

Examining the independent and joint effects of molecular genetic liability and environmental exposures in schizophrenia: results from the EUGEI study

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Schizophrenia is a heritable complex phenotype associated with a background risk involving multiple common genetic variants of small effect and a multitude of environmental exposures. Early twin and family studies using proxy-genetic liability measures suggest gene-environment interaction in the etiology of schizophrenia spectrum disorders, but the molecular evidence is scarce. Here, by analyzing the main and joint associations of polygenic risk score for schizophrenia (PRS-SCZ) and environmental exposures in 1,699 patients with a diagnosis of schizophrenia spectrum disorders and 1,542 unrelated controls with no lifetime history of a diagnosis of those disorders, we provide further evidence for gene-environment interaction in schizophrenia. Evidence was found for additive interaction of molecular genetic risk state for schizophrenia (binary mode of PRS-SCZ above 75% of the control distribution) with the presence of lifetime regular cannabis use and exposure to early-life adversities (sexual abuse, emotional abuse, emotional neglect, and bullying), but not with the presence of hearing impairment, season of birth (winter birth), and exposure to physical abuse or physical neglect in childhood. The sensitivity analyses replacing the a priori PRS-SCZ at 75% with alternative cut-points (50% and 25%) confirmed the additive interaction. Our results suggest that the etiopathogenesis of schizophrenia involves genetic underpinnings that act by making individuals more sensitive to the effects of some environmental exposures.

Key words: Schizophrenia, psychosis, genetics, environment, gene-environment interaction, polygenic risk, childhood trauma, cannabis, bullying

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Schizophrenia is a complex phenotype characterized by reality distortion, cognitive alteration and negative symptoms. Although the prevalence of schizophrenia spectrum disorders is relatively low – approximately 0.47% for schizophrenia (the poor outcome fraction) and 3.0% for other clinical diagnoses of psychotic disorders¹ – they account for a tremendous personal, economic and societal burden, with 218 disability adjusted life years (DALYs) per 100,000², making schizophrenia the fifth leading cause of DALYs in the age group of 15–44 years. These figures indicate that there is an urgent need for breakthroughs in prevention, diagnosis and management of

schizophrenia and related disorders, which can be achieved by increased understanding of etiopathology.

Decades of work consistently yielding high heritability estimates document the role of genetic background in the etiopathology of these disorders^{3,4}. In agreement with findings from early family-based studies, recent results from the Danish nationwide registers confirm that the heritability estimates range from 73% for schizophrenia spectrum disorders to 79% for narrow schizophrenia diagnosis⁵.

Based on these findings from the field of quantitative genetic epidemiology, molecular genetics has emerged as arguably

the most popular area of investigation in research targeting schizophrenia spectrum disorders. Easy and low-cost access to high-throughput techniques has increased genetic resolution. The Psychiatric Genomics Consortium⁶ was founded to achieve the power required to detect small effect sizes in a genome-wide association (GWA) analysis. The Schizophrenia Working Group of the Consortium identified 108 genome-wide significant loci⁷, and the number of novel genetic variants keeps growing as a function of sample size⁸. GWA findings, in line with the half-century-old polygenic theory of schizophrenia⁹, established that a large fraction of the genetic risk is explained by many common genetic variants with very small effects sizes.

However, the proportion of the genetic liability accounted for by single nucleotide polymorphisms (SNPs) detected in current GWA arrays represents only a fraction of the effect that was suggested by heritability estimates from twin studies. In other terms, there is a large “heritability gap” between twin and molecular genetics studies¹⁰. The most likely explanation for this gap is that part of the genetic effect documented by twin studies is contingent on environmental factors shared by individuals growing up in the same family¹⁰. The etiology of psychosis spectrum disorder is likely to involve genetic underpinnings that act by making individuals more sensitive to the effects of environmental exposures or by driving individuals to higher exposure rates¹¹.

In parallel to the growing knowledge base in genetics, environmental research into schizophrenia has produced consistent findings over years. Observational studies have identified various exposures associated with risk of psychosis spectrum disorder at different levels of evidence, with varying magnitude of the effect size estimates. These environmental risk factors include cannabis use, childhood adversities (e.g., sexual abuse, emotional neglect), peer-bullying, urban environment, proxies of social exclusion (e.g., ethnic minority, immigration, and hearing impairment), season of birth, and obstetric and pregnancy complications^{12,13}.

Although findings from empirical investigations relying on surrogates of genetic risk (i.e., familial history of schizophrenia) argue for a strong influence of environment in moderating genetic vulnerability¹¹, operationalizing and translating these findings by using molecular candidate-gene approaches have been challenging tasks¹⁴.

The utilization of polygenic risk score (PRS) as a single metric of molecular genetic risk has considerably increased the power to detect associations with phenotypes as well as gene-environment interactions. Currently, the PRS for schizophrenia (PRS-SCZ) of a subject can be estimated by summing the log odds ratios of individual SNPs multiplied by the number of risk alleles present at the corresponding loci¹⁵. PRS-SCZ has been shown to explain up to 7% of variation on the liability scale to schizophrenia, at least when using the latest release of the Psychiatric Genomics Consortium in patients with more chronic forms⁷.

We recently discussed the challenges of evaluating the role of environmental exposures in psychiatry and the need to use exposure-wide systematic approaches to separate genuine strong signals from selective reporting¹⁶. Guided by this, we aimed to analyze the main and joint associations of environmental exposures and PRS-SCZ in a cross-sectional sample that was specifically collected to test for gene-environment interactions in schizophrenia.

METHODS

Study population

This case-control gene-environment interaction study used data from the Work-package 6 (WP6) of the European Network of National Networks studying Gene-Environment Interactions in Schizophrenia (EUGEI)¹⁷ and the Genetic Risk and Outcome of Psychosis (GROUP) study within the EUGEI¹⁸. Data were collected between 2010 and 2015 in the Netherlands, Turkey, Spain and Serbia.

Patients were diagnosed with schizophrenia spectrum disorders according to the DSM-IV-TR (average duration of illness since age of first contact with mental health services = 9.9 years). The diagnosis was later confirmed by the Operational Criteria Checklist for Psychotic and Affective Illness¹⁹ in the EUGEI WP6, and the Schedules for Clinical Assessment in Neuropsychiatry²⁰ or the Comprehensive Assessment of Symptoms and History²¹ in the GROUP. Unrelated controls with no lifetime psychotic disorder were recruited from the same population as the cases. Exclusion criteria for all participants were a diagnosis of psychotic disorder due to another medical condition, a history of head injury with loss of consciousness, and an intelligence quotient <70.

A total of 1,866 patients and 1,583 healthy participants with genotype data available were included. As the predictive power of PRS-SCZ has not been established in people of non-white ethnic origin²², the present analyses were restricted to participants of Caucasian white ethnic origin. The final sample included 1,699 patients and 1,542 unrelated controls.

The projects were approved by the medical ethics committees of all participating sites and conducted in accordance with the Declaration of Helsinki. All respondents provided written informed consent. Participants below the age of 18 signed an assent; parent(s) also signed an informed consent.

To achieve high quality and homogeneity in clinical, experimental and environmental assessments, standardized instruments were administered by psychiatrists, psychologists or trained research assistants who completed mandatory on-site training sessions and online training modules, including interactive interview videos and self-assessment tools^{17,18}. Both on-site and online training sessions were repeated annually to maintain high inter-rater reliability throughout the study enrollment period.

Environmental exposures

Within the limits of data availability, we sought to examine all the environmental exposures that have previously been associated with schizophrenia spectrum disorders.

Childhood adversity was assessed using the Childhood Trauma Questionnaire Short Form (CTQ)²³. This consists of 28 items, rated on a 5-point Likert scale, measuring five domains of maltreatment (emotional and physical neglect; emotional, physical and sexual abuse). The psychometric characteristics of the translated versions (Spanish, Turkish, Dutch and Serbian) of the CTQ have been comprehensively studied^{24–26}. To dichotomize each childhood adversity domain (0=“absent” and 1=“present”), consistent with previous work in the EUGEI²⁷, we used the following cut-off scores for each domain: ≥ 9 for emotional abuse; ≥ 8 for physical abuse; ≥ 6 for sexual abuse; ≥ 10 for emotional neglect; and ≥ 8 for physical neglect.

Cannabis use was assessed by a modified version of the Cannabis Experiences Questionnaire²⁸ in the EUGEI WP6 (0=“none”; 1=“only once or twice”; 2=“a few times a year”; 3=“a few times a month”; 4=“once or more a week”; 5=“everyday”), and by the L section of the Composite International Diagnostic Interview (CIDI)²⁹ in the GROUP (0=“none”; 1=“less than weekly”; 2=“weekly”; 3=“daily”). Consistent with previous work^{30–32}, a binary regular cannabis use variable was constructed by using the cut-off value of once or more per week during the lifetime period of most frequent use.

In accordance with previous studies investigating the association between season of birth and schizophrenia in the Northern hemisphere sites³³, the high-risk birth period was defined based on the winter solstice (December–March), and a binary winter-birth exposure was constructed.

Hearing impairment was defined based on self-reported hearing impairment in the last 12 months (0=“absent” and 1=“present”).

The history of bullying by peers (emotional, psychological or physical violence) before 17 years of age was assessed using the short version of the Retrospective Bullying Questionnaire (RBQ)^{34,35}, that measures the severity of the bullying experience: 0=“none”; 1=“some (no physical injuries)”; 2=“moderate (minor injuries or transient emotional reactions)”; 3=“marked (severe and frequent physical or psychological harm)”. Exposure to childhood bullying was dichotomized using ≥ 1 as the cut-off point (0=“absent” and ≥ 1 =“present”).

Genetic data processing

Samples of all individuals were genotyped at Cardiff University Institute of Psychological Medicine and Clinical Neurology, using custom Illumina HumanCoreExome-24 BeadChip genotyping arrays containing probes for 570038 genetic variants (Illumina, San Diego, CA). Genotype data were called using the GenomeStudio package and transferred into PLINK format for further analysis.

Quality control was conducted in PLINK v1.07³⁶ or with custom Perl scripts. Variants with call rate $<98\%$ were excluded from the dataset. Hardy-Weinberg equilibrium p-value was calculated separately in Turkish, Northern European and Southern European samples. Variants with Hardy-Weinberg equilibrium p-value $<1\text{e-}6$ in any of these three regions were excluded from the dataset. After quality control, 559505 variants remained.

Samples with call rate $<98\%$ were excluded from the dataset. A linkage disequilibrium (LD) pruned set of variants was calculated using the `--indep-pairwise` command in PLINK (maximum $r^2=0.25$, window size=500 SNPs, window step size=50 SNPs) and used for further analyses. Homozygosity F values were calculated using the `--het` command in PLINK, and outlier samples ($F<-0.11$ or $F>0.15$) were excluded. The genotypic sex of samples was calculated from X chromosome data using the `--check-sex` command in PLINK, and samples with different genotypic sex to their database sex were excluded.

Identity-by-descent values were calculated for the sample in PLINK. Samples with one or more siblings among the genotyped samples according to the database but no identified genotypic siblings (defined as $\text{PI-HAT} >0.35$ and <0.65) were excluded. After these were removed from consideration, samples with two or more siblings in the database that were not supported by the genotypic data were also excluded.

After visually observing clustering of errors by genotyping chip, we decided to exclude chips with a high proportion of errors. All samples on chips with five or more sample exclusions due to heterozygosity or call rate (out of 12 possible samples) were excluded. All samples on chips with four or more sample exclusions due to sex or relative checks were also excluded, unless their identity was corroborated by concordance between database and genotypic relatedness data with a sample on another chip.

Principal components were calculated in PLINK using LD pruned variants after combining the dataset with the Thousand Genomes reference. Due to the inherently multi-population nature of the dataset and the variety of possible analyses, no exclusions were made to the whole dataset based on this analysis. Population effects were corrected for separately in individual analyses.

After quality control, genotypes were imputed on the Michigan Imputation Server using the Haplotype Reference Consortium reference panel (version 1.1) and the programs Eagle for haplotype phasing and Minimac3 for imputation^{37,38}. After imputation, variants with an imputation $r^2>0.6$, minor allele frequency (MAF) $>0.1\%$ and call rate $>99\%$ were retained (8277535 variants). Best-guess genotypes were generated from genotype probabilities using PLINK.

PRS-SCZ was constructed using summary statistics from the Psychiatric Genomics Consortium genome-wide association study, excluding samples present in the GROUP data⁷. Clumping was performed in imputed best-guess genotypes for each dataset using PLINK (maximum $r^2=0.2$, window size=500kb, minimum MAF=10%, minimum imputation information (INFO) score=0.7), and variants within regions of long-range LD around the genome (including the human ma-

for histocompatibility complex) were excluded³⁹. PRS-SCZ was then constructed from best-guess genotypes using PLINK at ten different p-value thresholds (1, 0.5, 0.3, 0.2, 0.1, 0.05, 0.01, 1×10^{-4} , 1×10^{-6} , 5×10^{-8}). Consistent with previous research in the field⁴⁰⁻⁴³, we used $p=0.05$ for our primary analysis, as this threshold explained most variation in the phenotype in the Psychiatric Genomics Consortium analysis⁷.

To be able to compare our estimates from the current sample with the previously reported estimates of the proportion of variance explained by PRS-SCZ, a logistic regression model was applied to test the association of PRS-SCZ with case-control status (adjusted for ancestry using the first ten principal components), and Nagelkerke's R^2 was calculated. PRS-SCZ discriminated cases from controls (odds ratio, OR=1.30; 95% CI: 1.25-1.34; $p<0.001$; Nagelkerke's $R^2=0.15$), after also controlling for age, sex and country (OR=1.30; 95% CI: 1.26-1.35; $p<0.001$; Nagelkerke's $R^2=0.20$).

PRS-SCZ was dichotomized using the quartile cut-off points based on the control distribution of PRS-SCZ within each country (to account for differences in PRS-SCZ between countries that may arise due to ethnic variation). The highest quartile (PRS-SCZ > 75% of the controls) was considered the binary genetic risk state for schizophrenia (hereafter: PRS-SCZ₇₅).

Statistical analyses

All analyses were carried out using the STATA version 15.0⁴⁴. Random intercept multilevel logistic regression models, taking into account clustering of participants within countries, were applied to test the univariate associations of exposures and PRS-SCZ₇₅ with case status. For each exposure, gene-environment correlation was tested using multilevel logistic regression models in the control sample. To test gene-environment interaction, additive models were chosen over multiplicative models prior to data collection (EUGEI consortium meeting, December 14, 2013), because they provide superior representation of biological synergy⁴⁵ and inform public health decisions within the sufficient cause framework^{46,47}.

To test the joint effects of environmental exposures and genetic score, we entered the four states occasioned by the combination of each exposure and binary PRS-SCZ risk state (PRS-SCZ₇₅) as independent variables (three dummy variables with no-risk state as the reference category), and case status as the dependent variable, in multilevel logistic regression models.

We tested for departure from additivity using the interaction contrast ratio, also called the relative excess risk due to interaction (RERI). The RERI is considered the standard measure for interaction on the additive scale in case-control studies⁴⁸. The RERI was estimated as $(OR_{\text{exposure} \& \text{PRS-SCZ}_{75}} - OR_{\text{exposure}} - OR_{\text{PRS-SCZ}_{75}} + 1)$ ⁴⁹. A RERI greater than zero was defined as a positive deviation from additivity, and considered significant when the 95% CI did not contain zero. Using the ORs derived from each model, the RERIs for each model were calculated using the delta method.

As a sensitivity measure, the alternative bootstrap percentile method⁵⁰ ($N=1,000$ bootstrap replications) was applied to esti-

mate the bootstrapped 95% CI for the RERI. All models were controlled for *a priori* covariates (age and sex), while models including PRS-SCZ₇₅ were additionally adjusted for ancestry, using the first ten principal components accommodating to the general recommendations. Following the extension to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) reporting guidelines⁴⁸, the interaction analyses were reported using a single reference category including the separate and joint effects of PRS-SCZ₇₅ and each exposure in strata of exposure and PRS-SCZ₇₅.

The analyses were also conducted on imputed data, given missing observations in environmental exposure assessments. Under the assumption of missing at random, the multiple imputation chained equation model⁵¹ with 20 imputations restricted to in-range values was applied (relative efficiency ranging between 97% to 99%). Imputed data were similar to observed values in the original dataset. All analyses were run on multiply imputed data, and estimates were pooled using Rubin's rules⁵².

To test the robustness of our findings, sensitivity analyses of binary genetic risk thresholds were conducted using the PRS-SCZ cut points at 50% and 25% of the controls. The nominal significance threshold was set at $p=0.05$.

RESULTS

Data concerning age, sex and environmental exposures in cases and controls are reported in Table 1.

All exposures except winter birth were associated with case status, also after adjusting for age and sex. Table 2 presents the unadjusted and adjusted ORs for PRS-SCZ₇₅ and each of the exposures associated with case status.

Except for physical abuse, there was no evidence for gene-environment correlation, as PRS-SCZ₇₅ was not associated strongly or significantly with exposures in the control group (Table 3). Physical abuse was associated with PRS-SCZ₇₅ (adjusted OR=1.84; 95% CI: 1.19-2.84; $p=0.006$).

Table 4 reports the interactive effects of PRS-SCZ₇₅ and the exposures on the case status. There was evidence for additive interaction between PRS-SCZ₇₅ and regular cannabis use (RERI=5.60; 95% CI: 0.88-10.33; $p=0.020$), childhood bullying (RERI=2.76; 95% CI: 0.29-5.23; $p=0.028$), emotional abuse (RERI=5.52; 95% CI: 2.29-8.75; $p<0.001$), sexual abuse (RERI=7.61; 95% CI: 2.05-13.17; $p=0.007$), and emotional neglect (RERI=2.46; 95% CI: 0.98-3.94; $p=0.001$), respectively. Figure 1 visualizes the significant interaction effects on an additive scale. No evidence was found for significant additive interaction effects between PRS-SCZ₇₅ and physical abuse, physical neglect, hearing impairment, and winter birth.

Analyses using the alternative bootstrap percentile method for estimating additive interactions yielded similar results (data not shown). The sensitivity analyses replacing the *a priori* set PRS-SCZ₇₅ as the genetic risk in the models with the alternative cut-points of PRS-SCZ (50% and 25%) confirmed that additive interaction was evident for regular cannabis use,

Table 1 Demographic variables and environmental exposures in cases and controls

	Total	Controls	Cases	Missing rates
Age (years, mean±SD)	32.4±9.8	33.4±10.6	31.5±9.0	
Sex				
Male	1,951 (60.2%)	762 (49.4%)	1,189 (70.0%)	
Female	1,290 (39.8%)	780 (50.6%)	510 (30.0%)	
Cannabis use				
No	2,390 (78.6%)	1,366 (91.2%)	1,024 (66.5%)	202 (6.2%)
Yes	649 (21.4%)	132 (8.8%)	517 (33.5%)	
Bullying				
No	1,947 (72.3%)	1,101 (83.7%)	846 (61.4%)	547 (16.9%)
Yes	747 (27.7%)	215 (16.3%)	532 (38.6%)	
Emotional abuse				
No	2,019 (73.0%)	1,230 (84.8%)	789 (60.0%)	475 (14.7%)
Yes	747 (27.0%)	221 (15.2%)	526 (40.0%)	
Physical abuse				
No	2,477 (88.7%)	1,362 (93.0%)	1,115 (84.0%)	450 (13.9%)
Yes	314 (11.3%)	102 (7.0%)	212 (16.0%)	
Sexual abuse				
No	2,269 (81.5%)	1,309 (90.1%)	960 (72.1%)	456 (14.1%)
Yes	516 (18.5%)	144 (9.9%)	372 (27.9%)	
Emotional neglect				
No	1,254 (45.3%)	789 (54.3%)	465 (35.4%)	473 (14.6%)
Yes	1,514 (54.7%)	664 (45.7%)	850 (64.6%)	
Physical neglect				
No	1,804 (64.8%)	1039 (71.3%)	765 (57.7%)	457 (14.1%)
Yes	980 (35.2%)	419 (28.7%)	561 (42.3%)	
Winter birth				
No	1,989 (63.2%)	951 (63.0%)	1,038 (63.4%)	94 (2.9%)
Yes	1,158 (36.8%)	559 (37.0%)	599 (36.6%)	
Hearing impairment				
No	2,869 (92.5%)	1,437 (95.6%)	1,432 (89.7%)	141 (4.4%)
Yes	231 (7.5%)	66 (4.4%)	165 (10.3%)	

childhood bullying, emotional abuse, sexual abuse, and emotional neglect across all PRS-SCZ cut-points (data not shown). The results from the analyses performed in the imputed data were similar (Table 5).

DISCUSSION

In this study examining the main and joint associations of environmental exposures and genetic liability with schizophrenia spectrum disorder, evidence emerged for a positive

Table 2 Main effects of environmental and genetic risk on case-control status

	Unadjusted main effects		Adjusted main effects ^a	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Cannabis use	4.85 (3.89-6.05)	<0.001	3.96 (3.16-4.97)	<0.001
Bullying	3.01 (2.48-3.65)	<0.001	3.06 (2.50-3.74)	<0.001
Emotional abuse	3.51 (2.93-4.22)	<0.001	3.77 (3.12-4.56)	<0.001
Physical abuse	2.70 (2.10-3.48)	<0.001	2.83 (2.18-3.67)	<0.001
Sexual abuse	3.66 (2.96-4.53)	<0.001	4.11 (3.30-5.13)	<0.001
Emotional neglect	2.52 (2.14-2.96)	<0.001	2.65 (2.24-3.13)	<0.001
Physical neglect	2.32 (1.96-2.75)	<0.001	2.33 (1.96-2.78)	<0.001
Winter birth	1.06 (0.92-1.23)	0.423	1.05 (0.91-1.23)	0.495
Hearing impairment	2.46 (1.82-3.31)	<0.001	2.67 (1.96-3.62)	<0.001
PRS-SCZ ₇₅ ^b	2.91 (2.48-3.40)	<0.001	2.85 (2.43-3.35)	<0.001

PRS-SCZ₇₅ – polygenic risk score for schizophrenia (75% cut-point)
^aadjusted for sex and age, ^badjusted for ten principal components

additive interaction of genetic liability with regular cannabis use and childhood adversity domains (sexual abuse, emotional abuse, emotional neglect, and childhood bullying).

To the best of our knowledge, our study is the first to report that the sensitivity to adverse life events during childhood and exposure to cannabis is moderated by genetic risk state for schizophrenia (PRS-SCZ₇₅). Put simply, the positive additive interaction between genetic liability and environmental exposure indicates synergy between gene and environment; that is, the combined influence of genetic liability and environmental exposure is larger than the sum of individual effects of each.

In line with previous findings, PRS-SCZ₇₅ discriminated cases from controls and all environmental exposures (except for winter birth) were associated with case status. However,

Table 3 Gene-environment correlation between PRS-SCZ₇₅ and environmental exposures

	Unadjusted effects		Adjusted effects ^a	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Cannabis use	0.98 (0.61-1.59)	0.949	0.93 (0.57-1.52)	0.771
Bullying	1.27 (0.86-1.86)	0.228	1.28 (0.87-1.89)	0.210
Emotional abuse	1.13 (0.80-1.58)	0.493	1.13 (0.81-1.59)	0.476
Physical abuse	1.82 (1.18-2.81)	0.007	1.84 (1.19-2.84)	0.006
Sexual abuse	0.79 (0.51-1.22)	0.287	0.79 (0.51-1.23)	0.292
Emotional neglect	1.18 (0.91-1.52)	0.212	1.16 (0.90-1.50)	0.258
Physical neglect	1.18 (0.89-1.56)	0.246	1.19 (0.90-1.58)	0.219
Winter birth	1.13 (0.88-1.45)	0.338	1.13 (0.88-1.45)	0.332
Hearing impairment	1.13 (0.63-2.02)	0.693	1.18 (0.65-2.13)	0.592

PRS-SCZ₇₅ – polygenic risk score for schizophrenia (75% cut-point)
^aadjusted for sex, age and ten principal components

Table 4 Interaction of environmental exposures and PRS-SCZ₇₅ on case-control status

	PRS-SCZ ₇₅ =0		PRS-SCZ ₇₅ =1		RERI (95% CI)
	N cases/controls	Odds ratio (95% CI) p<0.001	N cases/controls	Odds ratio (95% CI) p<0.001	
Cannabis use = 0	556/1042	1.0	468/324	2.84 (2.36-3.40) p<0.001	5.60 (0.88-10.33) p=0.020
Cannabis use = 1	296/102	4.10 (3.13-5.36) p<0.001	221/30	11.54 (7.60-17.51) p<0.001	
Bullying = 0	454/842	1.0	392/259	2.84 (2.31-3.47) p<0.001	2.76 (0.29-5.23) p=0.028
Bullying = 1	296/163	2.97 (2.34-3.76) p<0.001	236/52	7.56 (5.41-10.56) p<0.001	
Emotional abuse = 0	464/939	1.0	325/291	2.39 (1.95-2.94) p<0.001	5.52 (2.29-8.75) p<0.001
Emotional abuse = 1	273/166	3.26 (2.58-4.12) p<0.001	253/55	10.17 (7.33-14.10) p<0.001	
Physical abuse = 0	632/1049	1.0	483/313	2.71 (2.25-3.26) p<0.001	1.64 (-1.07 to 4.34) p=0.235
Physical abuse = 1	107/65	2.97 (2.11-4.17) p<0.001	105/37	6.31 (4.19-9.52) p<0.001	
Sexual abuse = 0	536/993	1.0	424/316	2.68 (2.21-3.25) p<0.001	7.61 (2.05-13.17) p=0.007
Sexual abuse = 1	208/114	3.89 (2.99-5.08) p<0.001	164/30	13.19 (8.60-20.22) p<0.001	
Emotional neglect = 0	273/610	1.0	192/179	2.64 (2.03-3.44) p<0.001	2.46 (0.98-3.94) p=0.001
Emotional neglect = 1	464/495	2.58 (2.10-3.17) p<0.001	386/169	6.69 (5.20-8.59) p<0.001	
Physical neglect = 0	438/804	1.0	327/235	2.81 (2.26-3.50) p<0.001	1.51 (0.00-3.03) p=0.051
Physical neglect = 1	308/306	2.42 (1.95 to 3.01) p<0.001	253/113	5.75 (4.36-7.58) p<0.001	
Winter birth = 0	562/733	1.0	476/218	3.11 (2.53-3.82) p<0.001	-0.55 (-1.36 to 0.27) p=0.186
Winter birth = 1	333/414	1.16 (0.96 to 1.41) p=0.123	266/145	2.72 (2.14-3.48) p<0.001	
Hearing impairment = 0	767/1098	1.0	665/339	2.97 (2.51-3.52) p<0.001	1.04 (-2.65 to 4.74) p=0.579
Hearing impairment = 1	107/50	3.11 (2.16 to 4.48) p<0.001	58/16	6.13 (3.43-10.95) p<0.001	

PRS-SCZ₇₅ – polygenic risk score for schizophrenia (75% cut-point), RERI – relative excess risk due to interaction
 Data adjusted for sex, age and ten principal components

no evidence for an additive interaction with PRS-SCZ₇₅ was observed for physical abuse, physical neglect, hearing impairment, or winter birth.

The proportion of variance explained by PRS-SCZ in our sample was comparable to previously reported estimates⁵³ and the most recent findings from the Psychiatric Genomics Consortium⁷. In this dataset, we strictly conformed to previous definitions of environmental exposures to improve reproducibility and allow comparability. In agreement with previous reports, our univariate analysis demonstrated that the exposures we tested were associated with case status to varying degrees, that were similar to meta-analytical estimates^{12,13}.

By taking advantage of direct molecular measures of genetic risk, we provided further support for the putative role of gene-environment interaction in schizophrenia spectrum disorder that was observed in previous studies applying indirect genetic liability estimates derived from family-based (e.g., twin, relative) samples⁵⁴. Our findings were corroborated by the results obtained from regression models using different genetic liability thresholds (PRS-SCZ cut-offs at 50% and 25%) and analyses ran in imputed data.

The RERIs and 95% CIs for emotional and sexual abuse were above 2, thereby suggesting a “mechanistic” interaction⁴⁹, i.e., that there are individuals who would develop schizophrenia

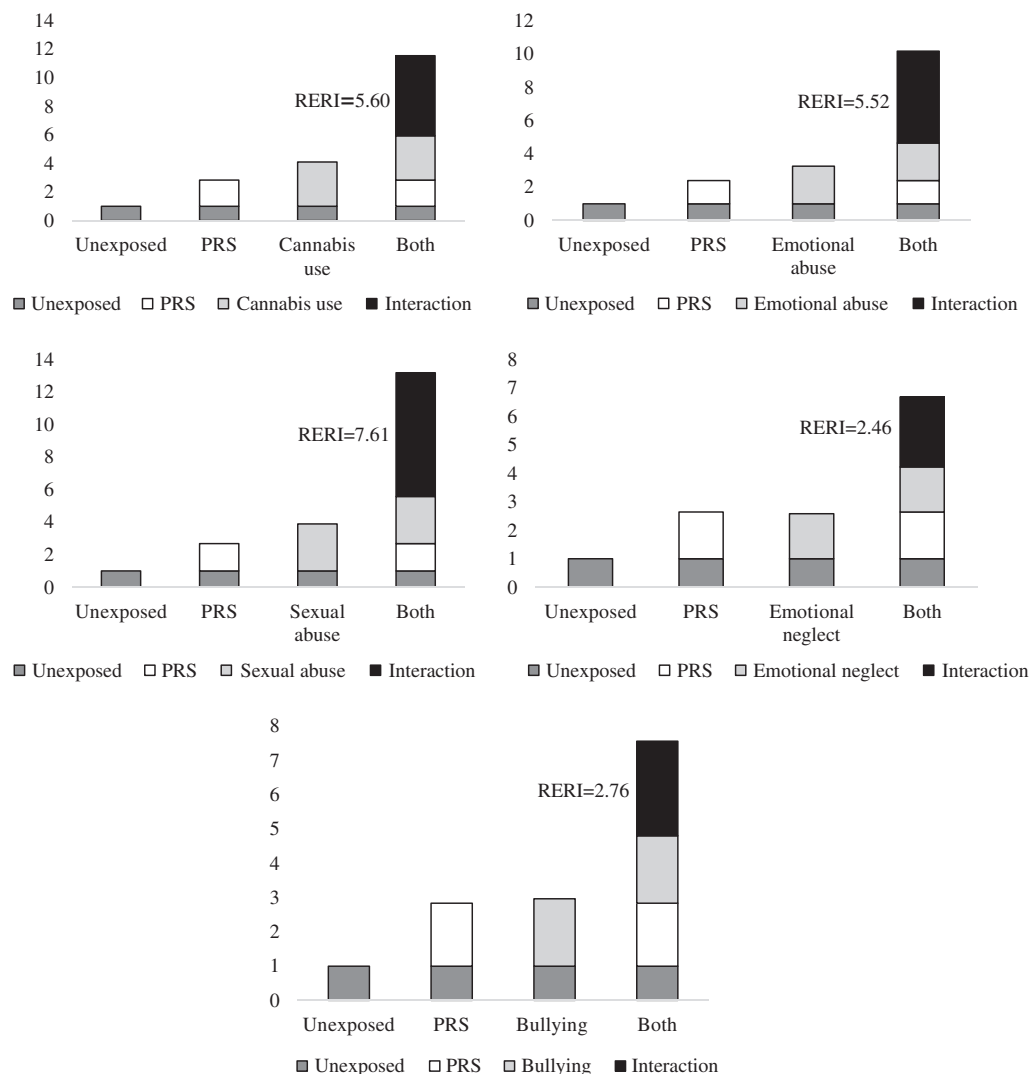


Figure 1 Additive effects of cannabis use, emotional abuse, sexual abuse, emotional neglect and bullying on the association between the polygenic risk score for schizophrenia, 75% cut-point (PRS) and case-control status, adjusted for sex, age and ten principal components; RERI – relative excess risk due to interaction

only when both genetic liability and environmental exposure (emotional or sexual abuse) are present, but would not develop schizophrenia when either genetic liability or environmental exposure is present alone.

PRS-based approaches have recently gained traction in detecting gene-environment interaction. Previously, studies investigated the possible interaction between some genetic polymorphisms possibly linked to the putative biological mechanisms underlying psychosis and cannabis use or childhood adversity. Although SNPs (in various genes) for genetic moderation (e.g., *AKT1*, *COMT*, *BDNF*) were identified, these findings were inconsistent across samples⁵⁵ and became secondary once the genome-wide approach took over the scene.

To date, a limited number of studies tested gene-environment interaction across the psychosis spectrum using PRS-SCZ. A pilot study of 80 patients with first-episode psychotic disorders

and 110 controls investigating whether PRS-SCZ moderates the association between childhood adversities and psychosis, although yielding main effects of both PRS-SCZ and childhood adversities, was considerably underpowered to detect gene-environment interaction⁵⁶. A recent study demonstrated that intra-uterine environment moderates the association between PRS-SCZ and schizophrenia, and further revealed in the pathway analysis that genes involved in cellular stress response were the main drivers of the gene-environment interaction⁵⁷. In our recent study of a general population twin cohort, we found evidence for positive interaction effects between PRS-SCZ and exposure to childhood adversities to pleiotropically influence momentary emotional regulation and psychosis proneness⁵⁸. Further, a multimodal study combining genetics and imaging techniques reported that the association between PRS-SCZ and cortical maturation in young male adults is moderated by

Table 5 Additive interaction effects of PRS-SCZ₇₅ and the environmental exposures on case-control status in the imputed data

	Main effects ^a		Interaction ^b	
	Odds ratio (95% CI)	p	RERI (95% CI)	p
Cannabis use	3.94 (3.15-4.93)	<0.001	5.18 (0.62-9.74)	0.026
Bullying	2.88 (2.36-3.51)	<0.001	2.88 (0.63-5.13)	0.012
Emotional abuse	3.49 (2.88-4.24)	<0.001	5.11 (2.10-8.13)	0.001
Physical abuse	2.65 (2.06-3.40)	<0.001	1.40 (-1.10 to 3.90)	0.272
Sexual abuse	3.74 (3.00-4.66)	<0.001	6.84 (1.77-11.92)	0.008
Emotional neglect	2.51 (2.14-2.95)	<0.001	2.37 (0.90-3.84)	0.002
Physical neglect	2.14 (1.79-2.57)	<0.001	1.42 (-0.05 to 2.88)	0.058
Winter birth	1.06 (0.91-1.23)	0.485	-0.53 (-1.36 to 0.30)	0.209
Hearing impairment	2.68 (1.97-3.66)	<0.001	1.24 (-2.51 to 5.00)	0.516

PRS-SCZ₇₅ – polygenic risk score for schizophrenia (75% cut-point), RERI – relative excess risk due to interaction

^aadjusted for sex and age, ^badjusted for sex, age and ten principal components

early-life exposure to cannabis⁵⁹. Taken together, while the area of gene-environment research is progressing rapidly toward a more replicable path informed by the use of GWA data, conclusive evidence has yet to emerge.

There are various ways in which our findings can move forward gene-environment interaction research in the GWA era. First, they are useful in providing direction for future pre-registered confirmatory studies. Second, they may open up promising research lines for further exploration of gene-environment interactions in the biological context, such as using biologically-informative pathway scores instead of an aggregate genetic risk score for disease phenotype. These studies may help us investigate both hypotheses for biologically plausible pathways impacted by distinct exposures (e.g., hypoxia-ischemia pathway x obstetric complications and childhood adversities x hypothalamic-pituitary-adrenal axis)^{60,61}, and putative common final pathways, such as the broad inflammatory pathway which may be influenced by many exposures cumulatively⁶².

However, there are important caveats: pathway scores may be less powerful than the overall polygenic scores for phenotypes, and there are almost endless options for selecting and constructing “putative” pathways. Therefore, gene-sets for pathways should be *a priori* defined and frozen at a central repository to avoid data-dredging. Further, study protocols for hypothesis-driven selective exposure and pathway analyses (e.g., regular cannabis use and endocannabinoid pathway) should ideally be either registered or, if this is not possible, agnostic data analyses should be followed through.

In our study, data were collected through extensive interviews by trained psychiatrists, psychologists and research assistants to specifically test the role of gene-environment interaction in schizophrenia. Further, our culturally and geographically diverse sample provided us with the advantage of

observing variations in environmental exposures, which increases the power to detect interaction effects⁶³.

However, some limitations should be acknowledged. First, the cross-sectional design informs only on temporal association and not causality. Nevertheless, cross-sectional analyses arguably remain an essential first step for identifying risk factors and pave the way for future longitudinal studies to investigate gene-environment interaction in evolutionary trajectories. Second, given the sample size and explorative nature of the study, we focused on main and interaction associations of previously established environmental factors and PRS-SCZ. However, the reality is much more complex than current statistical models can accommodate, involving dynamic interactions, causal and non-causal associations within the exposome (e.g., dense correlation matrix of environmental factors influenced by the timing, duration, severity and extent of repeated exposures over time)^{16,64}; the genome (e.g., epistasis, redundancy and pleiotropy)⁶⁵; and the phenome (multidimensional syndromal diversity)⁶⁶. Third, instead of the commonly-exercised selective reporting of one exposure at a time, we embraced a quasi-systematic approach to provide an overall picture of the gene-environment interactions findings from this dataset. However, we could not test some other known exposures (e.g., obstetric and pregnancy complications).

In conclusion, by using a molecular genetic risk measure, we have provided further evidence for the role of gene-environment interaction in schizophrenia. Our findings warrant further validation in pre-registered confirmatory research.

APPENDIX

GROUP investigators in EUGEI included: Behrooz Z. Alizadeh, Therese van Amelsvoort, Nico J. van Beveren, Richard Bruggeman, Wiepke Cahn, Lieuwe de Haan, Philippe Delespaul, Jurjen J. Luykx, Inez Myin-Germeys, Ruud van Winkel and Jim van Os.

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