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ORIGINAL ARTICLE

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Genetic and psychosocial influence on the association between early childhood infections and later psychiatric disorders

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Abstract

Objective: To evaluate the influence of extensive genetic and psychosocial confounding on the association between early childhood infection and five major psychiatric disorders

Methods: A case-cohort study including participants from the Danish iPSYCH2012 sample, a case-cohort sample where all cases born between May 1, 1981, and December 31, 2005, diagnosed with attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), bipolar affective disorder (BIP), Major Depressive Disorder (MDD) or schizophrenia (SCZ), were identified and pooled with a representative sample (subcohort) of the Danish population. We used Cox proportional hazards regression customized to the case-cohort setup to calculate hazard ratios of outcome with 95% confidence

intervals (CIs), following exposure to early childhood infection before the age of 5 years for ADHD and ASD, and before the age of 10 years for BIP, MDD, and SCZ. To evaluate psychosocial confounding we included sex, calendar period, sibling infections, urbanicity, parental socio-economic status, parental mental health information, and polygenic risk scores for all five disorders, as covariates. To estimate how liability for psychiatric disorders measured through the PRS influenced the risk of early childhood infection, we calculated odds ratios (ORs) with 95% CIs, using logistic regression

Results: Early childhood infection was associated with ADHD, ASD, MDD, and SCZ with number of childhood infections increasing the hazard. The HR was still significant in the model with full adjustments after 1 infection for ADHD (HR 1.29, 95% CI: 1.19–1.41), ASD (HR 1.28, 95% CI: 1.18–1.40), MDD (HR 1.23, 95% CI: 1.14–1.33), and SCZ (HR 1.21, 95% CI: 1.07–1.36), but not for BIP (HR1.17, 95% CI: 0.96–1.42). Probands exposed to sibling infections, but not own infection had an absolute risk of ADHD, BIP, MDD, and SCZ that closely approached the absolute risk for individuals exposed to own infections. We found evidence of gene–environment correlation with higher PRS of MDD and to some extent SCZ increasing the risk of infections and higher PRS of BIP associated with significantly decreased risk

Conclusion: Early childhood infection is significantly associated with ADHD, ASD, MDD, and SCZ and not explained by genetic or psychosocial confounding. Although we found evidence of gene–environment correlation, it had minor impact on the results

KEYWORDS

case-cohort study, early childhood infection, major psychiatric disorders, polygenic risk scores, sibling comparison

1 | INTRODUCTION

The association between childhood infection and later psychiatric disorders has been investigated and documented in several large Scandinavian cohort studies, but the evidence to substantiate the potential physiologic pathways is still lacking. Within the last two decades the idea of a compromised immune system in psychiatric disorders has gained strong support in especially schizophrenia where genome wide association studies (GWAS) have demonstrated the strongest associations with genes that play a role in the immune system.[1,2] However, evidence from epidemiologic studies has questioned the underlying mechanisms, as associations are reported after exposure to bacterial, viral, fungal, and parasitic infections for autism spectrum disorders (ASD),[3–5] schizophrenia (SCZ) and related psychoses,[6–8] major depressive disorder (MDD),[9,10] bipolar disorder (BIP)[10] and attention-deficit/hyperactivity disorder (ADHD).[10] Although all studies report on significant associations, several speculate that associations may be influenced by confounding, both genetic and psychosocial.[3–5,8] Associations between infections and psychiatric disorders have been shown to be influenced by temporal proximity and accumulation (individuals with more infections closer to onset have a higher risk),[6,7,9,10] they are observed across the entire childhood period, for admission with one infection only and also for noninfectious somatic diseases,[3,5] which raises the question of specificity of the exposure. While the findings can be interpreted to suggest that infection during childhood can contribute to development of psychiatric disorders by affecting neurodevelopment, they can also imply that hospital admission reflect an increased exposure and susceptibility to infection among individuals with a propensity to mental illness. Parental infection exposure before, during and after pregnancy, has been associated with mental illness in the child,[7,11,12] with the hypothesis being that either transmission of disease or genetic liability towards infection could be responsible for the association, and in an earlier study we have shown that familial liability for infection is robustly associated with schizophrenia,[8] suggesting

that factors other than the infection per se are involved in the association.

While it is practically impossible to directly confirm or reject the hypothesis using observational data, we use triangulation by including different epidemiologic study designs,[13] to increase the validity of our results. We incorporate extensive genetic and psychosocial data to study the association, first, in a large population-based case-cohort setup where we estimate hazard ratios of outcome following early childhood infection; second, by incorporating sibling childhood infection as a negative control variable[14] to aid in detecting unmeasured bias; third, we estimate and compare absolute risks following proband infection only versus sibling infection only; fourth, as people prone to psychiatric disorders may have higher risk of childhood infection, through gene–environment correlation, we estimate the odds of infection associated with genetic liability for the respective disorders.

1.1 | Aims of the study

To evaluate the influence of genetic and psychosocial confounding on the association between early childhood infection and later psychiatric disorders, by the use of different epidemiologic methods.

Significant outcomes

- Early childhood infection is associated with ADHD, ASD, MDD, and SCZ, but not BIP, independent of extensive adjustment for genetic and psychosocial confounders.
- Sibling childhood infections, independently increase risk of ADHD, MDD, and SCZ, suggesting that it is important to consider familial liability to infections when investigating associations between infections and psychiatric disorders.
- Liability for mental illness influences the risk of infection through gene–environment correlation where higher polygenic loading for MDD and to some extent SCZ is associated with higher risk of early childhood infections and higher polygenic loading of BIP is associated with decreased risk of early childhood infections.

Limitations

- The study was limited to early childhood infections, and the time span between infection and outcome may influence the association and limits generalization to early childhood infections.
- Familial clustering of infection was included as confounder, but we had no possibility to directly control for transmission of infection between siblings and transmission of infection could affect the results.

2 | MATERIALS AND METHODS

2.1 | Data sources

Data were obtained from the Danish iPSYCH2012 case-cohort study.[15] The sample has a case-cohort setup[16] and includes all individuals born in Denmark between 1981 and 2005 who received a diagnosis of ADHD, ASD, BIP, MDD, and SCZ in a Danish psychiatric hospital or inpatient clinic through December 31, 2012, and a random sample of 30,000 individuals drawn from the full Danish population born between 1981 and 2005, and who survived to their first birthday and had known mothers. We obtained information by linking Danish nationwide population registers, using the unique personal identification number, which is allocated to every individual with an address in Denmark and used across all national registers. The Danish Civil Registration System was established in 1968, and contains information on personal identification number, place of residence, sex, date and place of birth, and identity of parents.[17] Cases were identified in the Danish Psychiatric Central Research Register[18] which includes all psychiatric admission dates and diagnoses according to WHO ICD-8[19] and ICD-10.[20] It contains all psychiatric inpatient admissions since 1969 and outpatient contacts since 1994. Information about infections was obtained from The Danish National Patient Register which was established in 1977 and contains information on all nonpsychiatric hospital admissions.[21] In 1994, it was expanded to also include emergency room and outpatient contacts.

The Danish Newborn Screening Biobank stores dried blood spots taken few days after birth from nearly all infants born in Denmark after 1981.[22]

2.2 | Genotyping

Genotyping details have been described in detail elsewhere.[23] In brief, genotyping of members of the iPSYCH2012 case-cohort sample was done from blood

spots collected at birth as part of routine clinical practice and stored in the Danish Newborn Screening Biobank.[22] Blood spots were located for 93,3% of the original sample ($N = 80,422$) and 90% of the original sample passed quality control measures ($N = 77,639$).[15]

2.3 | Study sample

We defined a cohort of all individuals from the iPSYCH2012 case-cohort sample[15] who had received a diagnosis in a Danish psychiatric hospital of ADHD (F90) and ASD (F84.0–1, F84.5, F84.8–9) at age of ≥5 years, and BIP (F30-31), MDD (F32-33), and SCZ (F20) at age ≥ 10 years identified in the Psychiatric Central Research Register[18]; who were successfully genotyped, and passed quality-control measures; who were of European ancestry as determined by principal component analysis; and who were unrelated. The final study sample comprised 47,195 individuals with one or more of the five disorders (ADHD, $N = 14,823$; ASD, $N = 12,183$; BIP, $N = 1666$; MDD, $N = 22,246$; SCZ, $N = 4249$). The reason that the sum of individuals for the five disorders exceeds the total number of individuals, is because some individuals have received more than one of the five diagnoses over the course of the study period. For example, one individual could be diagnosed with MDD at the beginning of the study and later have a manic episode and be diagnosed with BIP. The iPSYCH2012 subcohort was selected according to the principle of case-cohort design,[16] and subcohort members were included regardless of when and if they received any of the five diagnoses. The total number of individuals in the subcohort was 22,153.

2.4 | Outcome

First diagnosis of ADHD or ASD at age ≥5 years, or BIP, MDD or SCZ at age ≥ 10 years.

2.5 | Exposure

All diagnoses of early childhood infection were identified according to ICD-8 and -10, and included viral, bacterial, fungal, and other infections. All codes that bore the subclassification "suspected" and "not found" were discarded. A chart of the diagnostic codes used to classify infections is provided in the Supporting Information (Supplementary Table 1). Diagnoses of hospital admission with infection during childhood were obtained from the National Patient Register[21] and were counted up till age <5 years (ADHD and ASD) or age <10 years (BIP, MDD, and SCZ).

2.6 | Polygenic risk score estimation

Polygenic risk scores (PRS) were first generated using LDpred[24] using GWAS summary statistics results for ADHD,[25] ASD,[26] BIP,[27] MDD,[28] and SCZ[29] from the Psychiatric Genomics Consortium (not including iPSYCH2012) as the discovery datasets on the overlapping set of SNPs with the iPSYCH genotypes.

Additionally, to get a better genetic predictor and because the iPSYCH2012 sample sizes are large for ADHD, ASD, and MDD, we have used two methods, LDpred and BOLT-LMM,[30] which we combined in a Meta-PRS which is simply a weighted sum of these (a detailed description of this has been published elsewhere).[31] Both models (LDpred and BOLT-LMM) employ a Bayesian framework, and do not use *p*-thresholds in the selection of variants included. However, they do have a polygenicity parameter that usually needs to be optimized. We used the infinitesimal model (polygenicity parameter = 1) for both LDpred and BOLT-LMM, which allowed us to avoid having to fit the polygenicity hyperparameter. Hence, according to the models used, we end up with just one PRS for every disorder.

2.7 | Covariates

Parental mental illness was categorized hierarchically as[2]; having a history of schizophrenia or bipolar affective disorder (ICD-8 code 295, 296.19, 296.39, and 298.19; ICD-10 code F20 and F30-F31), (1) any psychiatry, (ICD-8 codes 290–315; ICD-10 codes F00-F99) or (0) none, at age <5 years (ADHD and ASD) or <10 years (BIP, MDD, and SCZ) for the proband.

We generated a parental socio-economic status (SES) index for the year prior to the child's birth as a sum of six previously confirmed risk factors[32]: father's or mother's gross income in the lowest quintile, father or mother being unemployed or otherwise outside the labor market, and father's or mother's highest educational level less than high school completion.

Urban birth was classified as capital, capital suburb, provincial city, provincial town, or rural.[33]

2.8 | Family-averaged infection

To control for whether the individual effect of childhood infection was confounded by a familial propensity for infections, we included the average number of hospital-registered childhood infections among all maternal siblings as a covariate.

In the calculation of the family-averaged infection, the index person was not included, to minimize the

correlation between the individual effect of childhood infection and the family-averaged effect.[34] Siblings were identified through the Danish Civil Registration System[17] by identification of the mother and the number of hospital admissions with an infection diagnosis during childhood averaged among them. Infection diagnoses in the siblings had to be registered at age <5 (ADHD and ASD) or <10 years (BIP, MDD, and SCZ) of the sibling.

2.9 | Statistical analysis

Hazard ratios for the association between early childhood infection and any of the five disorders were calculated using Cox proportional hazards regression, with age in days as the underlying timescale. In contrast to traditional Cox regression models, the case-cohort study uses a weighted Cox regression model to account for the oversampling of cases in the estimation of hazard ratios.[16] For ADHD and ASD, individuals entered the analysis on their fifth birthday, and for BIP, MDD, and SCZ they entered on their 10th birthday (i.e., no earlier than 1995). Individuals were censored on the day of the first diagnosis of outcome, death, emigration, or December 31, 2012, whichever came first. In the Cox regression models, we estimated the association between number of early childhood infections (1, 2–3 or ≥4) and any of the five disorders. We estimated a sequence of four models with increasing degrees of control for confounding. The baseline models assessed the association between childhood infections and outcome for probands (Model 1a) and siblings (Model 1b), respectively, while only adjusting for sex and calendar period. In Model 2 proband and sibling infections were mutually adjusted. In Model 3 we also included SES of the parents, parental mental illness and urbanicity. Finally, in Model 4, we also included the PRS for the respective disorder. We used robust standard errors and Barlow weighting to account for oversampling of cases.[16] We estimated hazard ratios (HRs) with 95% confidence intervals (CIs).

To illustrate the potential confounding of probands own infections versus siblings' infections, we estimated the absolute risk of outcome from the Cox regression models after exposure to 0 proband childhood infections versus ≥4 sibling childhood infections, or ≥4 proband infections versus 0 sibling childhood infections. The absolute risk was calculated from the Cox regression with baseline adjustments (sex and calendar period) and mutual adjustment between proband and sibling infections. The parameter values (0 vs. ≥4 childhood infections) were chosen to maximize contrast. If there is a particular and individual effect of one's own infection, we would expect that to be maximized with the most infections, whereas it would be difficult to differ after, for example, only one childhood infection.

To explore how genetic liability for psychiatric disorders influences the risk of acquiring infections (gene–environment correlation), we estimated odds ratios (ORs) of ≥2 infections (vs. 0) for subcohort members, dependent upon the PRS divided into quartiles, using logistic regression. This analysis was adjusted for sex, calendar period and the first 10 principal components, to account for population stratification.

SAS 9.4 and R were used to manage and analyze data.

2.10 | Descriptive statistics

Descriptive statistics were based on the distribution of covariates in the individuals in the subcohort and in cases.

3 | RESULTS

Among the 22,153 individuals in the subcohort, consisting of randomly chosen population controls, a total of 4757 and 5706 individuals had been exposed to early childhood infections before the age of five and 10 years, respectively, and among the 47,195 individuals with any of the outcomes, the numbers were 12,716 and 15,090 individuals exposed before the age of five and 10 years, respectively. Among individuals in the subcohort, 3876 individuals had at least one sibling exposed to childhood infections before the age of five and 4806 individuals after the age of 10 years.

3.1 | Descriptive statistics

Descriptive statistics are shown for the subcohort and for cases in Table 1.

3.2 | PRS and associations with outcomes

For every PRS, we observed a significant association between PRS and outcome.

Each SD-increase in outcome-specific PRS was associated with a 53% increase in the hazard of ADHD (95% CI: 1.49–1.57), 34% increase in the hazard of ASD (95% CI, 1.30–1.38), 42% increase in the hazard of BIP (95% CI: 1.31. -1.53), 37% increase in the hazard of MDD (1.34–1.41) and 54% increase in the hazard of SCZ (95% CI: 1.45–1.65). For a graphical overview of association results for PRS for the five outcomes see Supplementary Figure 1.

TABLE 1 Descriptive statistics for the subcohort and cases for ADHD, ASD, BIP, MDD and SCZ. Display descriptive statistics for the subcohort and cases for the five disorders. Psychiatric family history was calculated as a cross sectional measure at age 5 years (for ADHD and ASD) and 10 years (for BIP, MDD, and SCZ).

	Subcohort	ADHD	ASD	BIP	MDD	SCZ
Sex						
Female	10,890	3966	2656	1013	15,184	2297
Male	11,263	10,857	9527	653	7062	1952
Psychiatric family history						
At age 10 years						
None	20,044	N/a	N/a	1354	19,053	3574
Any	1824	N/a	N/a	205	2659	591
Severe	285	N/a	N/a	107	534	188
At age 5 years						
None	20,734	127,35	10,754	N/a	N/a	N/a
Any	1229	1829	1209	N/a	N/a	N/a
Severe	190	259	220	N/a	N/a	N/a
SES index						
0	8756	3572	3997	490	6310	976
1	6183	3608	3282	466	6230	1137
2	3848	2959	2311	350	4688	945
3	1850	2129	1342	212	2806	688
4	1084	1631	793	120	1717	479
5	300	700	354	XXX[a]	406	110
6	132	224	104	XXX[a]	89	18
Urbanicity						
Capital	2340	1555	1809	193	2334	519
Capital suburb	2833	1896	1883	190	3093	638
Provincial city	2584	1577	1357	272	2583	458
Provincial town	6399	4572	3458	476	6610	1258
Rural area	7982	5209	3660	532	7610	1475

Note: The SES index was calculated as a sum of the following factor: father's or mother's gross income in the lowest quintile, father or mother being unemployed or otherwise outside the labor market, and father's or mother's highest educational level less than high school completion.
Note: By chance, some of the cases will also have been selected for the subcohort. The numbers are 289, 255, 37, 472, and 104 individuals for ADHD, ASD, BIP, MDD, and SCZ, respectively.
Abbreviations: ADHD, Attention deficit/hyperactivity disorder; ASD, autism spectrum disorder; BIP, bipolar disorder; MDD, major depressive disorder; SCZ, schizophrenia; N/a, not applicable.
[a]The XXX in the bipolar group are included to obtain the level of anonymity needed according to the Danish Data Protection Legislation.

3.3 | Childhood infection and hazard of outcome

The hazard of ADHD, ASD, MDD, and SCZ, and to a certain extent also BIP, increased in a dose-dependent manner with increasing number of childhood infections. Association results with baseline adjustments for sex and calendar period are presented in Table 2. In this model (Model 1a), the hazard was significantly increased after 1 infection for ADHD (HR 1.36, 95% CI 1.27–1.45), ASD (HR 1.35, 95% CI 1.25–1.45), BIP (HR 1.20, 95% CI 1.04–1.39), MDD (HR 1.28, 95% CI 1.20–1.36), and SCZ (HR 1.31, 95% CI, 1.19–1.44). Association results were attenuated with increasing degrees of control for confounding by parental mental illness, SES index, urbanicity (Model 3, Supplementary Table 2), and the polygenic risk score for the respective disorder (Model 4, Supplementary Table 2), but displayed the same overall pattern with the hazard increasing by number of infections for ADHD, ASD, MDD, and to a certain extent also SCZ. The hazard remained significant for ADHD (HR 1.29, 95% CI: 1.19–1.41), ASD (HR 1.28, 95% CI: 1.18–1.40), MDD (HR 1.23, 95% CI: 1.14–1.33), and SCZ (HR 1.21, 95%

TABLE 2 Association between early childhood infection and later psychiatric disorders for proband infections (Model 1a) and sibling infections (Model 1b).

		Model 1A[a]			Model 1B[a]			
Infections		**HR (95%CI)**			**HR (95%CI)**			***p*-value[b]**
ADHD								
Proband	1	1.36	1.27	1.45				$p < 0.0001$
Proband	2–3	1.52	1.41	1.64				
Proband	≥4	1.99	1.76	2.25				
Sibling	1				1.36	1.27	1.46	$p < 0.0001$
Sibling	2–3				1.44	1.31	1.58	
Sibling	≥4				1.72	1.47	2.02	
ASD								
Proband	1	1.35	1.25	1.45				$p < 0.0001$
Proband	2–3	1.37	1.25	1.49				
Proband	≥4	2.19	1.92	2.50				
Sibling	1				1.15	1.07	1.25	$p < 0.0001$
Sibling	2–3				1.17	1.05	1.30	
Sibling	≥4				1.47	1.23	1.76	
BIP								
Proband	1	1.20	1.04	1.39				$p < 0.0050$
Proband	2–3	1.15	0.95	1.40				
Proband	≥4	1.48	1.10	2.01				
Sibling	1				1.14	0.98	1.34	$p < 0.0426$
Sibling	2–3				1.16	0.93	1.43	
Sibling	≥4				1.18	0.78	1.78	
MDD								
Proband	1	1.28	1.20	1.36				$p < 0.0001$
Proband	2–3	1.38	1.27	1.49				
Proband	≥4	1.54	1.35	1.75				
Sibling	1				1.13	1.06	1.21	$p < 0.0001$
Sibling	2–3				1.27	1.17	1.39	
Sibling	≥4				1.58	1.35	1.85	
SCZ								
Proband	1	1.31	1.19	1.44				$p < 0.0001$
Proband	2–3	1.40	1.24	1.58				
Proband	≥4	1.73	1.43	2.10				
Sibling	1				1.19	1.07	1.32	$p < 0.0001$
Sibling	2–3				1.31	1.14	1.50	
Sibling	≥4				1.57	1.23	2.01	

Abbreviations: ADHD, attention deficit/hyperactivity disorder; ASD, autism spectrum disorder; BIP, bipolar disorder; MDD, major depressive disorder; SCZ, schizophrenia.

[a]This model is adjusted for sex and calendar year.

[b]This *p*-value tests for overall effect of early childhood infection by testing all hazards (including reference = 1) against each other at the same time.

CI: 1.07–1.36) after exposure to 1 infection in the fully adjusted model (Model 4, Supplementary Table 2), but not for BIP (HR 1.17, 95% CI 0.96–1.42). In the baseline model sibling childhood infection (Table 2, Model 1b) significantly increased the hazard of ADHD (HR 1.36, 95% CI: 1.27–1.46), ASD (HR 1.15, 95% CI: 1.07–1.25),

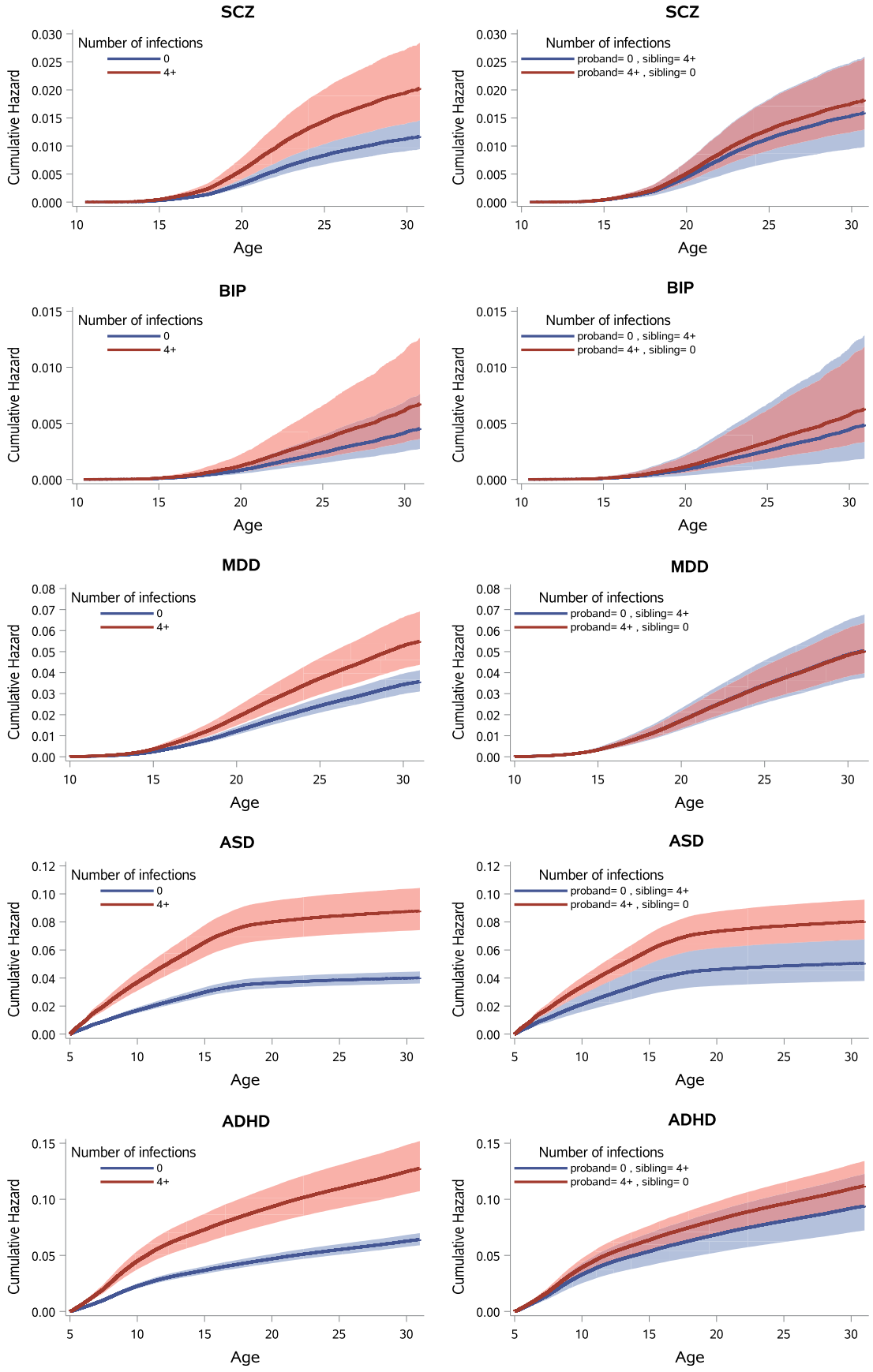

FIGURE 1 Legend on next page.

MDD (HR 1.13, 95% CI: 1.06–1.21), and SCZ (HR 1.19, 95% CI: 1.07–1.32) after 1 infection and followed the same pattern as for proband infection, with increasing hazard by number of infections. For BIP the hazard was not significantly increased following 1 infection (HR 1.10, 95% CI: 0.89–1.34). In the fully adjusted model (Model 4, Supplementary Table 2), the hazard of ADHD following sibling childhood infection remained significant after just 1 infection (HR 1.21, 95% CI: 1.11–1.33), whereas it was not significant for the other four disorders.

3.4 | Absolute risk and infections among siblings

The absolute risk associated with 0 versus ≥4 childhood infections is displayed in the left-hand column of Figure 1 for ADHD, ASD, BIP, MDD, and SCZ. As expected, we observe increments in the absolute risk for childhood psychiatric disorders ADHD and ASD, immediately following start of follow-up (fifth year birthday), whereas increase in absolute risk is observed after the age of 15 for BIP, MDD, and SCZ.

The right-hand column of Figure 1 illustrates the absolute risk of outcome in probands unexposed to own infections but exposed to ≥4 sibling infections (blue graph) versus probands exposed to ≥4 own infections but unexposed to sibling infections (red graph). When comparing the right-hand column to the left-hand column, we note that probands unexposed to own infections but exposed to sibling infections only (≥4 infections), have almost equal absolute risk compared with probands exposed to own infection (for ASD it was not the case, but for MDD the graphs were indistinguishable).

3.5 | Genetic liability for psychiatric disorder and odds of infection

Figure 2 display the association between polygenic liability for the respective disorders, here included as PRS quartiles, and odds of early childhood infections (≥2 infections vs. 0) for subcohort members. For MDD, we observed increasing odds of infections with increasing polygenic loading (higher PRS quartile) among subcohort members. For BIP, the direction of the association was reversed, with decreased odds of infections among subcohort members in any of the PRS quartiles. For ADHD, subcohort members in the 4th PRS quartile had increased odds of infection and for both ASD and SCZ, results were mixed.

4 | DISCUSSION

4.1 | Summary of main findings

In this large-scale population-based study, we found that after accounting for genetic and psychosocial confounding in terms of sex, calendar period, urbanicity, familial propensity for infection, SES, parental mental illness and PRS for the respective psychiatric disorder, exposure to childhood infections increased the hazard of ADHD, ASD, MDD, and SCZ in a dose-dependent manner by number of infections. For ASD, ADHD, MDD, and SCZ association results were attenuated but not explained by the adjustments. To a certain extent BIP displayed the same pattern as the other disorders, with increasing hazard by increasing number of infections in the baseline model but was more sensitive to adjustments. In the fully adjusted model, the hazard was only marginally significant for BIP following 2–3 infections.

For ADHD, ASD, MDD, and SCZ, but not BIP, the hazard was increased following sibling childhood infections. These results followed the same pattern with increasing hazard by increasing number of infections. The hazard of BIP following sibling childhood infections, was not significant at any point.

When comparing probands exposed to own infections (proband ≥4 infections, sibling infections 0) to probands unexposed to own infections but exposed to sibling infections only (proband infections 0 and sibling infections ≥4), the absolute risks approached each other closely for ADHD, MDD, and SCZ. If there is a particular and individual effect tied to one's own infection, we would expect

FIGURE 1 Absolute risks of ADHD, ASD, BIP, MDD, and SCZ, following ≥4 proband or sibling childhood infections only. The left-hand column illustrates the absolute risk for every outcome among probands exposed to 0 or ≥4 childhood infections. The right-hand column illustrates the absolute risk of outcome in probands unexposed to own infection but exposed to ≥4 sibling childhood infections, or probands exposed to ≥4 own childhood infections but unexposed to sibling childhood infections. The plots in both columns are adjusted for sex, calendar period and mutually adjusted for proband aninfections, and the plots on the right are adjusted for sibling childhood infections in addition. ADHD, Attention deficit/hyperactivity disorder; ASD, autism spectrum disorder; BIP, bipolar disorder; MDD, major depressive disorder; SCZ, schizophrenia. The plots illustrate the absolute risk for specified parameter values and are not based on actual counts. Also note the very different axis values for the respective outcomes.

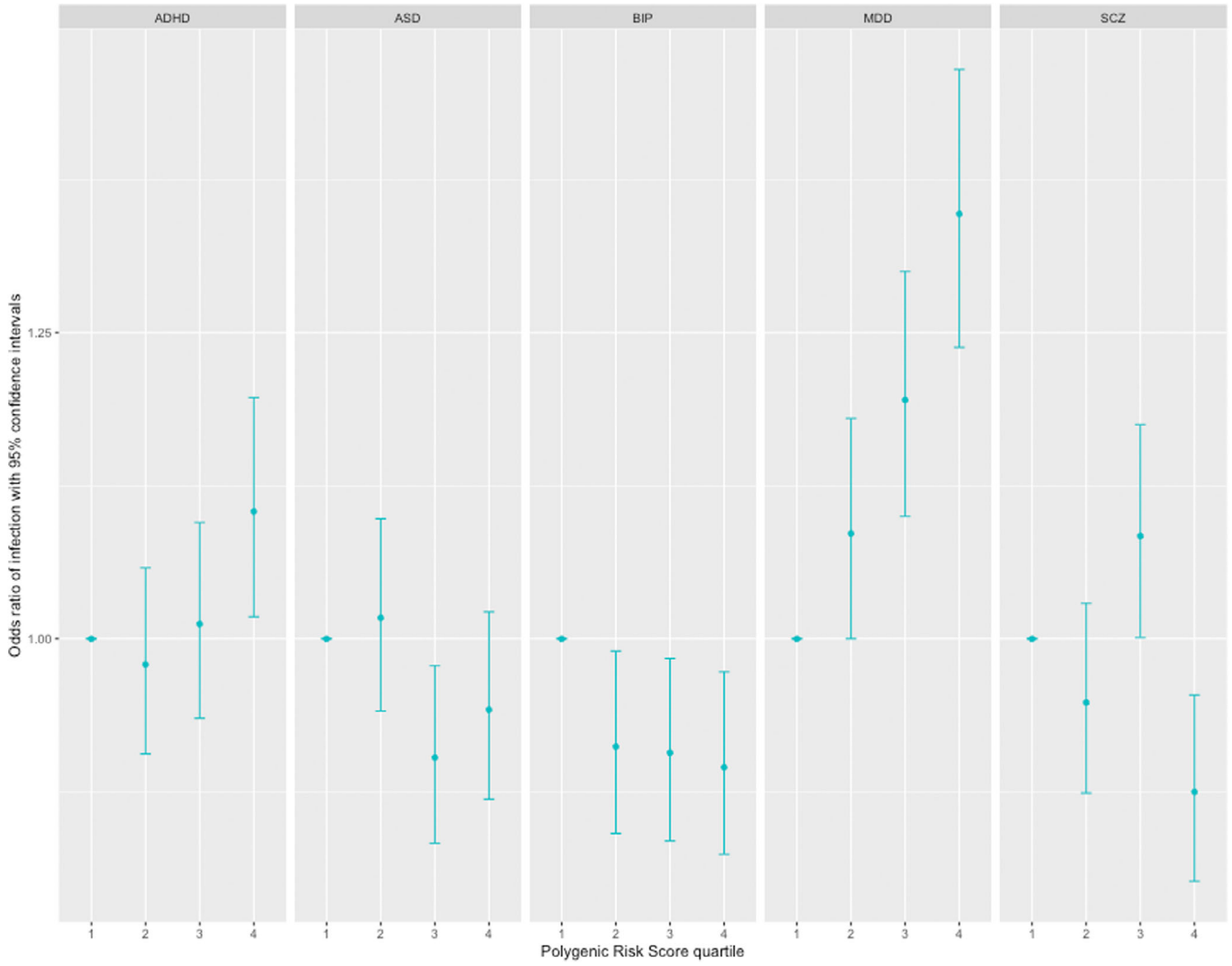

FIGURE 2 Odds ratios of infection associated with polygenic risk score for ADHD, ASD, BIP, MDD, and SCZ adjusted for sex, calendar period and first 10 principal components. Illustrates the odds ratio of childhood infection, dependent upon the polygenic risk score (PRS) divided into quartiles, for subcohort members. Results are adjusted for sex, calendar period and the first 10 principal components. ADHD, Attention deficit/hyperactivity disorder; ASD, autism spectrum disorder; BIP, bipolar disorder; MDD, major depressive disorder; SCZ, schizophrenia.

this effect to be most pronounced among those exposed to ≥4 childhood infections.

In the subcohort, we found evidence of gene–environment correlation with the PRS being associated with increased odds of acquiring childhood infections among individuals with higher polygenic loading for MDD. For ADHD the odds were increased following ≥4 infections. For BIP the odds were significantly decreased following any number of infections, and for ASD and SCZ results were mixed.

4.2 | Possible explanations

The hypothesis that childhood infection is associated with psychiatric disorders through a causal link, is practically impossible to test in observational epidemiology, but we aimed to elucidate the association by triangulation, using different methodology in the form of extensive confounder adjustment (genetic and psychosocial), sibling childhood infection as a negative control variable[14] and estimation of the correlation between genetic predisposition to psychiatric disorder and exposure to infections. We were unable to explain the association between early childhood infection and psychiatric disorder by confounding, or in terms of strong correlation between genetic predisposition to psychiatric disorders and exposure to infections. Association results were attenuated, but remained significant after 1 infection for ADHD, ASD, MDD, and SCZ after full adjustments. The association is attenuated but not explained by confounding or gene–environment correlation. The fact that the

association between infection and the respective disorder remains significant after adjustment for family-averaged infection (propensity to infections in the family), could be interpreted to suggest that factors specific to the individual (in contrast to factors shared in the family) are involved in the association. Subsequent adjustment for SES, parental mental illness, urbanicity, and genetic liability measured through the PRS attenuated the association but did not remove it. In general it is important to show caution when using hospital data to investigate the co-occurrence of diseases, as all medical contacts regardless of somatic disease, are associated with psychiatric morbidity.[5,35] If the increased rate of hospital contacts among individuals who later develop psychiatric disorders is not somehow considered, it may give rise to misleading conclusions. We addressed this issue by comparing proband only versus sibling only infections. Probands who did not have an infection but were exposed to sibling infections (proband 0, siblings ≥4) had an absolute risk of ADHD, MDD, and SCZ that was almost coinciding with probands exposed to own infections (proband ≥4, siblings 0). One interpretation could be that at the extremes (0 vs. ≥4), sibling childhood infections (registered) increase hazard of ADHD, MDD, and SCZ by its own right. It is difficult to draw any definitive conclusions on this as it is impossible to rule out transmission of infection between children in the same family and siblings often have antibodies to the same exposures.[36] However, as we were not concerned with confounding arising from a diagnosis of mental illness in the proband directly causing infections in the siblings, sibling infections were counted any time during the sibling's first 10 years of life (five for ADHD and ASD) irrespective of proband age. As we only included hospital-registered infections (and not infections diagnosed at general practitioners), infections will tend to be more severe, and if transmission between siblings occurred, it would be expected to lead to a higher degree of hospital contact for the other sibling(s) following infection.[37] As we observed an increase in hazard of ADHD, ASD, MDD, and SCZ irrespective of proband infection, and because we have a long period of follow-up we find it unlikely that sibling infections should increase the hazard purely because of transmission of infection.

As is already well established in the literature, this study confirms the presence of genetic risk for the outcomes measured through the PRS. We also demonstrate that genetic liability to BIP, MDD, and to a certain extent also SCZ affects exposure to the environment (gene–environment correlation). For MDD the risk of infection was increased with increased genetic liability measured through the PRS, for schizophrenia it was increased in the third quartile and decreased in the fourth, and for BIP it was significantly decreased. The mixed results do not lead to any obvious or uniform conclusion. The results for schizophrenia are in accordance with an earlier study on Danish data, but with a much smaller sample size, also showing increased risk among controls in the third PRS quartile.[38] For BIP, the significantly decreased risk of infection among subcohort members with higher polygenic loading, resembles findings on Danish data showing that genetic liability for BIP was associated with slightly better performance in school grades of Danish language.[39] However, concluding that subclinical genetic predisposition for BIP protects against early childhood infections, is extremely speculative, especially when compared with the results for MDD. More likely, this could be taken simply to indicate that PRS'es perform differently on the subcohort and hence express distinct genetic differences. Some of the differences observed between MDD and BIP could also be explained by the limited power for BIP analyses because a relatively young study population and later onset of the disorder.

In the present study we only investigated early childhood infection, and the proximity between infection and disease onset may influence the results, as seen in earlier studies,[6,9,10] where the association observed here with disorders typically diagnosed in adolescence or adulthood could be affected by the longer time span since exposure. Several meta-analyses have suggested a beneficial effect of anti-inflammatory treatment as add-on therapy for both affective and psychotic disorders,[40–42] further suggesting that proximity between inflammation and active disorder is important. However, these results may suggest that anti-inflammatory treatment can mitigate some of the negative consequences of active disorder, but not that they may actively influence development. Furthermore, most of the individual studies represent heterogenous populations and do not have long term follow-up, why it is difficult to draw any definitive conclusions in terms of the importance of timespan which is a relevant consideration in our study.

Overall, our study confirms the association between childhood infection and psychiatric disorders, but also show that exposure to sibling infection is associated with an almost equal absolute risk of ADHD, BIP, MDD, and SCZ compared to exposure to own infection. Genetic and psychosocial confounding did not explain the association. However, we found evidence of genetic control of the environment where population controls with higher polygenic loading for MDD and to some extent SCZ had a higher risk of infection, and those with higher polygenic loading for BIP had a significantly decreased risk. The contribution from common variants for mental disorders has been estimated to explain a very small amount of the variation in liability to infection in a sample of

Danish individuals (~4%),[43] and as the PRS was included in the analyses, we can state that genetic liability measured through the PRS only has minor influence on the association between early childhood infection and the five psychiatric disorders investigated. A GWAS for infection overall has earlier been done on the present sample (iPSYCH2012),[43] with the intention of creating a PRS for infection. Since it was done on the same study sample as the present one, we cannot use that information to create a PRS for infection, and to the best of our knowledge no other PRS for infection overall exists. In independent samples of differing psychiatric statuses, the authors showed that the PRS explained between 2% and 7% of the variation in susceptibility to infection,[43] and we conclude that such a genetic predictor will probably have very limited impact on the association between early childhood infection and psychiatric disorders.

4.3 | Strength and limitations

The present study had several key strengths, including the prospective design, large size of the national cohort and the minimization of selection, attrition and information biases commonly encountered in other studies, which are advantages arising from the use of interlinked national registers. Additionally, studies have shown high validity of both diagnoses of infections and mental disorders in the Danish registers.[44,45] In comparison to other studies, an earlier Danish study using an overlapping part of our sample, has investigated the influence of PRS on the association between infections and schizophrenia.[38] This former study employed a case–control design and was conducted on a much smaller sample size, and included older cases with more chronic conditions. The present study includes a random sample of the Danish population, and because of the design can be generalized to the entire population. In this study we can estimate absolute risks and compared to the former study, genotyping was conducted on an updated platform. However, there are also some important limitations of our study.

The main exposure was childhood infection in proband and siblings, respectively, but we cannot exclude the possibility that transmission between siblings influences the results. However, siblings will tend to have a higher degree of hospital contact following more severe childhood infection in the proband,[37] and as we only included hospital contact for infection, the less severe cases were not included. Furthermore, sibling infections were counted any time during the first 10 years of age of the sibling irrespective of proband age (5 years for ADHD and ASD). Hence, we find it very unlikely that the association would occur purely because of transmission between siblings.

The length of the exposure period was different for ADHD and ASD (5 years) compared to BIP, MDD, and SCZ (10 years), because follow-up started at different ages. This was done to accommodate a long enough exposure period on the one hand, and a period long enough for cases to accumulate on the other. As the study objective was to evaluate the influence of genetic and psychosocial confounding on the association, different length of the exposure period is less relevant to the interpretation of confounding effects on the association between infection and later psychiatric disorders.

We use the date for the first time the disorder was registered in the Danish Psychiatric Central Research Register to define onset of disorder. However, especially for ASD onset of symptoms has probably occurred sometime before this date. This means that exposure could occur after onset of the disorder. As infections are more common in individuals with registered psychiatric disorder, this could induce some degree of association as a form of reverse causality. However, psychosocial, and genetic confounding could as well influence this association irrespective of the direction of causality. Hence, the evaluation of confounding effects would remain unaffected by this.

Our definition of childhood infection could be considered heterogenous when each infection category or even ICD-10 diagnosis could be studied individually. In this study we wanted to investigate childhood infection as a general concept, and pooling the diagnostic codes allowed us to do so, while at the same time increasing statistical power as most of the categories only include a small number of cases. On the same time, earlier studies have confirmed equally strong associations between infection categories and mental disorders.[7,10,46] This also means that we cannot rule out any specific effect of infections.

We only included individuals with hospital contact for infection, meaning that the less severe cases of infection were not included. On one side, this could be advantageous because individuals requiring hospital contact for infection could represent a more well-defined group, but on the other side, it also makes it impossible to rule out the possibility of misclassification giving rise to the results.

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CONFLICT OF INTEREST

The authors report no financial relationships with commercial interests.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data can be obtained from Danish national health registers with obtained permission.

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SUPPORTING INFORMATION

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

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