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The relative and interactive impact of multiple risk factors in schizophrenia spectrum disorders: a combined register-based and clinical twin study

C. Lemvigh[1,2], R. Brouwer[3], R. Hilker[1,4], S. Anhøj[1], L. Baandrup[1,4,5], C. Pantelis[1,6], B. Glenthøj[1,4] and B. Fagerlund[1,2]

[1]Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS) and Center for Neuropsychiatric Schizophrenia Research (CNSR), Mental Health Center Glostrup, Glostrup, Denmark; [2]Department of Psychology, Faculty of Social Sciences, University of Copenhagen, Copenhagen, Denmark; [3]Department of Psychiatry, UMC Brain Center, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands; [4]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [5]Mental Health Center Copenhagen, Copenhagen NV, Denmark and [6]Department of Psychiatry, Melbourne Neuropsychiatry Centre, University of Melbourne and Melbourne Health, Carlton South, Victoria, Australia

Original Article

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Author for correspondence:
C. Lemvigh,
E-mail: cecilie.koldbaek.lemvigh@regionh.dk

Abstract

Background. Research has yielded evidence for genetic and environmental factors influencing the risk of schizophrenia. Numerous environmental factors have been identified; however, the individual effects are small. The additive and interactive effects of multiple risk factors are not well elucidated. Twin pairs discordant for schizophrenia offer a unique opportunity to identify factors that differ between patients and unaffected co-twins, who are perfectly matched for age, sex and genetic background.

Methods. Register data were combined with clinical data for 216 twins including monozygotic (MZ) and dizygotic (DZ) proband pairs (one or both twins having a schizophrenia spectrum diagnosis) and MZ/DZ healthy control (HC) pairs. Logistic regression models were applied to predict (1) illness vulnerability (being a proband *v.* HC pair) and (2) illness status (being the patient *v.* unaffected co-twin). Risk factors included: A polygenic risk score (PRS) for schizophrenia, birth complications, birth weight, Apgar scores, paternal age, maternal smoking, season of birth, parental socioeconomic status, urbanicity, childhood trauma, estimated premorbid intelligence and cannabis.

Results. The PRS [odds ratio (OR) 1.6 (1.1–2.3)], childhood trauma [OR 4.5 (2.3–8.8)], and regular cannabis use [OR 8.3 (2.1–32.7)] independently predicted illness vulnerability as did an interaction between childhood trauma and cannabis use [OR 0.17 (0.03–0.9)]. Only regular cannabis use predicted having a schizophrenia spectrum diagnosis between patients and unaffected co-twins [OR 3.3 (1.1–10.4)].

Conclusion. The findings suggest that several risk factors contribute to increasing schizophrenia spectrum vulnerability. Moreover, cannabis, a potentially completely avoidable environmental risk factor, seems to play a substantial role in schizophrenia pathology.

Introduction

Research has yielded evidence for both genetic and environmental factors influencing the risk of developing schizophrenia (Sullivan, Daly, & O'Donovan, 2012; van Os, Kenis, & Rutten, 2010). Strong evidence from family and twin studies has revealed a substantial genetic risk component, indicated by a clear relationship between closer familial relatedness and increased risk of developing the disorder. The risk of developing schizophrenia increases from 1% in the general population to 17% and 48% in dizygotic (DZ) and monozygotic (MZ) twin pairs, respectively (Gottesman, 1991). A recent population-based study using nationwide data from the Danish registers estimated the heritability to be 79% for schizophrenia and 73% for schizophrenia spectrum disorders (Hilker et al., 2017). The genetic architecture of schizophrenia is complex and the polygenic risk score (PRS) provides one way to summarize the genetic influences (Ripke et al., 2014). The PRS for schizophrenia has been shown to predict case-control status and symptom levels in independent samples (Calafat et al., 2018; Vassos et al., 2017).

Although genetic factors play an important role in schizophrenia, they do not fully explain the development of the disorder, evidenced by the average 50% disease-discordance in MZ twins (Cardno & Gottesman, 2000; Hilker et al., 2017). Environmental risk factors known to increase the risk of developing schizophrenia can be divided into developmentally early risk factors including maternal smoking during pregnancy, obstetric complications, low

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birth weight, advanced paternal age and winter/spring birth, and risk factors occurring later in development, such as urban living or growing up in a household of low socioeconomic status (SES), trauma during childhood or adolescence, and substance abuse, particularly of cannabis (Matheson, Shepherd, & Carr, 2014; Matheson, Shepherd, Laurens, & Carr, 2011; McDonald & Murray, 2000; Radua et al., 2018). Moreover, low premorbid intelligence has been demonstrated to increase the risk of schizophrenia in a dose–response fashion with an approximately 4% increase in risk per point decrease in IQ (Kendler, Ohlsson, Sundquist, & Sundquist, 2015; Khandaker, Barnett, White, & Jones, 2011). Some of these risks may in part be related to the genetic vulnerability for schizophrenia, indicated by impaired intelligence in unaffected first-degree family members of patients with schizophrenia (de Zwarte et al., 2020; Snitz, MacDonald, & Carter, 2006), andtwin studies indicating overlapping genetic factors for schizophrenia and cognition (Lemvigh et al., 2020a, 2020b; Toulopoulou et al., 2007).

Exposure to the above-mentioned environmental risk factors is relatively common, while the prevalence of schizophrenia spectrum disorders in comparison is low. Moreover, the magnitude of the effects of individual environmental risk factors on disease risk is typically small (Matheson et al., 2011). It is therefore likely that multiple risk factors acting additively or interactively during critical periods of neurodevelopment may be involved, leading to onset of symptoms once a critical threshold has been reached (Davis et al., 2016; Stilo & Murray, 2019).

A few studies have investigated multiple risk factors for schizophrenia simultaneously (Padmanabhan, Shah, Tandon, & Keshavan, 2017; Stepniak et al., 2014). One study reported an association between accumulating numbers of environmental risk factors and schizophrenia age of illness onset. These included perinatal complications, head injury, psychological trauma, cannabis use, urbanicity, migration, paternal age, and season of birth. Every additional risk factor worsened the outcome, so that patients exposed to four or more risk factors had an earlier age of onset compared to those exposed to three factors, etc. Patients exposed to four or more risk factors experienced disease onset almost 8 years earlier than those with no exposure to the investigated risk factors (Stepniak et al., 2014). An earlier onset is in turn associated with a poorer prognosis (Hollis, 2000; Juola, Miettunen, Veijola, Isohanni, & Jääskeläinen, 2013). Another study created a polyenviromic risk score (PERS) analogous to the PRS (Padmanabhan et al., 2017). They combined cannabis use, urbanicity, season of birth, paternal age, obstetric and perinatal complications, and various types of childhood adversity, each weighted by the odds ratio (OR) for its association with schizophrenia in the literature. A higher PERS was significantly associated with conversion to psychosis in young individuals at familial high risk for psychosis. Together these studies indicate cumulative effects of environmental risk factors. Moreover, environmental risk factors may interact with each other or with the genetic risk, such that a higher genetic liability may increase vulnerability to environmental insults (Misiak et al., 2018).

Discordant twin pairs offer a unique opportunity to identify factors that differ between patients and their unaffected co-twins, who are perfectly matched for age and sex, and partly matched for genetic vulnerability and early environmental influences (van Dongen, Slagboom, Draisma, Martin, & Boomsma, 2012). The aims of the current study were to examine the relative and interactive impact of genetic and environmental risk factors on (1) illness vulnerability and (2) having a schizophrenia spectrum diagnosis. This was done in a nation-wide, combined clinical- and register-based twin cohorts, including proband twin pairs (one or both twins have a diagnosis within the schizophrenia spectrum), and healthy control (HC) twin pairs (neither twin has a schizophrenia spectrum diagnosis).

Methods

The current study is part of the Vulnerability Indicators of Psychosis study, a combined clinical and register-based study that has been approved by The Danish National Committee on Health Research Ethics (H-2-2010-128), The Danish Health and Medicines Authority, and The Danish Data Protection Agency (2010-41-5468). Permission to link the clinical data with information from the Danish birth register was obtained from the Capital Region of Denmark and The Danish Data Protection Agency (CSU-FCFS-2017-012, I-Suite no. 05787). Previous results from this cohort are presented in Legind et al. (2019a, 2019b), Lemvigh et al. (2020a, 2020b), and Rasmussen et al. (2016).

Participants

In total, 216 individuals participated in the study including 32 complete MZ and 24 complete same-sex DZ proband pairs as well as 29 complete MZ and 20 complete same-sex DZ HC pairs. In addition, six individuals from proband pairs participated without their sibling.

To identify potential proband pairs defined by a main or secondary lifetime diagnosis of a schizophrenia spectrum disorder (ICD-8: 295, 297, 298.29, 298.39, 298.89, 298.99, 299.05, 299.09, 301.09, 301.29, or ICD-10: F20.0–F29[1]), the Danish Twin Register was linked with the Danish Psychiatric Central Research Register (Mors, Perto, & Mortensen, 2011) (refer to Table 1 for diagnoses of the included patients). This population was restricted to comprise twin pairs in the age range of 18–60 years, where both twins were alive and living in Denmark (MZ = 61, DZ = 143). First, the 61 MZ proband pairs were invited to participate in clinical examinations and subsequently, same-sex DZ proband pairs and MZ/DZ HC pairs were recruited, matched on age and sex according to the included MZ proband pairs. Exclusion criteria included severe head trauma (as verified in medical records), a diagnosis of addiction to drugs/alcohol, serious physical illness and pregnancy (due to magnetic resonance imaging scans). Additionally, HC twin pairs were excluded based on the presence of a psychosis diagnosis in first-degree family members.

Register diagnoses were validated clinically by trained personnel using the Schedules for Clinical Assessment in Neuropsychiatry interview (SCAN) (Wing et al., 1990). For patients with discrepancies between the register and project diagnosis, the project diagnosis was applied in analyses.

Genetic and environmental risk factors

Information on both early and late environmental risk factors was collected through participant interviews and self-report

[1]ICD-8 codes: 295 schizophrenia, 297 paranoid states, 298 other psychosis, 299 unspecified psychosis, 301.09 personality disorder paranoid, 301.29 personality disorder schizoid. ICD-10: F20 schizophrenia, F21 schizotypal disorder, F22 delusional disorders, F23 brief psychotic disorder, F24 shared psychotic disorder, F25 schizoaffective disorders, F28 other psychotic disorder not due to a substance or known physiological condition, F29 unspecified psychosis not due to a substance or known physiological condition.

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questionnaires. Participants were interviewed about whether their mother smoked during pregnancy, birth weight, obstetric complications, parental age at birth, drug use, education, parental SES, and childhood trauma. We compared the data provided by the two twins in a twin pair for the variables that should be identical to verify the information. In addition, the clinical information was supplemented by register data obtained from the Medical Birth Register (Bliddal, Broe, Pottegård, Olsen, & Langhoff-Roos, 2018) and the Danish Civil Registration System (Schmidt, Pedersen, & Sørensen, 2014), including parental age, birth weight, birth complications, birthplace, and Apgar scores. For variables with both clinical and register-based data, the information was combined to optimize the dataset and reduce the number of missing data. In cases of discrepancy, the register-based data were used to minimize recall bias.

The PRS for schizophrenia in the current sample was calculated at the Institute of Biological Psychiatry, Sct. Hans. The PRS was calculated using PRSice and the training set was the Psychiatric Genetics Consortium on Schizophrenia Genome wide Scanning (Ripke et al., 2014) minus the Danish sample. The samples were phased and imputed using the 1000 genomes phase 3 callset as a reference. SHAPEIT3 was used for phasing and IMPUTE2 for imputation. The p value threshold was 0.05.

Birth complications included e.g. bleeding, hypoxia, acute cesarean section, or premature delivery and were scored as either present or absent. The Apgar score is a quick test performed routinely on the newborn 1 and 5 min after birth, and includes assessment of activity (muscle tone), heart rate, grimace (reflex irritability), appearance (skin color), and breathing. Each category is scored from 0 to 2, with higher scores indicating better functioning (Watterberg et al., 2015). Parental SES was based on education and income level (high/moderate/low). Season of birth was divided into winter/spring (December–May) and summer/autumn (June–November) (Davies, Welham, Chant, Torrey, & McGrath, 2003). Lifetime use of cannabis was scored as: 0 = never, 1 = tried a few times/sporadic use, and 2 = regular use at any given time period, where 'tried a few times' was defined as sporadic experiences throughout the lifetime and 'regular use' covered reports of consistent/systematic use as well as cannabis use disorder and dependence. We also obtained information regarding the methods of use (smoking, edible, vaporizing, etc.), and none of the participants reported edible use. Register information regarding place of birth was used as a measure of urbanicity (urban = capital, suburban, or provincial city with >10 000 inhabitants, rural <10 000 inhabitants). Premorbid intelligence was estimated using the Danish version of the National Adult Reading Task (DART) (Hjorthoj, Vesterager, & Nordentoft, 2013; Nelson & Willinson, 1982). The Childhood Trauma Questionnaire (CTQ), a 28-item retrospective self-report questionnaire, was used to assess trauma during childhood by screening for five domains of maltreatment: emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect (Fink, Bernstein, Handelsman, Foote, & Lovejoy, 1995). Each item was scored on a 5-point Likert scale (1 = never true, 2 = rarely true, 3 = sometimes true, 4 = often true, 5 = very often true) with five items covering each domain (the remaining three items are used to examine minimization/denial), resulting in subscale scores ranging from 5 to 25. The five subscales were summarized into a total score (ranging from 25 to 100) reflecting the global level of self-reported childhood trauma. The Danish version of the CTQ has previously been validated in clinical samples (Kongerslev et al., 2019).

Statistical analyses

Statistical analyses were conducted using SPSS (version 25.0, SPSS Inc.) and R (version 3.6.1). As the continuous variables were on very different scales, to allow for direct comparisons of predictive values, all continuous variables were scaled (mean = 0, S.D. = 1). An unordered factor was created for cannabis to allow for potential differences in effects between categories. For CTQ, raw scores were used in statistical analyses, while cut-off scores based on the Danish norms calculated separately for males and females (Bernstein & Fink, 2011) were used to make figures.

The first aim was to predict illness vulnerability (being a proband pair *v.* a HC pair), and the second aim was to predict illness status (being the patient *v.* an unaffected co-twin). We conducted the same stepwise analyses for both aims. First, each variable was examined separately to identify the independent effect as a risk factor for schizophrenia. Group differences between proband pairs and HC pairs were examined using independent t test for continuous variables and the chi-squared test for ordinal variables. Group differences between patients and their unaffected co-twins (discordant pairs) were examined using paired t tests for continuous variables and paired Wilcoxon-signed rank tests for ordinal variables. Based on these initial analyses, variables showing a significant group effect were included in the models of multiple risk factors using logistic regression. Finally, interactions between significant predictors were explored one by one. Bonferroni corrections were applied to account for multiple testing. For the univariate analyses, the alpha-level was divided by the total number of variables tested in each aim. In the multivariate analyses, the overall model fit was adjusted for the number of models pr. aim.

For completeness, we also ran a model including all available risk factors, but due to a substantial amount of missing data, this resulted in a considerably smaller sample size due to listwise deletion (see online Supplementary Table S1). Finally, we also conducted subgroup analyses based on zygosity (see online Supplementary Table S2).

Results

Demographic and clinical information is presented in Table 1 for patients, their unaffected co-twins, and HC pairs. There were no significant differences in age, $F_{(2,213)} = 0.07$, $p = 0.928$, or sex, $\chi^2_{(2)} = 0.51$, $p = 0.774$, between the three groups.

Fig. 1 shows the number of risk factors reported for patients, unaffected co-twins and HC's ranging from no risk exposures up to six risk exposures. The distribution was not significantly different between groups, $\chi^2_{(12)} = 16.61$, $p = 0.165$. Online Supplementary Table S3 shows the number of twin pairs concordant for a history of obstetric complications.The included risk factors are presented in Table 2 for patients, unaffected co-twins and HC's.

Proband twin pairs v. HC twin pairs

There were significant group differences between proband pairs and HC pairs in self-reported levels of childhood trauma both on the CTQ total score: $t_{(202)} = 7.35$, $p < 0.001$, and all five subscales (emotional abuse: $t_{(202)} = 6.46$, $p < 0.001$; physical abuse: $t(202) = 3.69$, $p < 0.001$; sexual abuse: $t_{(202)} = 3.33$, $p = 0.001$; emotional neglect: $t_{(202)} = 7.07$, $p < 0.001$; and physical neglect: $t_{(202)} = 6.24$, $p < 0.001$). Online Supplementary Fig. S1 shows the distribution of trauma on the five CTQ subscales based on cut-off scores.

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Table 1. Demographic and clinical variables for patients, unaffected co-twins, and HCs

	Patients $N = 64$	Unaffected co-twins $N = 54$	HCs $N = 98$
Zygosity			
MZ, *N* (%)	38 (59.4)	28 (51.9)	58 (59.2)
DZ, *N* (%)	26 (40.6)	26 (48.1)	40 (40.8)
Age, mean (s.d.)	41.09 (10.53)	40.37 (10.51)	40.64 (10.20)
Sex, *N* (%)			
Females	29 (45.3)	28 (51.9)	48 (49.0)
Males	35 (54.7)	26 (48.1)	50 (51.0)
Years of education	13.1 (2.7)	14.0 (3.5)	15.9 (2.8)
Level of education			
Long-cycle higher education/self-employed	3 (4.8%)	7 (13.0%)	25 (25.5%)
Short-cycle higher education/skilled	29 (46.0%)	32 (59.3%)	58 (59.2%)
Unskilled	23 (36.5%)	10 (18.5%)	5 (5.1%)
Student	8 (12.7%)	5 (9.3%)	10 (10.2%)
PANSS, mean (s.d.)			
Positive	14.56 (5.98)	9.17 (3.45)	7.21 (0.72)
Negative	17.18 (7.62)	9.83 (3.49)	7.99 (2.56)
General	32.08 (9.33)	20.58 (5.30)	17.04 (2.20)
Total	63.81 (19.96)	39.57 (10.41)	32.24 (4.79)
F2x diagnosis, *N*			
Schizophrenia	39	0	0
Schizotypal disorder	11	0	0
Acute and transient psychotic disorders	9	0	0
Schizoaffective disorders	4	0	0
Unspecified nonorganic psychosis	1	0	0
Age at first F2x diagnosis, mean (s.d.)	26.88 (7.33)	–	–
Antipsychotics treatment, *N* (%)	39 (60.9)	0	0

Note: 11 individuals from proband pairs were from concordant MZ pairs and both twins were included in the patient group.

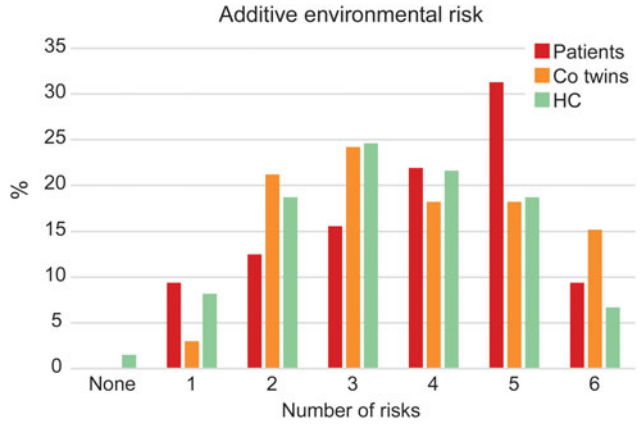

Fig. 1. Additive exposure to environmental risk factors. The figure shows the percentage of patients, unaffected co-twins, and HCs reporting exposure to none or up to six risk factors. The risk factors included in this figure are: childhood trauma (minimum one CTQ subscale above none, cannabis regular use, birth complications, birth weight <2500, paternal age >55, maternal smoking during pregnancy, urbanicity, and winter/spring birth) ($N = 134$).

Moreover, there was a significant group difference in the PRS, $t_{(206)} = 4.26$, $p < 0.001$, and cannabis use, $\chi^2_{(2)} = 21.30$, $p < 0.001$. These group differences all survived Bonferroni correction (Table 2).

There were no significant group differences in estimated premorbid intelligence, $t_{(203)} = 1.05$, $p = 0.296$; birth weight $t_{(169)} = 0.61$, $p = 0.541$; paternal age, $t_{(207)} = -0.47$, $p = 0.642$; Apgar scores (1 min: $t_{(68)} = -1.25$, $p = 0.215$; 5 min: $t_{(68)} = 0.23$, $p = 0.823$), birth complications, $\chi^2_{(1)} < 0.001$, $p = 0.993$, urbanicity, $\chi^2_{(1)} = 1.98$, $p = 0.159$, maternal smoking during pregnancy, $\chi^2_{(1)} = 1.38$, $p = 0.241$, parental SES, $\chi^2_{(2)} = 4.313$, $p = 0.116$, or season of birth, $\chi^2_{(1)} = 0.05$, $p = 0.824$.

The PRS, childhood trauma, and cannabis use were thus included in logistic regression on the 191 individuals with full datasets on these variables (Table 3). The overall model fit was significant, $\chi^2_{(4)} = 66.5$, $p < 0.001$, also after Bonferroni corrections and all three risk factors contributed significantly to the model [PRS: $p = 0.010$, OR 1.6 (1.1–2.3); childhood trauma: $p < 0.001$, OR 4.5 (2.3–8.8); regular cannabis use: $p = 0.003$, OR 8.3 (2.1–32.7)] (model 1). We observed no significant interaction between the PRS and either the CTQ total score or cannabis use (models 2

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Table 2. Risk factors for patients, unaffected co-twins, and HCs

	Patients		Unaffected co-twins		HCs	
	N	Mean (s.d.)	*N*	Mean (s.d.)	*N*	Mean (s.d.)
CTQ total	59	46.14 (18.70)	49	39.12 (12.78)	96	29.96 (5.46)
Emotional abuse		11.08 (6.19)		8.45 (4.27)		6.05 (1.87)
Physical abuse		6.86 (3.55)		6.02 (2.14)		5.28 (1.11)
Sexual abuse		7.14 (4.58)		6.02 (2.74)		5.27 (1.03)
Emotional neglect		12.95 (5.72)		11.10 (4.80)		7.73 (2.99)
Physical neglect		8.10 (3.43)		7.53 (3.05)		5.61 (1.36)
PRS (scaled)	58	0.26 (0.95)	53	0.27 (0.99)	97	−0.30 (0.95)
Birth weight	46	2537.17 (642.43)	39	2508.21 (735.86)	86	2464.55 (581.27)
Paternal age	60	30.97 (6.37)	51	30.63 (6.22)	98	31.22 (6.55)
Apgar scores						
1 min	18	8.89 (1.84)	18	8.39 (1.85)	34	9.18 (1.75)
5 min		9.89 (0.32)		9.61 (0.85)		9.71 (0.97)
Estimated premorbid intelligence (DART)	59	24.54 (8.61)	49	23.98 (7.36)	97	23.21 (6.56)
	N	Percent	*N*	Percent	*N*	Percent
Cannabis						
Never used	18	31.0	23	44.2	59	62.8
Tried a few times/sporadic use	22	37.9	22	42.3	32	34.0
Regular use	18	31.0	7	13.5	3	3.2
Birth complications						
Yes	26	43.3	23	45.1	41	44.1
No	34	56.7	28	54.9	52	55.9
Urbanicity						
Urban	50	86.2	42	84.0	87	91.6
Rural	8	13.8	8	16.0	8	8.4
Smoking during pregnancy[a]						
Yes	25	45.5	19	40.4	31	34.8
No	30	54.5	28	59.6	58	65.2
Parental SES						
High	18	43.9	16	40.0	23	46.0
Moderate	18	43.9	19	47.5	26	52.0
Low	5	12.2	5	12.5	1	2.0
Season of birth						
Winter/spring	32	50.0	24	44.4	48	49.0
Summer/autumn	32	50.0	30	55.6	50	51.0

CTQ, Childhood Trauma Questionnaire; PRS, Polygenic risk score; DART, Danish version of the National Adult Reading Task; SES, socioeconomic status.
Data from the CTQ, polygenic risk score, estimated premorbid intelligence, cannabis, smoking during pregnancy, and parental SES were collected as part of the clinical study. Birth weight, paternal age, birth complications, urbanicity, and season of birth were collected both as part of the clinical study and subsequent verified in the registers. Apgar scores were obtained from the registers.
[a]Maternal use of tobacco during pregnancy.

and 3). However, we did find a significant interaction between the CTQ total score and regular cannabis use [$p = 0.041$, OR 0.17 (0.03–0.93)] (model 4), although this did not survive Bonferroni correction. Figure 2 shows the percentage of patients, unaffected co-twins, and HCs reporting childhood trauma, cannabis use, or a combination of both. The distribution was significantly different between groups, $\chi^2_{(10)} = 49.34$, $p < 0.001$.

Inclusion of all available risk factors (except for Apgar scores and parental SES that were only available for a small number of participants) resulted in a sample size of 127 individuals (29

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Table 3. Logistic regression models predicting illness vulnerability (proband pairs *v*. HC pairs) and illness status (patients *v*. unaffected co-twins)

Models	*N*	Estimate	S.E.	*P* value	OR (CI 95%)	Pseudo-R^2	AIC
Proband pairs *v*. HC pairs							
Model 1							
Intercept	191	−0.01	0.26	0.961	0.99 (0.59–1.64)	0.25	208.02
CTQ total		1.50	0.34	<0.001**	4.49 (2.30–8.77)		
PRS		0.47	0.18	0.010**	1.61 (1.12–2.30)		
Cannabis factor 1		0.58	0.37	0.120	1.78 (0.86–3.66)		
Cannabis factor 2		2.11	0.70	0.003**	8.26 (2.09–32.74)		
Model 2							
Intercept	191	−0.01	0.26	0.961	0.99 (0.59–1.64)	0.25	210.02
CTQ total		1.50	0.34	<0.001**	4.49 (2.29–8.79)		
PRS		0.47	0.20	0.019*	1.61 (1.08–2.39)		
Cannabis factor 1		0.58	0.37	0.118	1.78 (0.86–3.67)		
Cannabis factor 2		2.11	0.71	0.003**	8.26 (2.07–32.91)		
PRS × CTQ		0.00	0.37	0.999	1.00 (0.48–2.07)		
Model 3							
Intercept	191	0.00	0.26	0.986	1.00 (0.60–1.67)	0.26	210.22
CTQ total		1.54	0.35	<0.001**	4.68 (2.36–9.30)		
PRS		0.41	0.25	0.103	1.50 (0.92–2.46)		
Cannabis factor 1		0.60	0.37	0.107	1.83 (0.88–3.81)		
Cannabis factor 2		2.04	0.69	0.003**	7.66 (1.98–29.65)		
PRS × Cannabis 1		0.26	0.39	0.513	1.29 (0.60–2.78)		
PRS × Cannabis 2		−0.97	0.96	0.312	0.38 (0.06–2.49)		
Model 4							
Intercept	191	0.16	0.32	0.610	1.17 (0.63–2.18)	0.26	209.00
CTQ total		1.99	0.56	<0.001**	7.35 (2.46–21.95)		
PRS		0.50	0.19	0.008**	1.64 (1.14–2.38)		
Cannabis factor 1		0.36	0.45	0.423	1.43 (0.60–3.43)		
Cannabis factor 2		1.67	0.70	0.018*	5.33 (1.34–21.22)		
CTQ × Cannabis 1		−0.65	0.74	0.380	0.52 (0.12–2.23)		
CTQ × Cannabis 2		−1.75	0.85	0.041*	0.17 (0.03–0.93)		
Patients *v*. unaffected co-twins							
Model 5							
Intercept	104	−0.31	0.32	0.341	0.73 (0.39–1.39)	0.07	144.15
CTQ emotional abuse		0.39	0.22	*0.069*	1.48 (0.97–2.26)		
CTQ sexual abuse		0.02	0.19	0.928	1.02 (0.70–1.48)		
Cannabis factor 1		0.16	0.46	0.722	1.18 (0.48–2.91)		
Cannabis factor 2		1.20	0.59	0.041*	3.31 (1.05–10.44)		

CTQ, Childhood Trauma Questionnaire; PRS, polygenic risk score; smoking preg, maternal smoking during pregnancy; cannabis factor 1, tried a few times/sporadic use; cannabis factor 2, regular use.
Note: Analyses conducted using R (version 3.6.1).
*Significant at the $p = 0.05$ level, **significant after Bonferroni corrections.

patients, 30 unaffected co-twins, and 68 HCs). Here, the CTQ total score and regular cannabis use were still significant predictors. Birth weight also reached significance, but as lower birth weight in the HC's and not due to an expected lower birth weight in probands (see online Supplementary Table S1).

Patients v. unaffected co-twins

The group averages of risk factors for patients and their unaffected co-twins are presented in Table 2. From univariate analyses of the discordant proband pairs, only CTQ total, $t_{(43)} = 2.68$, $p = 0.010$;

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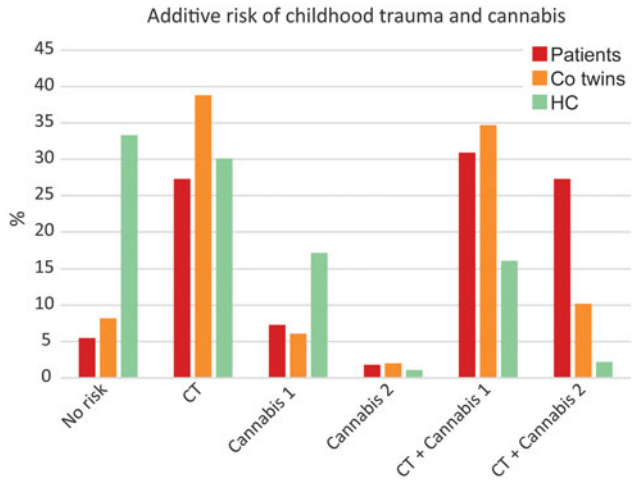

Fig. 2. Additive risk of childhood trauma and cannabis. Percentage of patients, unaffected co-twins, and HCs with exposure to childhood trauma and/or cannabis use. CT: childhood trauma (minimum one subscale above cut-off). Cannabis 1 = tried a few times/sporadic use, cannabis 2 = regular use.

emotional abuse, $t_{(43)} = 2.78$, $p = 0.008$; and sexual abuse, $t_{(43)} = 2.71$, $p = 0.010$, as well as cannabis use, $W = 164.5$, $p = 0.003$, differed significantly between patients and their unaffected co-twins, however only the difference in cannabis use survived Bonferroni corrections. There were no significant differences between patients and the unaffected co-twins on CTQ physical abuse, $t_{(43)} = 1.79$, $p = 0.080$; emotional neglect, $t_{(43)} = 1.66$, $p = 0.103$; or physical neglect, $t_{(43)} = 0.57$, $p = 0.570$. Moreover, there were no significant differences in estimated premorbid intelligence, $t_{(47)} = 0.84$, $p = 0.405$; the PRS, $t_{(46)} = -0.55$, $p = 0.585$; birth weight, $t_{(32)} = -0.08$, $p = 0.939$; Apgar scores (1 min: $t_{(16)} = 1.29$, $p = 0.216$; 5 min: $t_{(16)} = 1.57$, $p = 0.136$); or birth complications, $W = 18$, $p = 1.00$.

When CTQ emotional and sexual abuse as well as cannabis were included in logistic regression (Table 3), the model fit was significant, $\chi^2_{(4)} = 9.64$, $p = 0.05$, but only regular use of cannabis significantly contributed to the model, [$p = 0.041$, OR 3.3 (1.05–10.44)] (model 5). Including all risk factors that differed between patients and their unaffected co-twin resulted in only 65 observations and none of the predictors reached significance (see online Supplementary Table S1).

Discussion

The overall purpose of the study was to examine the relative and interactive effects of genetic and environmental risk factors in schizophrenia spectrum disorders in a large nation-wide twin cohort. The first aim was to examine whether the included risk factors predict illness vulnerability by comparing proband pairs with HC pairs. Here, we demonstrated that the PRS for schizophrenia, childhood trauma, and regular cannabis use significantly predicted illness vulnerability, indicating that these risk factors occur more frequently in proband pairs compared to HC pairs. We observed the highest ORs for childhood trauma and cannabis use, with exposure to childhood trauma resulting in a 4.5-fold increase in the risk of belonging to a proband pair and regular cannabis use resulting in an approximately eight-fold increase. The finding that the PRS for schizophrenia predicted illness vulnerability in our sample is consistent with the previous literature demonstrating that the PRS can predict case-control status and schizophrenia symptoms even in first-episode patients (Calafat et al., 2018; Mistry, Harrison, Smith, Escott-Price, & Zammit, 2017; Ripke et al., 2014; Trotta et al., 2016; Vassos et al., 2017). Moreover, substantial evidence supports the role of childhood trauma (Matheson, Shepherd, Pinchbeck, Laurens, & Carr, 2013) and cannabis use (Colizzi & Murray, 2018) as risk factors for schizophrenia.

In addition, we also observed an interaction between childhood trauma and regular cannabis use (OR of 0.17), although this did not survive Bonferroni correction. Previous studies have shown an interaction between childhood trauma and cannabis use (Setién-Suero et al., 2020), with some studies indicating that individuals with a history of childhood trauma are more likely to use cannabis (Fergusson, Boden, & Horwood, 2008; Hayatbakhsh et al., 2009), whereas others suggest that childhood trauma might make an individual more sensitive to the psychosis-inducing effects of cannabis (Harley et al., 2010; Konings, Stefanis, Kuepper, & De Graaf, 2012). Finally, we observed no interaction between the PRS for schizophrenia and either childhood trauma or cannabis use, suggesting that genetic liability and exposure to these environmental risk factors contribute independently to the development of schizophrenia. Together these findings support a multiple hit theory, where the risk of developing a schizophrenia spectrum disorder involves a genetic vulnerability in combination with early exposures to an adverse environment making an individual more susceptible to risk factors occurring later in life.

The second aim was to examine how risk factors predict diagnosis by comparing patients with their unaffected co-twins. Here, we found that cannabis was the only significant predictor of illness status, indicating that regular use of cannabis may be important in the clinical manifestation of the illness. This is consistent with longitudinal studies in the general population demonstrating that individuals who use cannabis have an increased risk of subsequent development of psychotic symptoms and schizophrenia spectrum disorders (Murray et al., 2017). Moreover, the association between cannabis and psychosis has been found in individuals who are not genetically vulnerable to schizophrenia (as indicated by PRS) (Di Forti, Vassos, Lynskey, Craig, & Murray, 2015; Murray, Quigley, Quattrone, Englund, & Di Forti, 2016). Unfortunately, our measure of cannabis use did not involve an indication of the specific time period, and we do not know whether the individuals started using cannabis before or after the diagnosis of a schizophrenia spectrum disorder. It is therefore not possible to determine causality from the current findings. Some patients may self-medicate with cannabis, although this notion has been refuted by previous research (Murray et al., 2017). The fact that regular cannabis use, a potentially completely avoidable risk factor, was the only significant risk factor for having a schizophrenia spectrum diagnosis within proband pairs, points to an important target for illness prevention strategies and treatment (Murray, David, & Ajnakina, 2020). In line with this, a recent study comparing incidence rates of psychotic disorders across Europe estimated that 12% of all first-episode psychosis cases could have been prevented if high-potency cannabis was made unavailable (Di Forti et al., 2019).

In addition, we observed differences within proband pairs in emotional and sexual abuse, with patients reporting higher levels compared to their unaffected co-twins, although these did not survive Bonferroni correction. Here, it is important to note that we assessed the subjective experience of childhood trauma. All twin pairs in our study grew up in the same household [all

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participants reported living in the same home >10 years (missing data from 10 participants), and 174 reported living in the same home >17 years]. It is entirely possible that only one twin was exposed to trauma, yet an alternative explanation is that the twin, who later developed a schizophrenia spectrum disorder, may have perceived exposures as more traumatic or been more sensitive to such experiences during childhood.

In contrast to previous findings, in this sample of twins, birth weight, birth complications, paternal age, maternal smoking during pregnancy, season of birth, urbanicity, and premorbid intelligence did not significantly increase the risk of schizophrenia vulnerability or manifest illness. However, the observed ORs for these variables are in line with reports from a recent review (Radua et al., 2018). Several issues may explain why these risk factors were not significant predictors in our study. For urbanicity, we used register-based information regarding birthplace. There was very little variation in this measure, i.e. only approximately 10% of the sample was classified as rural. Moreover, place of birth may not be an appropriate measure of urbanicity as this includes no information about where the individual spent most of their lifetime.

Other findings may be explained by the twin design of our study. Twins are exposed to different intrauterine and postnatal environments compared to singletons, in ways that may be related to the future risk of schizophrenia. For example, multiple births in general are associated with lower birth weight and more obstetric complications compared to singleton births (Alexander, Kogan, Martin, & Papiernik, 1998; Campbell & Templeton, 2004; Muhlhausler, Hancock, Bloomfield, & Harding, 2011; Umstad & Gronow, 2003). Indeed evidence suggests that being a twin in itself increases the risk of schizophrenia (Kläning, Mortensen, & Kyvik, 1996; Kleinhaus et al., 2008).

Our categorization of birth complications was broad (present/absent) due to the data available, and more detailed information about the type of complication might have yielded different results.

Finally, we did not observe any significant effects of estimated premorbid intelligence or group differences between patients, unaffected co-twins, and HCs. This is surprising given the substantial literature demonstrating low intelligence as a risk factor for schizophrenia (Dickson, Laurens, Cullen, & Hodgins, 2012; Kendler et al., 2015; Khandaker et al., 2011), but may in part be explained by the use of a word-reading task. We did not have a measure of intelligence obtained before onset of psychosis and therefore had to estimate the premorbid levels. Even though the National Adult Reading Task has been validated as an estimate of premorbid intelligence in schizophrenia; and the Danish version DART has shown good test–retest reliability in patients with psychosis (Hjorthoj et al., 2013), other evidence indicates that word-reading tests may overestimate premorbid intelligence, especially for levels below average, and may therefore not be a sufficiently sensitive measure of premorbid intelligence in our sample (Russell et al., 2000).

The current results should be considered within the limitations of the study. One potential limitation concerns the participants with missing data on one or more risk factor variables, which made it difficult to compare models with different numbers of predictors. We tried to optimize the data by combining register and clinical information, but for some variables the number of participants was very low. In line with this, another potential limitation is the number of participants included in the study. Even though we were able to identify all eligible twin pairs nationwide through the registers, the scarcity of twins with a schizophrenia spectrum disorder in combination with the fact that this patient group is typically difficult to recruit, resulted in a sample size where we did not have sufficient power to conduct subgroup analyses. It is also possible that some of the negative findings in our study may be explained by the lack of power. Moreover, the inclusion of twins means that the data observations are not independent and should theoretically be corrected for familial relatedness. However, we observed too little variation in the included risk factors to apply such corrections reliably. Finally, a diagnosis of drug/alcohol dependency, severe head trauma, and serious physical illness were applied as exclusion criteria for the study, which may be considered a limitation given that these can be associated with the risk for psychosis. Particularly, this means that we would have missed individuals with a current heavy cannabis use. Moreover, we did not have any information regarding the amount and potency of the cannabis used by our participants or the precise time period of use. Nevertheless, we were able to detect an effect of cannabis using this coarse classification. Future studies should apply more detailed measures of cannabis use including the time period of use in relation to the illness onset either through a more comprehensive retrospective interview or a prospective longitudinal design. In addition, information regarding maternal use of cannabis during pregnancy could also potentially provide novel insights into the role of cannabis use in the development of psychosis.

In sum, the PRS, self-reported childhood trauma, regular cannabis use, and an interaction between childhood trauma and cannabis use predicted illness vulnerability, whereas only regular cannabis use distinguished between the patient and unaffected co-twin within proband pairs. The findings suggest that the risk for schizophrenia is influenced by complex processes involving multiple cumulating and potentially interacting risk factors. Moreover, cannabis use seems to play a substantial role in the manifest illness.

Both childhood trauma and cannabis use are traditionally viewed as modifiable environmental risk factors. However, one could question whether trauma and cannabis are truly environmental as the effects of trauma and cannabis use may be inherited, e.g. through epigenetic events across generations. Nevertheless, the current findings underscore the importance of discouraging and targeting cannabis use in children and adolescents at high risk for psychosis, especially those with a history of abuse, to help prevent the development of manifest illness. Future studies should examine factors that could confer resilience to the development of a schizophrenia spectrum disorder. Here, the unaffected co-twins may be a valuable source of information given that they manage to stay well despite an underlying vulnerability to the disorder. Although research on protective factors is sparse, there is some evidence suggesting that a positive family environment (González-Pinto et al., 2011; McMahon et al., 2020) and positive parenting (Whittle et al., 2017), better health and functioning of the mother (Keskinen et al., 2016), high levels of intelligence (Khandaker et al., 2011), and physical activity (Brokmeier et al., 2020) may confer resilience to schizophrenia and reduce the risk of later development of the disorder.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291721002749

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