Accumulated environmental risk determining age at schizophrenia onset: a deep phenotyping-based study

Beata Stepniak, Sergi Papiol, Christian Hammer, Anna Ramin, Sarah Everts, Lena Hennig, Martin Begemann, Hannelore Ehrenreich

Summary

Background **Schizophrenia is caused by a combination of genetic and environmental factors, as first evidenced by twin studies. Extensive efforts have been made to identify the genetic roots of schizophrenia, including large genome-wide association studies, but these yielded very small effect sizes for individual markers. In this study, we aimed to assess the relative contribution of genome-wide association study-derived genetic versus environmental risk factors to crucial determinants of schizophrenia severity: disease onset, disease severity, and socioeconomic measures.**

Methods **In this parallel analysis, we studied 750 male patients from the Göttingen Research Association for Schizophrenia (GRAS) dataset (Germany) with schizophrenia for whom both genome-wide coverage of single-nucleotide polymorphisms and deep clinical phenotyping data were available. Specifically, we investigated the potential effect of schizophrenia risk alleles as identified in the most recent large genome-wide association study versus the effects of environmental hazards (ie, perinatal brain insults, cannabis use, neurotrauma, psychotrauma, urbanicity, and migration), alone and upon accumulation, on age at disease onset, age at prodrome, symptom expression, and socioeconomic parameters.**

Findings **In this study, we could show that frequent environmental factors become a major risk for early schizophrenia onset when accumulated (prodrome begins up to 9 years earlier; $p=2\cdot9\times10^{-10}$). In particular, cannabis use—an avoidable environmental risk factor—is highly significantly associated with earlier age at prodrome ($p=3\cdot8\times10^{-20}$). By contrast, polygenic genome-wide association study risk scores did not have any detectable effects on schizophrenia phenotypes.**

Interpretation **These findings should be translated to preventive measures to reduce environmental risk factors, since age at onset of schizophrenia is a crucial determinant of an affected individual's fate and the total socioeconomic cost of the illness.**

Funding **German Research Foundation (Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain), Max Planck Society, Max Planck Förderstiftung, EXTRABRAIN EU-FP7, ERA-NET NEURON.**

Lancet Psychiatry 2014

Published Online
October 22, 2014
http://dx.doi.org/10.1016/S2215-0366(14)70379-7

See Online/Comment
http://dx.doi.org/10.1016/S2215-0366(14)70310-4

Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Göttingen, Germany (B Stepniak MSc, S Papiol PhD, C Hammer PhD, A Ramin BSc, S Everts BSc, L Hennig BSc, M Begemann MD, Prof H Ehrenreich MD); **and DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany** (S Papiol, Prof H Ehrenreich)

Correspondence to:
Prof Hannelore Ehrenreich, Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Hermann-Rein-Str. 3, 37075 Göttingen, Germany
ehrenreich@em.mpg.de

Introduction

Substantial efforts have been made to identify the genetic roots of schizophrenia, in view of heritability estimates of up to 80%.[1] However, awareness is increasing that so-called disease genes of general significance do not exist for the biologically highly heterogeneous, purely clinical construct of schizophrenia. This absence of shared disease genes is supported by the consistently very low odds ratios (ORs) for individual markers derived from genome-wide association studies that are based on ever-increasing numbers of individuals.[1,2]

The most recently published large Psychiatric Genomics Consortium (PGC) genome-wide association study, comprising 36 989 patients with schizophrenia and 113 075 healthy controls, identified 108 genetic loci with genome-wide associations.[2] In addition to these loci, a substantial proportion of schizophrenia risk has been suggested to lie in markers that do not achieve genome-wide significance. Therefore, quantitative polygenic schizophrenia risk scores were calculated on the basis of nominal allele effects. These risk scores now explain up to 7% of variance in the diagnosis of schizophrenia in independent samples.[2] Based on previous genome-wide association studies, the effects of polygenic schizophrenia risk scores on various disease-relevant phenotypes have been explored with variable degrees of success.[3,4]

Importantly, genes alone cannot explain the development of schizophrenia, as indicated by a roughly 50% concordance rate in monozygotic twins.[5] Therefore, intensified research into environmental risk factors is pivotal, also with respect to its inherent preventive potential. Perinatal brain insults, cannabis use, neurotrauma, psychotrauma, urbanicity, and migration are among the most prominently discussed environmental hazards associated with the risk of schizophrenia development.[6–9]

By contrast with most previous work that assessed the effect of different environmental factors on the risk of schizophrenia development, the aim of this study was to assess in patients with schizophrenia the effects of these factors alone and upon accumulation on disease onset, symptom expression, and socioeconomic parameters. Specifically, we retrospectively assessed environmental risk exposure before disease onset. Moreover, we aimed to do back-to-back comparisons of the effects of environmental risk factors and genome-wide association study-derived risk genotypes (grouped into polygenic

schizophrenia risk scores) on the same outcome measures within the same population. Owing to the known differences between male and female patients with schizophrenia in terms of age at onset, psychopathological symptom clusters, vulnerability, and exposure to environmental stressors, we focused on male patients only.[10,11] Similar analyses with female patients remain to be done.

Methods

Study design and participants

The study population for environmental risk assessment consisted of a total of 750 male patients with schizophrenia and schizoaffective disorder (according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision* [DSM-IV-TR]) from the Göttingen Research Association for Schizophrenia (GRAS) dataset.[12,13] To make a polygenic schizophrenia risk score-based case–control status prediction including both men and women, 1067 patients with schizophrenia (according to DSM-IV-TR) from the GRAS sample (including the 750 male patients in whom we assessed environmental risk) and 1169 healthy controls (anonymous blood donors) were analysed.[12] The ethics committees of the Georg-August-University (Göttingen, Germany) and of the 23 centres participating in GRAS throughout Germany approved the study, which complies with the Declaration of Helsinki. All participants (and/or authorised legal representatives) provided written informed consent.

Phenotyping procedures

A meticulous description of the GRAS data collection standard operating procedures is provided elsewhere.[13] In brief, comprehensive information regarding the prodrome (which precedes schizophrenia onset and is characterised by cognitive decline, social withdrawal, and depression), disease onset (onset of first psychotic episode), symptom expression, and socioeconomic functioning was acquired from a very detailed examination. These assessments included positive and negative syndrome scale (PANSS) rating, assessment based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and other semi-structured interviews (assessing suicidality, education history, present employment status, and history of admissions to hospital), telephone consultations, questionnaires, and an essentially complete collection of hospital discharge letters. For example, for all outcome measures of socioeconomic functioning assessed here, patients' self-reports were always double-checked against hospital letters. Education was measured as total years spent in school, further education, and professional training based on the highest qualification achieved. Present employment status and livelihood were assessed and patients were classified as either unemployed or not unemployed (in full-time or part-time employment, retired, or in education). For number of admissions to hospital, all admissions due to psychiatric diagnoses were counted. To assess cognitive symptoms, a composite score was calculated from different neuropsychological tests, comprising reasoning (Leistungsprüfsystem subtest 3 [LPS3]), executive function (Trail-Making Test, part B [TMT-B]), and verbal learning and memory (Verbal Learning and Memory Test [VLMT]).[12] To estimate family mental illness burden, history of any severe mental illness (schizophrenia, psychosis, depression, or mania) in first-degree relatives was recorded.

Environmental risk exposure

To assess environmental risk exposure, specific information about perinatal complications, neurotrauma, psychotrauma, cannabis use, and migration was derived from the patient's history and extensive semi-structured interviews with patients and relatives or caregivers (GRAS Manual[13]) and from SCID-I. Every patient was dichotomously (yes/no) classified as having or not having been exposed (before disease onset and up to 18 years of age) to perinatal complications, neurotrauma, cannabis, psychotrauma, and migration. To measure urbanicity from birth until 18 years of age, information about place of residence and relocation was collected from hospital discharge letters and social history. If information was missing, patients were contacted by telephone or post with an urbanicity questionnaire. In cases of contradictory information or if the missing data could not be obtained, patients were excluded from the respective analysis. For paternal age at birth and season of birth, information was obtained from files.

Perinatal complications were defined as any deviations from normality during pregnancy (eg, alcohol or substance use, infections, pre-eclampsia, or diabetes), delivery (eg, premature or protracted birth, or hypoxia), and in early postnatal life (eg, pronounced jaundice). Neurotrauma was classified as documented head trauma of any grade of severity, from mild trauma to concussion and contusion. For cannabis use, in addition to the dichotomous classification of all patients into cannabis users and non-users, all people who had used cannabis before 18 years of age and before disease onset were grouped regarding frequency of use into: infrequent users (from five times in life up to six times per year); regular users (from once per month up to every other day); and daily use. In this classification, regular use and daily use referred to the 6-month period in life when use was heaviest. Cases of psychotrauma included loss of a first-degree relative or close attachment figure of high importance (severity of loss ≥5 on a rating scale of 0–10), sexual abuse, severe physical abuse (comprising unpredictability of violence, injury caused by physical reprimand, or use of objects for corporal punishment), or any combination thereof. To measure urbanicity, each city the patients lived in was allocated to one of four categories according to its total population (category 1: ≤10 000, category 2: 10 001–50 000, category 3: 50 001–100 000, or category 4: >100 000 inhabitants). The category was then

multiplied by the number of years that the person had spent living there. In cases of relocation, the same procedure was repeated for each new place of residence and values were added up to obtain one urbanicity score per individual. For further calculations, the urbanicity score was dichotomously grouped into rural (score 18–45) and urban (score 46–72) places of residence. To obtain clean data for the risk of 18 years of urban exposure, patients with a schizophrenia onset before 18 years of age were excluded (n=77). For the risk factor of migration, information from sociodemographic interviews was used to classify any patient who immigrated to Germany up to age 18 years as a migrant.

Statistical analysis of environmental risk

To assess group differences in continuous variables, we used the Mann-Whitney *U* test or, in cases of normal distribution of dependent continuous variables, the *t* test. We used logistic regression analysis to study the effects of more than one variable on dichotomous outcome categories. To compare means of more than two groups, we used the Kruskal-Wallis *H* test if the data followed a non-parametric distribution; otherwise, we used ANOVA if data followed a normal distribution. To assess frequency differences between groups we used the χ^2 test. To assess whether medians of more than two groups ascend or descend, we applied the Jonckheere-Terpstra trend test. Trends in frequency distributions were calculated with the Cochran-Armitage test. Covariate correction was done through calculation of linear regression-based standardised residuals when duration of disease or age, chlorpromazine equivalents of current antipsychotic medication, and PANSS negative subscale score were used as independent variables. We used a linear regression (forced entry) model to calculate the variance explained for age at prodrome and age at onset by either cannabis use alone or all other risk factors (perinatal complications, neurotrauma, psychotrauma, urbanicity, and migration). We generated Kaplan–Meier survival curves for different cannabis use frequencies, with age at prodromal onset as the endpoint. We used the log-rank test to make pairwise comparisons of different curves. For all analyses, statistical significance was set to the 0·05 level. We applied Bonferroni correction as a very conservative method to account for multiple testing. P values that withstand this correction are underlined in the respective tables. Statistical analyses were done using SPSS for Windows version 17.0, except for the Cochran-Armitage test for trend, for which R version 2.15.1 was used.

Genotyping, quality control, population structure, and relatedness

Genotyping of the GRAS patients and control sample was done with a semi-custom Axiom myDesign genotyping array (Affymetrix, Santa Clara, CA, USA), based on a CEU (Caucasian residents of European ancestry from Utah, USA) marker backbone including 518 722 single-nucleotide polymorphisms (SNPs), and a custom marker set including 102 537 SNPs. The array was designed using the Axiom Design center, applying diverse selection criteria.[14] Genotyping was done by Affymetrix on a GeneTitan platform. Several quality control steps were used (SNP call rate >97%, Fisher's linear discriminant >3·6, heterozygous cluster strength offset >–0·1, and homozygote ratio offset >–0·9). These steps were completed with use of either genotyping console software (Affymetrix) or R. In a subsequent step, markers in X, Y, and mitochondrial chromosomes and those with Hardy–Weinberg equilibrium $p<1\times10^{-6}$ (healthy controls) or $p<1\times10^{-10}$ (GRAS patients) were removed, leaving 589 921 SNPs available for analyses. We used this dataset to do a principal components analysis of the whole sample in EIGENSTRAT (SmartPCA module) to identify and exclude ancestral outliers in our participant collection, for which we used a sigma threshold of 5·0.[15] This SNP dataset was pruned further, with minor allele frequency (minor allele frequency ≥0·05) and linkage disequilibrium between markers ($r^2 \leq 0·05$) as inclusion criteria, leaving 33 311 markers ready for relatedness ascertainment. Relatedness was ascertained with PLINK version 1.07 through calculation of a genome-wide pairwise identity-by-descent estimation.[16] In those participant pairs with a PI-HAT relatedness coefficient score greater than 0·2, one of the members of the pair was randomly excluded from analyses, resulting in exclusion of a total of 12 participants (in whom PI-HAT ranged from 0·2706 to 0·9996). This pairwise identity-by-descent estimation was also used to calculate multidimensional scaling components to control for population stratification in polygenic analyses. Similarly, the inbreeding coefficient was calculated from the previously mentioned 33 311 SNPs dataset. PLINK was also used to calculate multidimensional scaling components and inbreeding coefficients.[16]

For more on the **Axiom Design center** see www.affymetrix.com

Imputation

Genotype imputation was done with the prephasing or imputation approach implemented in IMPUTE2 and SHAPEIT (chunk size 3 Mb).[17,18] A version of the phase 1 integrated variant set release (v3) from the full 1000 Genomes Project dataset (March, 2012)[19] that is limited to variants with more than one minor allele copy ("macGT1"; Aug 26, 2012) was used as imputation reference dataset (INFO value >0·1 and minor allele frequency >0·005).

Derivation of polygenic schizophrenia risk scores

Polygenic schizophrenia risk scores were calculated as described in the most recent international collaborative genome-wide association study of schizophrenia.[2] Briefly, insertions/deletions, low-frequency genetic variants (minor allele frequency <10%), low imputation quality variants (INFO value <0·9), and extended major histocompatibility complex region genetic variants were excluded for these calculations. Variants in r^2 of 0·1 or

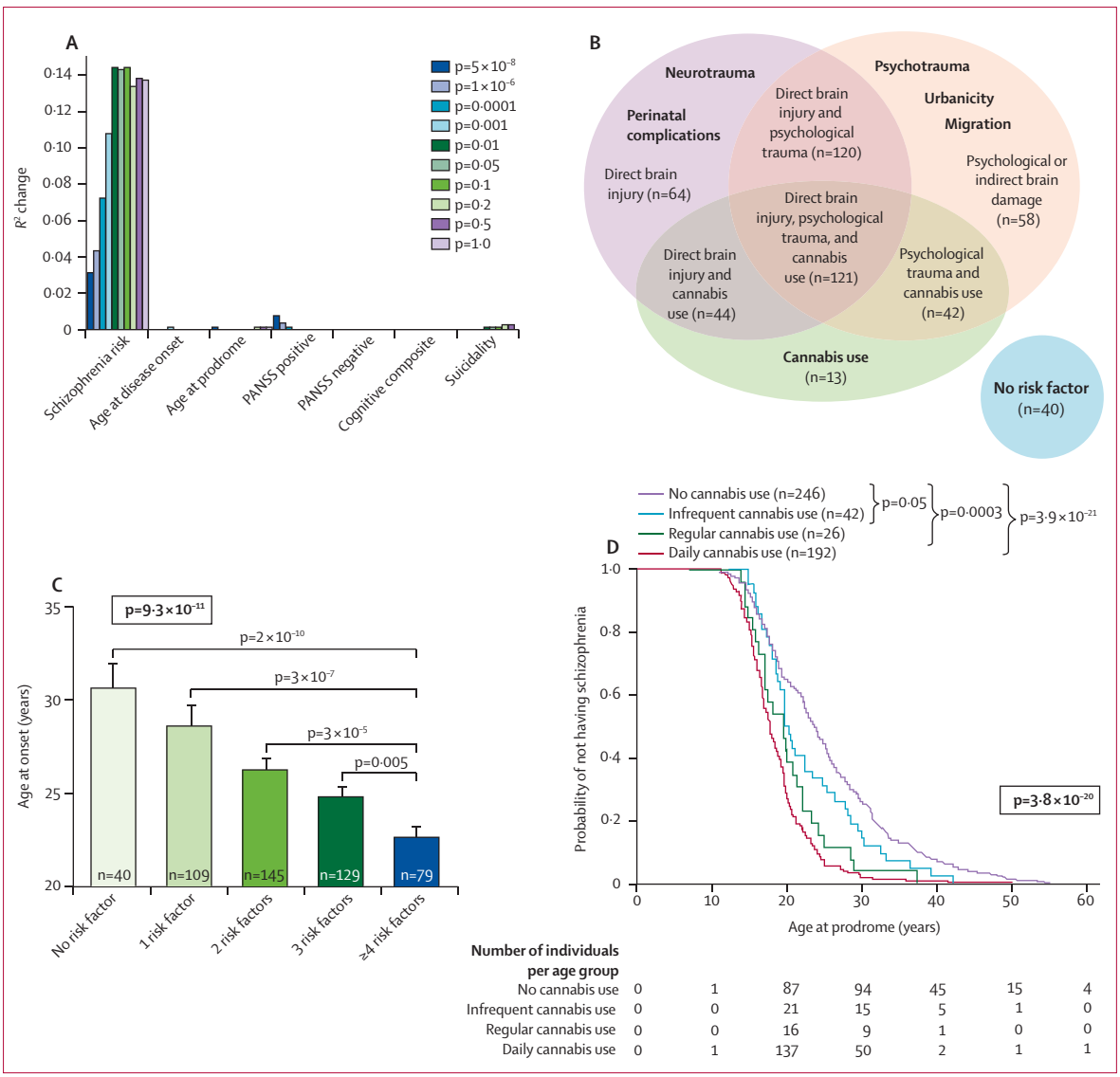


Figure: **Environmental risk contribution to disease onset in schizophrenia versus absence of genetic effects**

(A) Proportion of variance explained (change in adjusted R^2 or Nagelkerke pseudo-R^2) by polygenic genome-wide association study-derived schizophrenia risk scores at increasing p value thresholds. Note that the schizophrenia risk data displayed on the left of the graph are based on 1067 patients (of both sexes) with schizophrenia from the Göttingen Research Association for Schizophrenia sample and 1169 healthy controls (of both sexes), whereas the association with disease phenotypes has been calculated in the male subset of patients with schizophrenia assessed for environmental effects (also see appendix p 3). (B) Overlap of environmental risk exposure in 502 male patients with schizophrenia for whom complete information about all factors was available. (C) Effects of accumulated environmental risk on age at disease onset for the same 502 male patients. The overall p value is shown (in the box) and individual pairwise comparisons of the group with four or more risk factors versus all other groups are presented. The Kruskal-Wallis *H* test and Mann-Whitney *U* test were used for analysis; data are mean (SE) (Bonferroni-corrected significance level: p<0·01). (D) Kaplan–Meier dose–response curves for patients with different frequencies of cannabis use before disease onset and at up to 18 years of age compared with those who have never used cannabis. Regular use ranges from once per month up to every other day, whereas infrequent use includes frequencies from five times in life up to six times per year. The log-rank test was used for group and pairwise intergroup comparisons (Bonferroni-corrected significance level: p<0·02). PANSS=positive and negative syndrome scale.

See Online for appendix

greater and within 500 kb of another marker with stronger effects were discarded, eventually leaving a set of 102 375 SNPs ready for profile scoring. Polygenic schizophrenia risk scores were calculated by application of the *–score function* in PLINK[16] using subsets of SNPs below different p value cutoffs (5×10^{-8}, 1×10^{-6}, 0·0001, 0·001, 0·01, 0·05, 0·1, 0·2, 0·5, 1·0). For each SNP included under these subsets (amounting to a total of 60, 239, 1229, 3359, 10479, 24460, 35486, 51691, 81137, 102375, respectively), the imputation probability for the risk allele was weighted by its respective logarithm of the OR. The individual SNP values were added for each individual participant, leading to the calculation of ten polygenic schizophrenia scores for each person in the target sample.

	Perinatal complications*			Neurotrauma			Cannabis use*		
	No (n=373–424)†	Yes (n=251–284)†	p value (χ^2/Z/t)	No— before first psychotic episode and age ≤18 years (n=292–332)†	Yes— before first psychotic episode and age ≤18 years (n=333–379)†	p value (χ^2/Z/t)	No— before first psychotic episode and age ≤18 years (n=197–217)†	Yes— before first psychotic episode and age ≤18 years (n=188–215)†	p value (χ^2/Z/t)
Disease variables									
Age at disease onset (years)	25·53 (8·01)	23·68 (6·65)	p=0·003 (Z=–2·97)	26·05 (8·25)	24·32 (7·32)	p=0·001 (Z=–3·21)	25·29 (7·50)	23·20 (5·81)	p=0·002 (Z=–3·15)
Age at prodrome (years)	22·71 (7·94)	20·52 (6·57)	p=0·0003 (Z=–3·62)	23·37 (8·20)	21·32 (7·26)	p=0·001 (Z=–3·30)	22·40 (7·19)	20·21 (6·07)	p=0·003 (Z=–2·97)
Positive score on PANSS‡	13·36 (5·94)	13·86 (6·24)	p=0·436 (Z=–0·78)	13·11 (5·89)	13·90 (6·22)	p=0·154 (Z=–1·43)	13·70 (6·56)	13·12 (5·37)	p=0·741 (Z=–0·33)
Negative score on PANSS‡	18·42 (7·70)	17·78 (7·27)	p=0·242 (Z=–1·17)	18·07 (7·75)	18·33 (7·64)	p=0·536 (Z=–0·62)	18·90 (7·88)	17·49 (7·11)	p=0·091 (Z=–1·69)
Cognitive composite§	0·03 (0·81)	0·11 (0·86)	p=0·363 (t=–0·91)	–0·06 (0·87)	0·09 (0·80)	p=0·061 (t=–1·88)	0·06 (0·84)	0·19 (0·74)	p=0·575 (t=–0·56)
Suicidality¶	142 (34·4%)	109 (39·2%)	p=0·196 (χ^2=1·67)	129 (39·7%)	130 (34·7%)	p=0·170 (χ^2=1·89)	74 (35·1%)	88 (41·3%)	p=0·186 (χ^2=1·75)
Socioeconomic variables									
Education (years)‖	11·97 (2·93)	11·68 (2·92)	p=0·095 (Z=–1·67)	12·11 (3·03)	11·86 (2·97)	p=0·357 (Z=–0·92)	12·75 (3·24)	11·18 (2·44)	p<0·00001 (Z=–5·12)
Unemployment**	173 (41·6%)	128 (45·6%)	p=0·300 (χ^2=1·08)	123 (37·4%)	171 (45·6%)	p=0·027 (χ^2=4·86)	79 (37·3%)	104 (48·6%)	p=0·018 (χ^2=5·58)
Number of hospital admissions‡	7·41 (9·45)	8·68 (9·33)	p=0·003 (Z=–2·96)	8·34 (10·93)	8·11 (8·49)	p=0·256 (Z=–1·14)	6·57 (7·98)	10·17 (11·52)	p<0·00001 (Z=–4·69)

Data are uncorrected means (SD) or n (%). For statistical analysis, the Mann-Whitney *U* test or χ^2 test was used, and for normally distributed variables the *t* test was used. Significance values are displayed uncorrected, and p values withstanding Bonferroni correction are underlined. PANSS=positive and negative syndrome scale.*Groups matched for age.†Because of missing data, sample sizes vary. ‡Corrected for duration of disease (standardised residuals after linear regression). §Corrected for age, PANSS negative, and chlorpromazine equivalent (standardised residuals after linear regression). ¶Suicidality=individuals who have attempted suicide in the past. ‖Education refers to the total number of years spent to achieve the highest individual qualification; people presently in education excluded. **Significant results for unemployment were re-examined adding duration of disease as a covariate in a logistic regression model. Neurotrauma (no/yes) did remain a significant predictor of unemployment (no/yes) in the model (Wald test=4·90, p=0·027, odds ratio 1·43). Cannabis use (no/yes) remained a significant predictor of unemployment (no/yes) in the model (Wald test=6·08, p=0·014, odds ratio 1·65).

Table 1: **Effect of environmental risk factors (direct brain injury and cannabis) on measures of disease severity and socioeconomic functioning in male patients with schizophrenia**

Statistical analysis of polygenic schizophrenia risk scores

As dependent variables in a linear regression model, quantitative traits (eg, age at onset and cognitive composite) were used to analyse the effects of polygenic schizophrenia scores. These trait values were corrected when applicable as indicated in the table footnotes. Ten multidimensional scaling components and the inbreeding coefficient were selected as covariates of potential relevance. Adjusted R^2 values derived from a model including all of these covariates were subtracted from adjusted R^2 values from a model including covariates plus the respective polygenic schizophrenia scores. The difference between the adjusted R^2 represents the increase in the variance explained attributable to the score. For dichotomous variables (case–control study and suicidality), in an analogous fashion, Nagelkerke's pseudo-R^2 from a logistic regression containing only covariates (10 multidimensional scaling components and inbreeding coefficient) was compared against the one obtained in a model containing covariates and polygenic schizophrenia scores to estimate the proportion of variance of case–control status explained by the polygenic schizophrenia risk score. The potential effect of an interaction between the risk score and environmental load on phenotypes of interest was also assessed. For this purpose, adjusted R^2 of a model containing only environmental load as predictor was set as the baseline R^2 for comparisons with two models: a model containing environment plus covariates (multidimensional scaling components plus inbreeding coefficient) plus polygenic schizophrenia risk score; and a model containing environment plus covariates (multidimensional scaling components plus inbreeding coefficient) plus polygenic schizophrenia risk score plus the interaction between environment and polygenic schizophrenia risk score (G×E). All calculations were done with SPSS version 17.0.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We studied comprehensively genotyped and phenotyped male patients from our GRAS dataset of patients with schizophrenia.[12–14] We calculated polygenic schizophrenia

	Psychotrauma			Urbanicity			Migration*		
	No—before first psychotic episode and age ≤18 years (n=383–436)†	Yes—before first psychotic episode and age ≤18 years (n=228–257)†	p value (χ^2/Z/t)	Rural upbringing (0–18 years of age) before first psychotic episode (n=299–330)†	Urban upbringing (0–18 years of age) before first psychotic episode (n=213–246)†	p value (χ^2/Z/t)	No—before first psychotic episode and age ≤18 years (n=437–491)†	Yes—before first psychotic episode and age ≤18 years (n=61–71)†	p value (χ^2/Z/t)
Disease variables									
Age at disease onset (years)	25·10 (7·60)	25·02 (8·09)	p=0·567 (Z=–0·57)	26·48 (8·24)	25·90 (6·59)	p=0·640 (Z=–0·47)	23·53 (6·21)	22·56 (4·37)	p=0·595 (Z=–0·53)
Age at prodrome (years)	22·38 (7·44)	21·85 (8·32)	p=0·146 (Z=–1·45)	23·71 (8·50)	22·69 (7·08)	p=0·470 (Z=–0·72)	20·70 (6·20)	19·85 (4·09)	p=0·902 (Z=–0·12)
Positive score on PANSS‡	13·92 (6·44)	13·17 (5·59)	p=0·272 (Z=–1·10)	12·95 (5·72)	13·96 (6·51)	p=0·108 (Z=–1·61)	13·33 (5·85)	12·22 (5·97)	p=0·041 (Z=–2·04)
Negative score on PANSS‡	18·78 (7·64)	17·45 (7·50)	p=0·017 (Z=–2·38)	18·09 (7·55)	18·02 (7·76)	p=0·666 (Z=–0·43)	17·81 (7·32)	17·18 (6·75)	p=0·718 (Z=–0·36)
Cognitive composite§	0·02 (0·86)	0·04 (0·81)	p=0·941 (t=–0·07)	0·03 (0·82)	0·10 (0·82)	p=0·295 (t=–1·05)	0·23 (0·76)	0·01 (0·69)	p=0·0004 (t=3·58)
Suicidality¶‖	145 (33·9%)	103 (40·6%)	p=0·080 (χ^2=3·07)	102 (31·3%)	95 (39·6%)	p=0·041 (χ^2=4·19)	174 (36·1%)	26 (37·1%)	p=0·865 (χ^2=0·03)
Socioeconomic variables									
Education (years)**	12·23 (3·08)	11·57 (2·79)	p=0·006 (Z=–2·75)	12·31 (2·91)	12·23 (3·27)	p=0·547 (Z=–0·60)	11·91 (2·80)	10·18 (2·10)	p<0·00001 (Z=–5·01)
Unemployment‖	185 (42·0%)	104 (40·5%)	p=0·683 (χ^2=0·17)	114 (35·1%)	115 (47·3%)	p=0·003 (χ^2=8·67)	243 (50·0%)	35 (50·7%)	p=0·910 (χ^2=0·01)
Number of hospital admissions‡	8·1 1 (10·28)	7·85 (7·85)	p=0·927 (Z=–0·09)	7·92 (9·25)	8·42 (10·34)	p=0·877 (Z=–0·15)	7·54 (8·51)	7·17 (12·91)	p=0·147 (Z=–1·45)

Data are uncorrected means (SD) or n (%). For statistical analysis, the Mann-Whitney *U* test or χ^2 test was used, and for normally distributed variables the *t* test was used. Significance values are displayed uncorrected, and p values withstanding Bonferroni correction are underlined. PANSS=positive and negative syndrome scale. *Groups matched for age. †Because of missing data, sample sizes vary. ‡Corrected for duration of disease (standardised residuals after linear regression). §Corrected for age, PANSS negative, and chlorpromazine equivalent (standardised residuals after linear regression). ¶Suicidality=individuals who have attempted suicide in the past. ‖Significant results for suicidality and unemployment were re-examined adding duration of disease as a covariate in a logistic regression model. Urbanicity (rural/urban) remained a significant predictor of suicidality (no/yes) (Wald test=3·95, p=0·047, odds ratio 1·43) and unemployment (no/yes) (Wald test=9·77, p=0·002, odds ratio 1·77). **Education refers to the total number of years spent to achieve the highest individual qualification; people presently in education excluded.

***Table 2*: Effect of environmental risk factors (psychological damage) on measures of disease severity and socioeconomic functioning in male patients with schizophrenia**

risk scores for each participant on the basis of p values and ORs available from the latest genome-wide association study of the PGC.[2] Every patient's exposure to selected environmental risk factors up to the age of 18 years and before the onset of psychosis was established on the basis of SCID-I, semi-structured interviews, telephone consultations, questionnaires, and a comprehensive collection of hospitalisation letters.

As figure A and appendix p 3 show, polygenic schizophrenia risk scores based on different levels of significance highly significantly (up to $p=1{\cdot}15\times10^{-50}$) reproduced the association with schizophrenia diagnosis in the GRAS sample, which supports the validity of the study population. By contrast, the same approach did not show any effect on schizophrenia-relevant phenotypes, thus questioning an appreciable role of polygenic schizophrenia risk score-grouped genome-wide association study-derived genotypes for co-determining lead illness features.

With a focus on the environment, we questioned whether the experience of single risk factors during a vulnerable time of brain development, ie, up to the age of 18 years and before the first psychotic episode, has an effect on the time of illness or prodrome onset. Prodrome is a period of several years, typically preceding schizophrenia onset, which is clinically characterised by cognitive decline, social withdrawal, and depression.[20] A comparison of individuals who had suffered a particular environmental insult of interest, to the remaining patients without this insult (table 1), showed significant results for perinatal complications (defined as any kind of deviation from normality that occurred during pregnancy or around birth), neurotrauma (comprising all levels of severity), and cannabis (ranging from any consumption up to regular use). All these risk factors were associated with younger age at disease onset (first psychotic episode) and at prodrome start (all p values ≤0·003, withstanding Bonferroni correction) (table 1). We note that age at onset co-determines overall prognosis.[20] The earlier individuals develop schizophrenia, the less progressed are their levels of education and socialisation. These factors in turn affect individual social functioning and social role performance and, consequently, societal costs.[20] We did not record any effects on age at disease onset or age at prodrome for psychotrauma, urbanicity, and migration (table 2).

Next, we studied whether risk factor exposure had an effect on schizophrenia severity and socioeconomic

	No risk factor (n=37–40)*	One risk factor (n=93–109)*	Two risk factors (n=132–145)*	Three risk factors (n=115–129)*	Four or more risk factors (n=70–79)*	p value (χ^2, *H*, *F*)†	p value (χ^2, *J*)‡
Disease variables							
Age at disease onset (years)	30·66 (7·68)	28·72 (9·34)	26·20 (7·42)	24·84 (5·64)	22·59 (5·21)	$\underline{p=9{\cdot}3\times10^{-11}}$ (*H*=52·82)	$\underline{p=5{\cdot}2\times10^{-12}}$ (*J*=36068·0)
Age at prodrome (years)	28·34 (7·97)	26·07 (9·32)	23·28 (7·82)	21·58 (6·15)	19·40 (5·26)	$\underline{p=2{\cdot}9\times10^{-10}}$ (*H*=50·46)	$\underline{p=5{\cdot}6\times10^{-12}}$ (*J*=28005·5)
Positive score on PANSS§	13·05 (6·32)	13·50 (6·50)	13·16 (5·61)	13·84 (6·24)	12·63 (5·94)	p=0·650 (*H*=2·47)	NA
Negative score on PANSS§	18·31 (7·37)	17·71 (7·87)	18·88 (7·58)	17·48 (6·66)	16·37 (7·29)	p=0·189 (*H*=6·13)	NA
Cognitive composite¶	–0·10 (0·85)	–0·02 (0·88)	–0·02 (0·85)	0·19 (0·77)	0·26 (0·74)	p=0·916 (*F*=0·24)	NA
Suicidality‖	8 (20·0%)	36 (33·6%)	50 (35·2%)	48 (37·5%)	32 (41·0%)	p=0·229 (χ^2=5·63)	p=0·039 (χ^2=4·27)
Sociodemographic variables							
Education (years)**	13·71 (3·03)	13·48 (3·38)	12·24 (3·11)	11·93 (2·67)	10·93 (2·56)	$\underline{p=2{\cdot}3\times10^{-8}}$ (*H*=41·34)	$\underline{p=7{\cdot}7\times10^{-10}}$ (*J*=34112·5)
Unemployment	7 (17·5%)	33 (30·8%)	60 (42·3%)	55 (43·0%)	45 (57·0%)	$\underline{p=0{\cdot}0002}$ (χ^2=22·34)	$\underline{p=6{\cdot}8\times10^{-6}}$ (χ^2=20·24)
Number of hospital admissions§	6·95 (9·80)	7·32 (7·77)	7·39 (7·38)	8·75 (12·05)	8·15 (7·94)	$\underline{p=0{\cdot}001}$ (*H*=19·08)	$\underline{p=2{\cdot}1\times10^{-5}}$ (*J*=56421·0)

Data are uncorrected means (SD) or n (%). NA=not applicable (no trend in data). *Because of missing data, sample sizes vary. †For statistical analysis, the Kruskal-Wallis *H* test or χ^2 test was used, and for normally distributed data ANOVA was used. Significance values are displayed uncorrected, and p values withstanding Bonferroni correction are underlined. ‡To test for statistical trends, the Cochran–Armitage test (qualitative traits) or the Jonckheere-Terpstra test (quantitative traits) was used. Significance values are displayed uncorrected, and p values withstanding Bonferroni correction are underlined. §Corrected for duration of disease (standardised residuals after linear regression). ¶Corrected for age, PANSS negative, and chlorpromazine equivalent (standardised residuals after linear regression). ‖Suicidality=individuals who have attempted suicide in the past. **Education refers to the total number of years spent to achieve the highest individual qualification; people presently in education excluded.

***Table* 3: Effect of an accumulation of environmental risk factors on measures of disease severity and socioeconomic functioning in male patients with schizophrenia**

readouts. Indeed, patients with a history of perinatal complications and those who had started to use cannabis before illness onset had more admissions to hospital (all p values ≤0·003, withstanding Bonferroni correction). Neurotrauma, urbanicity, and cannabis use tended to be associated with higher unemployment rates (tables 1, 2). Psychotrauma, including loss of a close attachment figure and physical and sexual abuse, was associated with fewer years of education (p=0·006; table 2), as were cannabis use and migration (both with p values <0·00001, withstanding Bonferroni correction) (tables 1, 2). Season of birth[21,22] and paternal age at birth,[23] also previously discussed as schizophrenia risk factors, did not show associations with any outcome parameters (appendix pp 4–5); except for paternal age, which was significantly associated with years spent in education (p=0·002, withstanding Bonferroni correction; appendix p 5).

The logical question of whether or not an accumulation of up to four or more risk factors would lead to a more severe disease expression has, to our knowledge, never previously been addressed. Figure B exemplifies the complexity of environmental risk exposure in the male sample group, which is categorised for this illustration into three major domains: direct brain injury (perinatal complications and neurotrauma), psychological or indirect brain damage (psychotrauma, urbanicity, or migration), and cannabis use. To systematically study cumulative effects, we compared patients without risk factor exposure and those with one to four or more environmental risks. We recorded highly significant group differences (all withstanding Bonferroni correction) for age at disease onset and prodromal onset (p values around 1×10^{-10}), years of education ($p=2{\cdot}3\times10^{-8}$), unemployment (p=0·0002), and number of admissions to psychiatric hospital (p=0·001) (table 3). Remarkably, every additional risk factor worsens the outcome further, as emphasised by highly significant trend tests (table 3, figure C). Patients with none or one risk factor experience prodromal onset about 8 years later than do those with four or more environmental insults ($p=3{\cdot}7\times10^{-10}$; Cohen's d=0·99; OR for prodrome before age 23 years versus after: OR 10 [95% CI 4·27–21·70, χ^2=36·63]). The strength of these associations could offset potential concerns regarding false-positive results.

Importantly, the effect of cannabis as a preventable environmental risk factor on age at onset ($p=3{\cdot}8\times10^{-20}$) is enormous (figure D). Cannabis use alone can explain 10·2% of variance in age at disease onset, compared with 4·7% explained by all other environmental risks together (linear regression model). This result calls for public education that targets prevention.

As an internal control (accounting for the unavailability worldwide of an adequate replicate sample), we split the male GRAS population into two equally sized samples according to recruitment date. This split-sample approach provided similar results for both halves of the population (appendix p 6).

No appreciable associations between any environmental factor tested here—alone or upon accumulation—with positive or negative symptom load or cognitive performance were detectable. Within some environmental risk constellations, secondary factors predisposing to mental illness might be hidden, such as social status, societal integration, peer group pressure, access to drugs including cannabis, or family history of psychiatric disorders. For family load of mental disease, no differences were noted between risk factor groups (p>0·05; data not shown). Gene–environment interaction analyses based on genome-wide association study-derived polygenic schizophrenia risk scores and individual environmental burden did not show associations withstanding multiple testing

Panel: Research in context

Systematic review

Between Jan 1, 2005, and Dec 31, 2012, we did a cross-sectional study—the Göttingen Research Association for Schizophrenia (GRAS) data collection—of patients with schizophrenia, recruited from throughout Germany.[12,13] We aimed to collect a disease population with a level of phenotyping accuracy unprecedented until now, complemented by comprehensive genotype and serological analyses.[14] The deep phenotyping provided the basis for all outcome measures and items used in the present study. Systematic literature searches (on Medline and Google Scholar) in preparation for this work showed an accumulation of four or more environmental risk factors in the same population has never previously been studied, and in reports about more than one risk factor, assessed in the same group of individuals, no comparable numbers of comprehensively phenotyped and genotyped patients were evaluated. However, published studies about single environmental risk factors point to them having an important role not only in disease outbreak (schizophrenia risk),[6–9,21–23] but also in disease severity or modulation.[30,35,36] So far, no study has provided data about accumulated environmental risk factors back-to-back with genetic data. However, these facts are less surprising when we consider that although funding for, and public awareness of, genetic trials have been substantial during the past decade, appreciable support for research into environmental risk factors has developed only very recently.

Interpretation

Our study is the first to show that the effect of accumulated environmental risk factors on age at schizophrenia onset is huge, as indicated by the fact that the disease develops nearly a decade earlier in individuals with four or more environmental risk factors than in those with no environmental risk. The environmental effect will exert its share of damage in any individual genetically predisposed to schizophrenia. Not all risk factors are avoidable but some, such as cannabis use, certainly are. Here, awareness among clinicians and in the general public needs to grow. Other risk factors, such as migration and urbanicity, could be alleviated by psychosocial and sociopolitical actions. However, some factors (perinatal complications, neurotrauma, and psychotrauma) might not be easily avoidable. Yet even for these, prophylactic measures might apply (eg, better management of at-risk pregnancies, wearing a helmet when cycling, and early post-trauma intervention). Support for controlled studies of the efficiency of these potential prophylactic measures and interventions should be encouraged by our work.

corrections (appendix pp 7–8). However, this finding does not exclude the possibility that interactions might exist between particular environmental risk factors and specific genetic loci that cannot be detected in aggregate.

Discussion

We used a large sample of thoroughly phenotyped male patients with schizophrenia to investigate for the first time back-to-back in the same population the effects of genome-wide association study-derived genetic markers and of environmental risk factors on disease phenotypes (panel). In this population, we show: a qualitatively and quantitatively different effect of defined single environmental hazards on disease onset and socio-economic burden; a substantial effect of accumulated environmental risk factors on age at prodrome and schizophrenia onset; and an absence of detectable effects of genome-wide association study-derived polygenic schizophrenia risk scores on disease-relevant phenotypes. We also show that the relative significance of cannabis as avoidable environmental risk on age at prodrome is substantial.

The absence of detectable effects of case-control genome-wide association study-derived polygenic schizophrenia risk scores on disease-relevant phenotypes might be less surprising when we consider the tremendous heterogeneity of people who fall into the diagnostic category of schizophrenia.[24] Furthermore, detection of any risk score effects on disease variables, if at all relevant, might need huge sample sizes. On the other hand, SNP variants associated with disease risk as aggregated into genome-wide association study-derived polygenic schizophrenia risk scores might not always overlap with risk variants associated with specific syndrome domains (eg, see reference 25). Moreover, schizophrenia risk score-based analyses might not be optimised for study of association with disease-relevant phenotypes.

Environmental hazards differ greatly in time and pattern of occurrence. Whereas perinatal complications happen early during development, neurotrauma and psychotrauma can occur at any point during childhood or adolescence, even repeatedly, and with variable intensity and individual perception. Urbanicity affects people continuously from birth until adulthood. Moreover, environmental hazards differ in their initial mechanisms of action on the brain, even though they might share final common deleterious pathways downstream. As shown in this study, environmental insults that directly—ie, physically or through specific drug effects—affect brain development, maturation, and integrity of cerebral structures have major effects on crucial outcome elements such as age at disease onset. Psychotrauma and migration act indirectly, probably by inducing high amounts of negative psychosocial stress.[26] Urbanicity is often referred to as a proxy for chronic inevitable, and therefore negative, everyday stress.[27]

A substantial amount of published work exists about early cannabis use and the raised risk of developing schizophrenia (eg, see references 28, 29), whereas work analysing the effect of cannabis on age at onset of schizophrenia is less abundant (eg, see a recent meta-analysis[30]). Age at first cannabis use has been falling[31] and the harmful effects, especially of consumption during the teenage years on cognition, development of social competence, and education, have long been known. This specific window of vulnerability could indicate the crucial developmental role of the endogenous cannabinoid system.[32,33] Interference with this system through exogenous cannabinoids has detrimental consequences on consolidation of maturing brain networks as shown in experimental studies.[7,34] We noted that even minor consumption of cannabis has significant effects on age at onset, indicating that the timepoint of exogenous cannabinoid influence is critical, rather than the dose.

Perinatal complications have previously been described to be associated with earlier age at onset of schizophrenia.[35]

Regarding psychotrauma, sexual but also physical abuse has been linked to intensified hallucinations.[36] However, this finding was not replicable in our purely male population.

To obtain a clean and comparable dataset, environmental risk factors were assessed only when they occurred up to the age of 18 years. This timeframe was used to consider the most relevant time of brain development[37] and thus most important susceptibility to environmental effects.[32,33,38] Of course, the effect of environmental factors (eg, adult life events as disease trigger[39]), and certainly disease onset, can also take place much later.

So far, no assessment of environmental risk accumulation regarding schizophrenia onset or severity that was based on more than two risk factors in an appreciable number of patients has been done. Although psychotrauma, urbanicity, or migration per se did not affect age at onset in our study, our accumulation approach suggests that they still contribute to its reduction. This finding would support potential interaction effects, adding to our overall results. Similarly, although the genetic approach in our study using polygenic schizophrenia risk scores did not show any association with age at onset, some published studies indicate that it is determined by a combination of genetic and—to a greater extent—environmental factors.[40]

Patients with up to one environmental risk factor experienced prodromal onset roughly 8 years later than did those with four or more environmental insults. This time difference is highly relevant regarding chances of outcome, since early adulthood is the most crucial time in life during which the groundwork is laid for occupational integration and success, and social inclusion and stability. These processes will all be negatively affected by early onset of prodrome or psychosis.[20]

To summarise, we obtained in the same cohort of male individuals with schizophrenia robust effects of accumulated environmental risk on age-at-onset of schizophrenia or its prodrome, critical determinants of individual prognosis and socioeconomic burden, in contrast to non-detectable effect of accumulated genome-wide association study-derived risk variants (as assessed by the application of polygenic schizophrenia risk scores) on lead phenotypes of schizophrenia. Several important points can be emphasised here: first, increased public awareness about the risks of early schizophrenia onset is needed, especially regarding the effects of cannabis. Second, after the tremendous interest in genome-wide association studies, the present study will hopefully lead to increased support of intensified research into environmental risk factors and their mechanisms of action. Third, the genetic effect is probably highly specific, and definition of biological disease subgroups or syndromes rather than building on the heterogeneous clinical construct "schizophrenia" will be indispensable for successful genome-wide association studies in the future. By contrast, the effect of environmental factors is enormous but rather non-specific, and will exert its share of damage in any genetically predisposed individual.

Contributors

BS, AR, SE, and LH worked on the establishment of the database including information about the environmental risk factors of Göttingen Research Association for Schizophrenia patients. SP and CH did the genetic analyses. BS, SP, and HE analysed and interpreted the data. MB recruited, diagnosed, and assessed patients. HE planned, supervised, and coordinated the project. BS and HE wrote the report. All authors contributed to the final version of the report.

Declaration of interests

We declare no competing interests.

Acknowledgments

This work was supported by the Max Planck Society, Max Planck Förderstiftung, the German Research Foundation (Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain), EXTRABRAIN EU-FP7, and ERA-Net NEURON (grant no. 01EW1106). We thank all Göttingen Research Association for Schizophrenia (GRAS) patients for participating in the study, and all the many colleagues who have contributed to the GRAS data collection during the past 10 years.

References

1 Giusti-Rodriguez P, Sullivan PF. The genomics of schizophrenia: update and implications. *J Clin Invest* 2013; **123:** 4557–63.
2 Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014; **511:** 421–27.
3 Papiol S, Mitjans M, Assogna F, et al. Polygenic determinants of white matter volume derived from GWAS lack reproducibility in a replicate sample. *Transl Psychiatry* 2014; **4:** e362.
4 Terwisscha van Scheltinga AF, Bakker SC, van Haren NE, et al. Genetic schizophrenia risk variants jointly modulate total brain and white matter volume. *Biol Psychiatry* 2013; **73:** 525–31.
5 Cardno AG, Gottesman II. Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. *Am J Med Genet* 2000; **97:** 12–17.
6 Brown AS. The environment and susceptibility to schizophrenia. *Prog Neurobiol* 2011; **93:** 23–58.
7 Di Forti M, Morrison PD, Butt A, Murray RM. Cannabis use and psychiatric and cogitive disorders: the chicken or the egg? *Curr Opin Psychiatry* 2007; **20:** 228–34.
8 McDonald C, Murray RM. Early and late environmental risk factors for schizophrenia. *Brain Res Brain Res Rev* 2000; **31:** 130–37.
9 van Os J, Kenis G, Rutten BP. The environment and schizophrenia. *Nature* 2010; **468:** 203–12.
10 Abel KM, Drake R, Goldstein JM. Sex differences in schizophrenia. *Int Rev Psychiatry* 2010; **22:** 417–28.
11 Korosi A, Naninck EF, Oomen CA, et al. Early-life stress mediated modulation of adult neurogenesis and behavior. *Behav Brain Res* 2012; **227:** 400–09.
12 Begemann M, Grube S, Papiol S, et al. Modification of cognitive performance in schizophrenia by complexin 2 gene polymorphisms. *Arch Gen Psychiatry* 2010; **67:** 879–88.
13 Ribbe K, Friedrichs H, Begemann M, et al. The cross-sectional GRAS sample: a comprehensive phenotypical data collection of schizophrenic patients. *BMC Psychiatry* 2010; **10:** 91.
14 Hammer C, Stepniak B, Schneider A, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood–brain barrier integrity. *Mol Psychiatry* 2013; published online Sept 3. DOI:10.1038/mp.2013.110.
15 Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; **38:** 904–09.
16 Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81:** 559–75.
17 Delaneau O, Zagury JF, Marchini J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat Methods* 2013; **10:** 5–6.

18 Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009; **5:** e1000529.

19 Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature* 2012; **491:** 56–65.

20 Häfner H, an der Heiden W. Course and outcome of schizophrenia. In: Hirsch SR, Weinberger SR, eds. Schizophrenia, 2nd edn. Oxford: Blackwell Publishing, 2003: 101–41.

21 Messias E, Kirkpatrick B, Bromet E, et al. Summer birth and deficit schizophrenia: a pooled analysis from 6 countries. *Arch Gen Psychiatry* 2004; **61:** 985–89.

22 Mortensen PB, Pedersen CB, Westergaard T, et al. Effects of family history and place and season of birth on the risk of schizophrenia. *N Engl J Med* 1999; **340:** 603–08.

23 Miller B, Messias E, Miettunen J, et al. Meta-analysis of paternal age and schizophrenia risk in male versus female offspring. *Schizophr Bull* 2011; **37:** 1039–47.

24 Ehrenreich H, Nave KA. Phenotype-based genetic association studies (PGAS)—towards understanding the contribution of common genetic variants to schizophrenia subphenotypes. *Genes* 2014; **5:** 97–105.

25 Fanous AH, Zhou B, Aggen SH, et al. Genome-wide association study of clinical dimensions of schizophrenia: polygenic effect on disorganized symptoms. *Am J Psychiatry* 2012; **169:** 1309–17.

26 Glaser JP, van Os J, Portegijs PJ, Myin-Germeys I. Childhood trauma and emotional reactivity to daily life stress in adult frequent attenders of general practitioners. *J Psychosom Res* 2006; **61:** 229–36.

27 Lederbogen F, Kirsch P, Haddad L, et al. City living and urban upbringing affect neural social stress processing in humans. *Nature* 2011; **474:** 498–501.

28 Andreasson S, Allebeck P, Engstrom A, Rydberg U. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet* 1987; **2:** 1483–86.

29 Semple DM, McIntosh AM, Lawrie SM. Cannabis as a risk factor for psychosis: systematic review. *J Psychopharmacol* 2005; **19:** 187–94.

30 Large M, Sharma S, Compton MT, Slade T, Nielssen O. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. *Arch Gen Psychiatry* 2011; **68:** 555–61.

31 Monshouwer K, Smit F, de Graaf R, van Os J, Vollebergh W. First cannabis use: does onset shift to younger ages? Findings from 1988 to 2003 from the Dutch National School Survey on Substance Use. *Addiction* 2005; **100:** 963–70.

32 Ehrenreich H, Rinn T, Kunert HJ, et al. Specific attentional dysfunction in adults following early start of cannabis use. *Psychopharmacology* 1999; **142:** 295–301.

33 Schneider M. Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addict Biol* 2008; **13:** 253–63.

34 Malone DT, Hill MN, Rubino T. Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. *Br J Pharmacol* 2010; **160:** 511–22.

35 Verdoux H, Geddes JR, Takei N, et al. Obstetric complications and age at onset in schizophrenia: an international collaborative meta-analysis of individual patient data. *Am J Psychiatry* 1997; **154:** 1220–27.

36 Read J, van Os J, Morrison AP, Ross CA. Childhood trauma, psychosis and schizophrenia: a literature review with theoretical and clinical implications. *Acta Psychiatr Scand* 2005; **112:** 330–50.

37 Sturman DA, Moghaddam B. The neurobiology of adolescence: changes in brain architecture, functional dynamics, and behavioral tendencies. *Neurosci Biobehav Rev* 2011; **35:** 1704–12.

38 Romeo RD. Adolescence: a central event in shaping stress reactivity. *Dev Psychobiol* 2010; **52:** 244–53.

39 Beards S, Gayer-Anderson C, Borges S, Dewey ME, Fisher HL, Morgan C. Life events and psychosis: a review and meta-analysis. *Schizophr Bull* 2013; **39:** 740–47.

40 Hare E, Glahn DC, Dassori A, et al. Heritability of age of onset of psychosis in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B:** 298–302.

Supplementary Materials

Accumulated environmental risk determining age at schizophrenia onset

Beata Stepniak, MSc[1], Sergi Papiol, PhD[1,2], Christian Hammer, PhD[1], Anna Ramin, BSc[1], Sarah Everts, BSc[1], Lena Hennig, BSc[1], Martin Begemann, MD[1], and Hannelore Ehrenreich, MD, DVM[1,2,*]

[1]Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Göttingen, Germany

[2]DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany

*Correspondence:

Hannelore Ehrenreich, MD, DVM
Clinical Neuroscience
Max Planck Institute of Experimental Medicine
Göttingen, Germany
Phone +49-551-3899615
Fax +49-551-3899670
Email: ehrenreich@em.mpg.de

TABLE OF CONTENTS

Table S1: Association of polygenic schizophrenia scores (PSS) derived from GWAS with disease phenotypes and amount of variance explained (R^2 change) in male schizophrenic patients of the GRAS data collection *(note: PSS case-control study is based on males and females of GRAS)*

		PSS according to different *p*-value thresholds									
		PSS≤5x10^{-8}	**PSS≤1x10^{-6}**	**PSS≤0.0001**	**PSS≤0.001**	**PSS≤0.01**	**PSS≤0.05**	**PSS≤0.1**	**PSS≤0.2**	**PSS≤0.5**	**PSS≤1.0**
Case-control study [a]											
Schizophrenia risk [b]	***p*-value**	**3.31E-13**	**1.67E-17**	**1.14E-27**	**1.88E-39**	**2.56E-50**	**2.17E-50**	**1.15E-50**	**1.66E-47**	**1.32E-48**	**2.66E-48**
(N= 1067 SCZ & 1169 HC)	**R^2 change**	**0.031**	**0.043**	**0.072**	**0.108**	**0.144**	**0.143**	**0.144**	**0.134**	**0.138**	**0.137**
Disease variables											
Age at disease onset [c]	***p*-value**	0.517	0.835	0.396	0.224	0.303	0.733	0.557	0.454	0.448	0.441
(N=700 SCZ males)	**R^2 change**	-0.001	-0.001	0.000	0.001	0.000	-0.001	-0.001	-0.001	-0.001	-0.001
Age at prodrome [c]	***p*-value**	0.168	0.741	0.916	0.827	0.521	0.483	0.340	0.253	0.242	0.228
(N=621 SCZ males)	**R^2 change**	0.001	-0.001	-0.002	-0.002	-0.001	-0.001	0.000	0.001	0.001	0.001
PANSS positive [c]	***p*-value**	**0.015**	0.070	0.183	0.563	0.490	0.329	0.417	0.583	0.396	0.351
(N=683 SCZ males)	**R^2 change**	**0.007**	0.003	0.001	-0.001	-0.001	0.000	-0.001	-0.001	0.000	0.000
PANSS negative [c]	***p*-value**	0.478	0.323	0.576	0.823	0.317	0.430	0.306	0.515	0.426	0.443
(N=678 SCZ males)	**R^2 change**	-0.001	0.000	-0.001	-0.001	0.000	-0.001	0.000	-0.001	-0.001	-0.001
Cognitive composite [c]	***p*-value**	0.687	0.763	0.886	0.937	0.659	0.608	0.403	0.393	0.311	0.280
(N=663 SCZ males)	**R^2 change**	-0.001	-0.001	-0.001	-0.002	-0.001	-0.001	0.000	0.000	0.000	0.000
Suicidality [b]	***p*-value**	0.944	0.664	0.989	0.722	0.581	0.546	0.431	0.264	0.268	0.282
N=(684 SCZ males)	**R^2 change**	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.002	0.002	0.002

For all analyses 10 population stratification dimensions and inbreeding coefficient were used as covariates. [a] For case-control study both genders were included in the analyses. [b] Logistic regression was performed for dichotomous phenotypes; Nagelkerke's pseudo R^2 between a model containing only covariates and a model containing covariates+PSS were compared in order the address the effect of PSS. [c] Linear regression was used for continuous variables; adjusted R^2 between a model containing only covariates and a model containing covariates+PSS were compared in order the address the effect of PSS. SCZ, Schizophrenia cases; HC, Healthy controls.

Table S2: Impact of season of birth on readouts of disease severity and socioeconomic functioning in male schizophrenic individuals

	Season of birth			
	February & March**	June & July**	other months	p [a] ($\chi^2/Z/T$) [a]
	n=104-117[b]	n=113-126[b]	n=439-503[b]	
Disease variables				
Age at disease onset, mean±SD	25.89±7.73	25.61±8.42	24.75±7.61	p=0.179 (H=3.44)
Age at prodrome, mean±SD	22.80±7.98	22.74±8.25	21.91±7.63	p=0.301 (H=2.40)
PANSS positive, mean±SD [c]	14.22±5.78	13.01±6.08	13.64±6.18	p=0.194 (H=3.28)
PANSS negative, mean±SD [c]	19.04±8.13	18.14±7.83	18.13±7.50	p=0.557 (H=1.17)
Cognitive composite, mean±SD [d]	0.03±0.76	-0.04±0.82	0.05±0.86	p=0.370 (F=1.00)
Suicidality, n (%) [e]	36 (32.1)	48 (39.3)	182 (36.6)	p=0.511 (χ^2=1.34)
Socioeconomic variables				
Education, years, mean±SD [f]	12.30±3.31	11.91±3.09	11.84±2.89	p=0.593 (H=1.04)
Unemployment, n (%)	44 (38.6)	51 (41.1)	213 (42.8)	p=0.706 (χ^2=0.70)
Number of hospitalizations [c]	8.67±11.63	8.23±10.47	8.14±9.08	p=0.678 (H=0.78)

**Previous work reported highest risk for schizophrenia in individuals born in February and March[22] or associations with deficit schizophrenia upon summer birth (June, July)[21].

Uncorrected means±standard deviations (SD) displayed. [a] For statistical methods, Kruskal-Wallis-H or χ^2-test and for normally distributed data ANOVA was used. Bolded values, p<0.05. Significance values are displayed uncorrected with p-values withstanding Bonferroni correction underlined. [b] Due to missing data, sample sizes vary. [c] Corrected for duration of disease (standardized residuals after linear regression). [d] Corrected for age, PANSS negative, chlorpromazine equivalents (standardized residuals after linear regression). [e] Suicidality = individuals with history of suicide attempts. [f] Education refers to total of years spent to achieve the highest individual qualification; individuals currently in education excluded.

Table S3: Impact of paternal age on readouts of disease severity and socioeconomic functioning in male schizophrenic subjects

	Paternal age (at birth)*			
	age < 25**	age 25-35	age > 35**	p [a] (χ^2/Z/T) [a]
	n=122-137[b]	n=340-381[b]	n=106-118[b]	
Disease variables				
Age at disease onset, mean±SD	25.53±8.96	25.08±7.58	25.19±7.74	p=0.896 (H=0.22)
Age at prodrome, mean±SD	22.25±9.21	22.33±7.40	22.46±8.28	p=0.387 (H=1.90)
PANSS positive, mean±SD [c]	13.09±5.77	13.36±6.12	14.36±6.31	p=0.357 (H=2.06)
PANSS negative, mean±SD [c]	18.70±7.36	17.53±7.71	18.49±7.32	p=0.132 (H=4.05)
Cognitive composite, mean±SD [d]	0.05±0.84	0.11±0.81	0.00±0.79	p=0.802 (F=0.22)
Suicidality, n (%) [e]	51 (38.6)	131 (34.8)	41 (35.0)	p=0.727 (χ^2=0.64)
Socioeconomic variables				
Education, years, mean±SD [f]	11.56±2.62	12.53±3.12	11.70±3.00	**p=0.002** (H=12.69)
Unemployment, n (%)	57 (42.2)	148 (39.2)	50 (42.4)	p=0.733 (χ^2=0.62)
Number of hospitalizations [c]	9.20±12.40	7.75±8.77	8.19±7.37	p=0.249 (H=2.78)

**High and low paternal age has previously been associated with increased risk for schizophrenia[23].
Uncorrected means±standard deviations (SD) displayed. [a] For statistical methods, Kruskal-Wallis-H or χ^2-test and for normally distributed data ANOVA was used. Bolded values, p<0.05. Significance values are displayed uncorrected with p-values withstanding Bonferroni correction underlined. [b] Due to missing data, sample sizes vary. [c] Corrected for duration of disease (standardized residuals after linear regression). [d] Corrected for age, PANSS negative, chlorpromazine equivalents (standardized residuals after linear regression). [e] Suicidality = individuals with history of suicide attempts. [f] Education refers to total of years spent to achieve the highest individual qualification; individuals currently in education excluded.

Table S4: Impact of accumulation of environmental factors on disease and prodrome onset in male schizophrenic individuals: Internal control via group split by recruitment date

GROUP 1	No risk factor	One risk factor	Two risk factors	Three risk factors	Four or more risk factors	p [a] (H) [a]	p [b] (J) [b]
	n=18-20[c]	n=47-54[c]	n=62-72[c]	n=51-64[c]	n=32-39[c]		
Disease variables							
Age at disease onset, mean±SD	29.20±6.53	28.92±9.21	26.50±8.01	25.05±5.38	22.18±5.44	**$p=4.5\times10^{-6}$** (H=30.19)	**$p=3.8\times10^{-7}$** (J=8730.5)
Age at prodrome, mean±SD	26.90±7.31	26.37±9.24	24.47±8.81	21.27±6.14	18.73±5.90	**$p=9.8\times10^{-6}$** (H=28.52)	**$p=1.5\times10^{-7}$** (J=5927.5)

Uncorrected means and standard deviations (SD) displayed, bolded values, $p<0.05$ (two-tailed).
[a] For statistical methods, Kruskal-Wallis-H test was used. [b] To test for statistical trend Jonckheere-Terpstra test (quantitative traits) was used. [c] Due to missing data, sample sizes vary.

GROUP 2	No risk factor	One risk factor	Two risk factors	Three risk factors	Four or more risk factors	p [a] (H) [a]	p [b] (J) [b]
	n=19-20[c]	n=46-55[c]	n=70-73[c]	n=64-65[c]	n=38-40[c]		
Disease variables							
Age at disease onset, mean±SD	32.13±8.60	28.52±9.56	25.91±6.84	24.64±5.96	22.99±5.01	**$p=6.1\times10^{-5}$** (H=24.58)	**$p=3.1\times10^{-6}$** (J=9312.0)
Age at prodrome, mean±SD	29.71±8.52	25.78±9.50	22.23±6.72	21.83±6.19	19.96±4.66	**$p=7.8\times10^{-5}$** (H=24.04)	**$p=1.7\times10^{-5}$** (J=8269.0)

Uncorrected means and standard deviations (SD) displayed, bolded values, $p<0.05$ (two-tailed). Significance values are displayed uncorrected with p-values withstanding Bonferroni correction underlined.
[a] For statistical methods, Kruskal-Wallis-H test was used. [b] To test for statistical trend Jonckheere-Terpstra test (quantitative traits) was used. [c] Due to missing data, sample sizes vary.

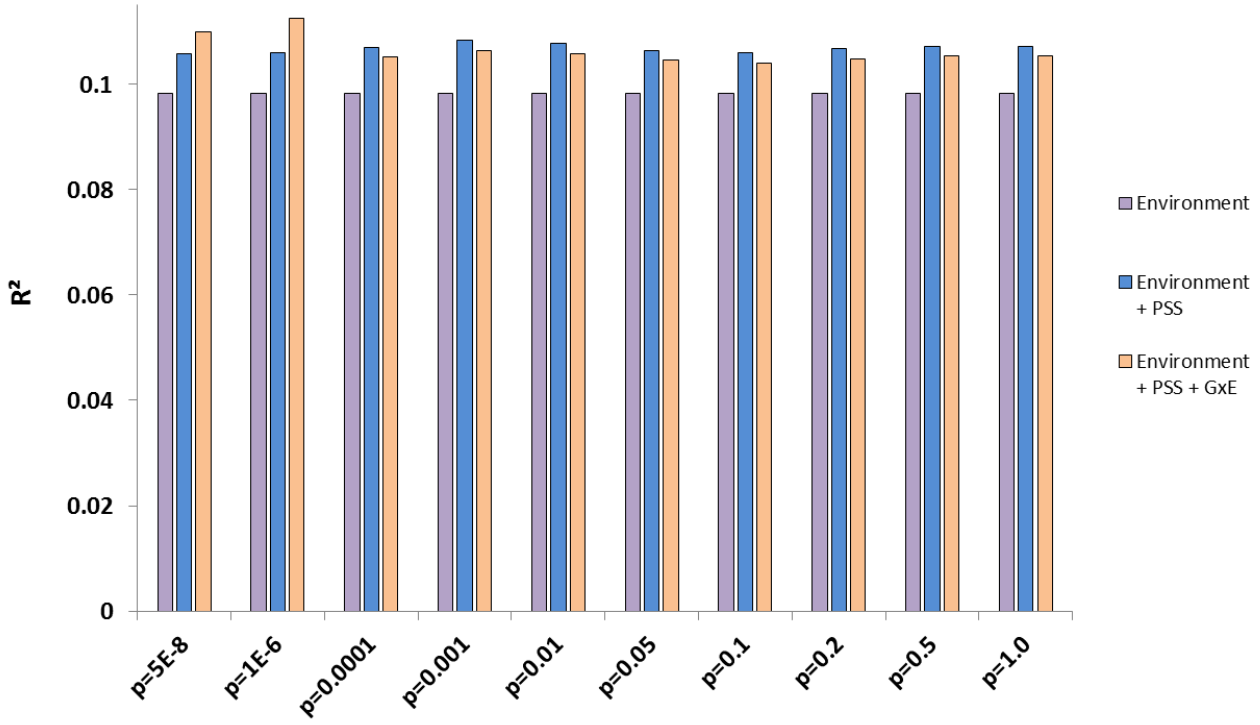
Table S5: Association of polygenic schizophrenia scores (PSS) or their interaction with environmental factors (GxE) with disease phenotypes and amount of variance explained (R^2 change) in male schizophrenic patients. *Models containing only environmental load as predictor were used to set the baseline R^2 in order to estimate the potential additional effect of PSS or GxE.*

Disease variables			PSS according to different *p*-value thresholds									
			PSS≤5x10^{-8}	**PSS≤1x10^{-6}**	**PSS≤0.0001**	**PSS≤0.001**	**PSS≤0.01**	**PSS≤0.05**	**PSS≤0.1**	**PSS≤0.2**	**PSS≤0.5**	**PSS≤1.0**
Age at disease onset [a]	PSS	***p*-value**	0.524	0.459	0.308	0.189	0.243	0.415	0.497	0.346	0.288	0.289
		R^2 change	0.008	0.008	0.009	0.010	0.009	0.008	0.008	0.008	0.009	0.009
	GxE	***p*-value**	0.080	**0.039**	0.860	0.950	0.834	0.683	0.897	0.844	0.768	0.768
		R^2 change	0.012	0.014	0.007	0.008	0.008	0.006	0.006	0.007	0.007	0.007
Age at prodrome [a]	PSS	***p*-value**	0.145	0.943	0.802	0.731	0.427	0.444	0.451	0.286	0.262	0.257
		R^2 change	0.010	0.005	0.006	0.006	0.007	0.007	0.007	0.008	0.008	0.008
	GxE	***p*-value**	0.126	**0.014**	0.178	0.399	0.531	0.454	0.683	0.736	0.638	0.669
		R^2 change	0.013	0.017	0.007	0.005	0.006	0.006	0.005	0.006	0.007	0.007
PANSS positive [a]	PSS	***p*-value**	0.104	0.388	0.192	0.472	0.921	0.891	0.997	0.844	0.979	0.997
		R^2 change	0.001	-0.003	-0.001	-0.004	-0.005	-0.005	-0.005	-0.005	-0.005	-0.005
	GxE	***p*-value**	0.427	0.643	0.989	0.865	0.637	0.777	0.925	0.839	0.927	0.995
		R^2 change	0.000	-0.005	-0.004	-0.006	-0.007	-0.007	-0.007	-0.007	-0.007	-0.007
PANSS negative [a]	PSS	***p*-value**	0.687	0.715	0.582	0.521	0.991	0.997	0.874	0.863	0.896	0.916
		R^2 change	0.001	0.001	0.002	0.002	0.001	0.001	0.001	0.001	0.001	0.001
	GxE	***p*-value**	0.147	0.904	0.261	0.654	0.983	0.810	0.930	0.856	0.906	0.855
		R^2 change	0.004	-0.001	0.002	0.000	-0.001	-0.001	-0.001	-0.001	-0.001	-0.001
Cognitive composite [a]	PSS	***p*-value**	0.853	0.595	0.613	0.336	0.694	0.928	0.891	0.885	0.890	0.888
		R^2 change	0.004	0.005	0.005	0.006	0.005	0.004	0.004	0.004	0.004	0.004
	GxE	***p*-value**	**0.015**	0.096	0.145	0.310	0.332	0.380	0.805	0.616	0.736	0.745
		R^2 change	0.016	0.009	0.007	0.006	0.004	0.004	0.002	0.002	0.002	0.002
Suicidality [b]	PSS	***p*-value**	0.191	0.308	0.900	0.460	0.588	0.794	0.745	0.629	0.618	0.691
		R^2 change	0.042	0.041	0.038	0.039	0.038	0.038	0.038	0.038	0.038	0.038
	GxE	***p*-value**	0.457	0.910	0.424	0.260	0.256	0.875	0.837	0.980	0.936	0.829
		R^2 change	0.044	0.041	0.039	0.043	0.042	0.038	0.038	0.038	0.038	0.038

These analyses included only those male schizophrenic patients with full genetic and environmental data (N=472). For all analyses involving genetic data 10 population stratification dimensions and inbreeding coefficient were used as covariates. Bolded values, $p<0.05$. [a] Linear regression was used for continuous variables; adjusted R^2 of a model containing only environmental load as predictor was set as the baseline R^2 for comparisons with the additional effect of PSS or GxE. [b] Logistic regression was performed for dichotomous phenotypes; Nagelkerke's pseudo R^2 of a model containing only environmental load as predictor was set as the baseline for comparisons with the additional effect of PSS or GxE.

Figure S1: Evaluation of environmental and genetic contributions to age at disease onset in male schizophrenic individuals

The amount of variance (adjusted R^2) explained by different models regarding age at disease onset is shown. Purple model contains only environmental load as predictor. Blue model contains environmental load and polygenic schizophrenia score (PSS) as predictors. Finally, orange model contains environmental load, PSS and the interaction between them (GxE) as predictors. Overall, in the sample under analysis, genetic effects (either as main factor or in interaction with environment) are not statistically significant and do not significantly improve the original (purple) environmental model. A very similar picture emerges if other schizophrenia-related phenotypes are considered (see Table S5).



X axis shows the different *p*-value thresholds used to define the polygenic scores. *Y* axis shows the adjusted R^2 value. These analyses included only those male schizophrenic patients with full genetic and environmental data (N=472). For all analyses involving genetic data (Environment + PSS or Environment + PSS + GxE) 10 population stratification dimensions and inbreeding coefficient were used as covariates.

REFERENCES

1. Giusti-Rodriguez P, Sullivan PF. The genomics of schizophrenia: update and implications. J Clin Invest. 2013 Nov 1;123(11):4557-63.
2. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014 Jul 24;511(7510):421-7.
3. Papiol S, Mitjans M, Assogna F, Piras F, Hammer C, Caltagirone C, et al. Polygenic determinants of white matter volume derived from GWAS lack reproducibility in a replicate sample. Transl Psychiatry. 2014;4:e362.
4. Terwisscha van Scheltinga AF, Bakker SC, van Haren NE, Derks EM, Buizer-Voskamp JE, Boos HB, et al. Genetic schizophrenia risk variants jointly modulate total brain and white matter volume. Biol Psychiatry. 2013 Mar 15;73(6):525-31.
5. Cardno AG, Gottesman, II. Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. Am J Med Genet. 2000 Spring;97(1):12-7.
6. Brown AS. The environment and susceptibility to schizophrenia. Prog Neurobiol. 2011 Jan;93(1):23-58.
7. Di Forti M, Morrison PD, Butt A, Murray RM. Cannabis use and psychiatric and cogitive disorders: the chicken or the egg? Curr Opin Psychiatry. 2007 May;20(3):228-34.
8. McDonald C, Murray RM. Early and late environmental risk factors for schizophrenia. Brain Res Brain Res Rev. 2000 Mar;31(2-3):130-7.
9. van Os J, Kenis G, Rutten BP. The environment and schizophrenia. Nature. 2010 Nov 11;468(7321):203-12.
10. Abel KM, Drake R, Goldstein JM. Sex differences in schizophrenia. Int Rev Psychiatry. 2010;22(5):417-28.
11. Korosi A, Naninck EF, Oomen CA, Schouten M, Krugers H, Fitzsimons C, et al. Early-life stress mediated modulation of adult neurogenesis and behavior. Behav Brain Res. 2012 Feb 14;227(2):400-9.
12. Begemann M, Grube S, Papiol S, Malzahn D, Krampe H, Ribbe K, et al. Modification of cognitive performance in schizophrenia by complexin 2 gene polymorphisms. Arch Gen Psychiatry. 2010 Sep;67(9):879-88.
13. Ribbe K, Friedrichs H, Begemann M, Grube S, Papiol S, Kastner A, et al. The cross-sectional GRAS sample: a comprehensive phenotypical data collection of schizophrenic patients. BMC Psychiatry. 2010;10:91.
14. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. Mol Psychiatry. 2013 Sep 3.
15. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006 Aug;38(8):904-9.
16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007 Sep;81(3):559-75.
17. Delaneau O, Zagury JF, Marchini J. Improved whole-chromosome phasing for disease and population genetic studies. Nat Methods. 2013 Jan;10(1):5-6.
18. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. 2009 Jun;5(6):e1000529.
19. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012 Nov 1;491(7422):56-65.
20. Häfner H, an der Heiden W. Course and outcome of schizophrenia. In: Hirsch SR, Weinberger SR, editors. Schizophrenia. 2. ed. Oxford: Blackwell Publishing; 2003:101-141.
21. Messias E, Kirkpatrick B, Bromet E, Ross D, Buchanan RW, Carpenter WT, Jr., et al. Summer birth and deficit schizophrenia: a pooled analysis from 6 countries. Arch Gen Psychiatry. 2004 Oct;61(10):985-9.
22. Mortensen PB, Pedersen CB, Westergaard T, Wohlfahrt J, Ewald H, Mors O, et al. Effects of family history and place and season of birth on the risk of schizophrenia. N Engl J Med. 1999 Feb 25;340(8):603-8.
23. Miller B, Messias E, Miettunen J, Alaraisanen A, Jarvelin MR, Koponen H, et al. Meta-analysis of paternal age and schizophrenia risk in male versus female offspring. Schizophr Bull. 2011 Sep;37(5):1039-47.
24. Ehrenreich H, Nave KA. Phenotype-Based Genetic Association Studies (PGAS)-Towards Understanding the Contribution of Common Genetic Variants to Schizophrenia Subphenotypes. Genes (Basel). 2014;5(1):97-105.
25. Fanous AH, Zhou B, Aggen SH, Bergen SE, Amdur RL, Duan J, et al. Genome-wide association study of clinical dimensions of schizophrenia: polygenic effect on disorganized symptoms. Am J Psychiatry. 2012 Dec 1;169(12):1309-17.
26. Glaser JP, van Os J, Portegijs PJ, Myin-Germeys I. Childhood trauma and emotional reactivity to daily life stress in adult frequent attenders of general practitioners. J Psychosom Res. 2006 Aug;61(2):229-36.
27. Lederbogen F, Kirsch P, Haddad L, Streit F, Tost H, Schuch P, et al. City living and urban upbringing affect neural social stress processing in humans. Nature. 2011 Jun 23;474(7352):498-501.

28. Andreasson S, Allebeck P, Engstrom A, Rydberg U. Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet. 1987 Dec 26;2(8574):1483-6.
29. Semple DM, McIntosh AM, Lawrie SM. Cannabis as a risk factor for psychosis: systematic review. J Psychopharmacol. 2005 Mar;19(2):187-94.
30. Large M, Sharma S, Compton MT, Slade T, Nielssen O. Cannabis use and earlier onset of psychosis: a systematic meta-analysis. Arch Gen Psychiatry. 2011 Jun;68(6):555-61.
31. Monshouwer K, Smit F, de Graaf R, van Os J, Vollebergh W. First cannabis use: does onset shift to younger ages? Findings from 1988 to 2003 from the Dutch National School Survey on Substance Use. Addiction. 2005 Jul;100(7):963-70.
32. Ehrenreich H, Rinn T, Kunert HJ, Moeller MR, Poser W, Schilling L, et al. Specific attentional dysfunction in adults following early start of cannabis use. Psychopharmacology (Berl). 1999 Mar;142(3):295-301.
33. Schneider M. Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. Addict Biol. 2008 Jun;13(2):253-63.
34. Malone DT, Hill MN, Rubino T. Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. Br J Pharmacol. 2010 Jun;160(3):511-22.
35. Verdoux H, Geddes JR, Takei N, Lawrie SM, Bovet P, Eagles JM, et al. Obstetric complications and age at onset in schizophrenia: an international collaborative meta-analysis of individual patient data. Am J Psychiatry. 1997 Sep;154(9):1220-7.
36. Read J, van Os J, Morrison AP, Ross CA. Childhood trauma, psychosis and schizophrenia: a literature review with theoretical and clinical implications. Acta Psychiatr Scand. 2005 Nov;112(5):330-50.
37. Sturman DA, Moghaddam B. The neurobiology of adolescence: changes in brain architecture, functional dynamics, and behavioral tendencies. Neurosci Biobehav Rev. 2011 Aug;35(8):1704-12.
38. Romeo RD. Adolescence: a central event in shaping stress reactivity. Dev Psychobiol. 2010 Apr;52(3):244-53.
39. Beards S, Gayer-Anderson C, Borges S, Dewey ME, Fisher HL, Morgan C. Life events and psychosis: a review and meta-analysis. Schizophr Bull. 2013 Jul;39(4):740-7.
40. Hare E, Glahn DC, Dassori A, Raventos H, Nicolini H, Ontiveros A, et al. Heritability of age of onset of psychosis in schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 2010 Jan 5;153B(1):298-302.