and access is restricted to projects approved by the relevant research committees (stockholm@rdn.jordbruksverket.se).

GWAS summary data: GWAS summary data for IBD, ulcerative colitis, Crohn's disease and autism used in the LD score regression, polygenic risk score and Mendelian randomization analyses, are publicly available (IBD, UC, Crohn's: ftp://ftp.sanger.ac.uk/pub/project/humgen/summary_statistics/human/2016-11-07/ ; Autism: https://www.med.unc.edu/pgc/download-results/). GWAS summary data for autism without intellectual disabilities are not publicly available and can be accessed after correspondence with the iPSYCH: https://ipsych.dk/ .

ALSPAC data: Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Individual-level data from the ALSPAC birth cohort are not publicly available and can be accessed after application to the ALSPAC executive team: http://www.bristol.ac.uk/alspac/researchers/access/

CODE AVAILABILITY

Analyses were conducted using established protocols for each analytic approach used in the present study. Specifically in the case of LD score regression, the protocol described at: https://github.com/bulik/ldsc/wiki/Heritability-and-Genetic-Correlation , was used. In the case of polygenic risk score calculation, the approach described at: https://www.nature.com/articles/nature13595 , was applied. Finally, for two-sample Mendelian randomization, the approach described at: https://mrcieu.github.io/TwoSampleMR/articles/introduction.html , was applied.

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AUTHOR CONTRIBUTIONS

Research idea; CD; Study design and supervision; CD, RG, DR; Data analysis: AS, CD, RG; Interpretation of results: All authors; Drafting of manuscript: AS, CD, RG, DR; Critical comments and editing of manuscript drafts: All Authors; Approval of final submitted manuscript: All authors.

COMPETING INTERESTS

The authors declare no competing interests.

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FIGURE LEGENDS

Figure1. Summary of studies conducted in the present study, aiming at investigating the links between parental diagnoses of IBD and offspring autism and elucidating their underlying aetiology. GWAS: Genome-wide association study; IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease.

TABLES

Table 1. Summary of research question, data sources used as well as key strengths and limitations of each methodological approach applied in the present study.

Method	Research question	Data sources	Key strengths	Key limitations
Nationwide registry-based cohort study in Sweden	Are parental diagnoses of IBD associated with autism in the offspring?	Medical & administrative registers	• Large diverse total population, intergenerational sample. • Prospective recording of data. • Low rate of loss to follow up. • Large availability of confounder data.	• Unmeasured confounding. • Exposure misclassification.
Linkage Disequilibrium score regression	Is there a shared genetic background between IBD and autism?	GWAS summary data	• Use of GWAS summary data instead of twin data or individual level data maximizes sample sizes and power. • Indicates genetic correlation due to linkage disequilibrium or pleiotropy.	• Cannot assess causality.
Polygenic risk score analysis in the ALSPAC cohort	Is maternal genetic liability for IBD associated with childhood broad autism phenotype?	GWAS summary data and individual level genotype and phenotype data	• Estimates the underlying genetic liability for IBD in each genotyped mother of the cohort, regardless of diagnosis. This overcomes limitations of observational studies, such as measurement error in the exposure. • Can provide indication on potentially genetically transmitted vs in utero effects through the assessment of the maternal vs offspring underlying genetic liability for IBD. • Large birth cohort. • Prospectively collected information on the outcome phenotype.	• Cannot decipher whether the identified associations are causal or instead due to pleiotropy. • Polygenic risk scores at lower p-value thresholds might not capture adequately the exposure phenotype. • Attrition can influence association estimates.
Two-sample Mendelian randomization	Does genetic liability to IBD have a causal effect on autism?	GWAS summary data, exposure proxied by variants robustly associated with the exposure	• Using common genetic variants as instruments for IBD, allows the assessment of causal effects. • Allows the assessment of reverse causation. • Allows the assessment of the influence of pleiotropy.	• Cannot decipher whether the identified causal effect is of parental origin. • Can be biased by dynastic effects and assortative mating.

Table 2. Associations between maternal or paternal diagnosis for any inflammatory bowel disease (IBD), ulcerative colitis, Crohn's disease, other IBD and offspring diagnosis of autism.

	Maternal diagnoses							Paternal diagnoses						
Exposure	n ASD/n total (% ASD)[a]	Model1[b] OR (95% CIs)	P	Model2[c] OR (95% CIs)	P	Model3[d] OR (95% CIs)	P	n ASD/n total (% ASD)[a]	Model1[b] OR (95% CIs)	P	Model2[c] OR (95% CIs)	P	Model3[d] OR (95% CIs)	P
No IBD	43,568/2,272,606 (1.92%)	Ref		Ref		Ref		43,989/2,281,119 (1.93%)	Ref		Ref		Ref	
Any IBD	1,361/51,621 (2.64%)	1.39 (1.31,1.47)	<0.001[e]	1.32 (1.24,1.40)	<0.001[e]	1.32 (1.25,1.40)	<0.001[e]	940/43,108 (2.18%)	1.14 (1.06,1.22)	<0.001[e]	1.11 (1.03,1.18)	0.004	1.09 (1.02,1.17)	0.012
Crohn's Disease	422/17,832 (2.37%)	1.23 (1.09,1.40)	0.001[e]	1.19 (1.05,1.35)	0.006	1.20 (1.06,1.36)	0.004	346/18,290 (1.89%)	1.18 (1.04,1.35)	0.013	1.16 (1.02,1.33)	0.023	1.16 (1.01,1.32)	0.031
Ulcerative Colitis	292/12,390 (2.36%)	1.24 (1.12,1.38)	<0.001[e]	1.22 (1.10,1.35)	<0.001[e]	1.22 (1.10,1.36)	0.001	254/11,274 (2.25%)	0.99 (0.88,1.10)	0.806	0.98 (0.87,1.09)	0.662	0.97 (0.86,1.08)	0.575
Other IBD[f]	722/24,865 (2.90%)	1.53 (1.42,1.66)	<0.001[e]	1.42 (1.32,1.54)	<0.001[e]	1.43 (1.32,1.55)	<0.001[e]	407/16,958 (2.40%)	1.25 (1.12,1.38)	<0.001[e]	1.19 (1.07,1.32)	0.001[e]	1.17 (1.05,1.30)	0.003
Crohn's or Ulcerative Colitis[g]	639/26,756 (2.39%)	1.25 (1.15,1.35)	<0.001[e]	1.21 (1.11,1.32)	<0.001[e]	1.22 (1.12,1.32)	<0.001[e]	533/26,150 (2.04%)	1.06 (0.97,1.16)	0.187	1.05 (0.96,1.15)	0.312	1.04 (0.95,1.14)	0.408

[a] The total numbers for those exposed to maternal or paternal Crohn's Disease, Ulcerative Colitis, or Other IBD do not sum to the total exposed to any IBD because some mothers or fathers received both a Crohn's Disease and an Ulcerative Colitis diagnosis Please see supplementary Figure S2 for details on the prevalence and overlap in diagnoses in the study sample.

[b] Crude models.

[c] Models adjusted for child's sex, year of birth, birth order, maternal/paternal age, migrant status, education level, family income and parental psychiatric history.

[d] Mutually adjusted models for maternal/paternal IBD diagnoses, child's sex, year of birth, birth order, maternal/paternal age, migrant status, education level, family income and parental psychiatric history.

[e] p-value is less than Bonferroni-corrected value of 0.0012, accounting for 42 models used within Study 1.

[f] Excluding Crohn's and Ulcerative Colitis and including ICD-9 558 "Other and unspecified non-infectious gastroenteritis and colitis" and ICD-10 K52.3 "Indeterminate colitis" and K52.9 "Noninfective gastroenteritis and colitis". Please see supplementary methods S1 for details on the diagnostic codes.

[g] Including Crohn's and ulcerative colitis diagnoses and excluding ICD-9 558 "Other and unspecified non-infectious gastroenteritis and colitis" and ICD-10 K52.3 "Indeterminate colitis" and K52.9 "Noninfective gastroenteritis and colitis". Please see supplementary methods S1 for details on the diagnostic codes.

Table 3. LD-score regression coefficients (r_g), 95% confidence intervals (95% CIs) and p-values for the analyses investigating the genetic correlation between genetic liability to autism, Inflammatory Bowel Disease (IBD), ulcerative colitis and Crohn's disease.

Trait 1	Trait 2	r_g (95% CIs)	P
Autism	IBD	-0.0615 (-0.15, 0.02)	0.158
Autism	Ulcerative colitis	-0.0656 (-0.17, 0.04)	0.2064
Autism	Crohn's disease	-0.0403 (-0.13, 0.05)	0.3551

Table 4. Associations between child and maternal PRS for inflammatory bowel disease (IBD), ulcerative colitis, Crohn's disease at p-value threshold 0.05, and autism factor mean score in the children of the ALSPAC birth cohort.

	IBD PRS				Ulcerative colitis PRS				Crohn's disease PRS			
	Mother N= 7,348		Child N= 7,503		Mother N= 7,348		Child N= 7,503		Mother N= 7,348		Child N= 7,503	
	β (95% CIs)	P	β (95% CIs)	P	β (95% CIs)	P	β (95% CIs)	P	β (95% CIs)	P	β (95% CIs)	P
Autism factor mean score*	0.02 (-0.004, 0.04)	0.1	0.003 (-0.02, 0.02)	0.79	0.02 (0.003, 0.05)	0.03	0.001 (-0.02, 0.02)	0.89	0.03 (0.01, 0.05)	0.004	0.007 (-0.01, 0.03)	0.49

*Standardised score, with mean = 0, standard deviation = 1 and higher scores reflecting more autism related difficulties.

Table 5. Mendelian randomisation IVW estimates, 95% confidence intervals and p-values for the effect of genetic liability to inflammatory bowel disease (IBD), Crohn's disease (Crohn's), ulcerative colitis (UC) on autism and vice versa.

Exposure	Outcome	OR (95% CIs)	P
Genetic liability to IBD	Autism	1.02 (1.0, 1.05)	0.1
Genetic liability to ulcerative colitis	Autism	1.04 (1.01, 1.07)	0.006
Genetic liability to Crohn's disease	Autism	1.01 (1.0, 1.04)	0.2
Genetic liability to autism	IBD	0.90 (0.73, 1.11)	0.32
Genetic liability to autism	Ulcerative colitis	0.95 (0.77, 1.18)	0.65
Genetic liability to autism	Crohn's disease	0.85 (0.63, 1.15)	0.29

FIGURES

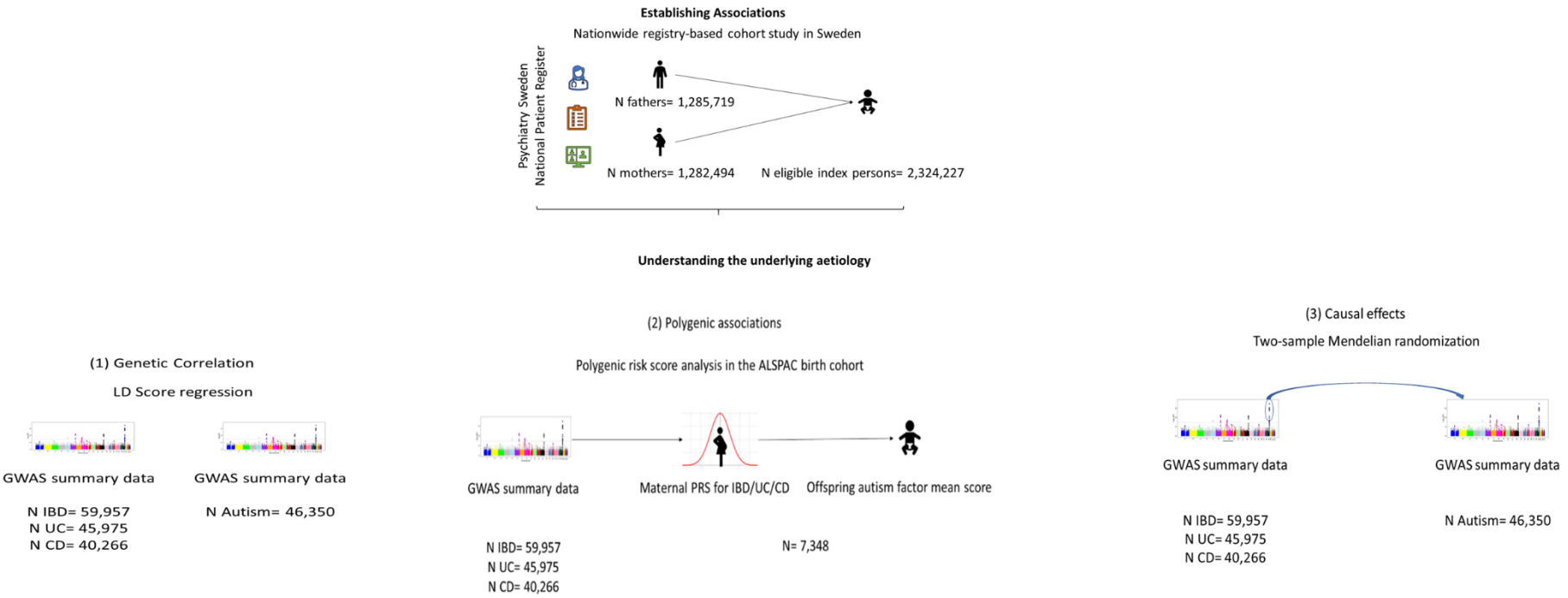

Figure 1.