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Evidence that endophenotypic expression of schizophrenia polygenic risk is greater in healthy siblings of patients compared to controls, suggesting gene-environment interaction. The EUGEI study.

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Abstract

BACKGROUND.

First-degree relatives of patients with psychotic disorder have higher levels of polygenic risk (PRS) for schizophrenia and higher levels of intermediate phenotypes.

METHODS

We conducted, using two different samples for discovery (n=336 controls and 649 siblings of patients with psychotic disorder) and replication (n=1208 controls and 1106 siblings), an analysis of association between PRS on the one hand and psychopathological and cognitive intermediate phenotypes of schizophrenia on the other in a sample at average genetic risk (healthy controls) and a sample at higher than average risk (healthy siblings of patients). Two subthreshold psychosis phenotypes, as well as a standardized measure of cognitive ability, based on a short version of the WAIS-III short form, were used. In addition, a measure of jumping to conclusion bias (replication sample only) was tested for association with PRS.

RESULTS

In both discovery and replication sample, evidence for association between PRS and subthreshold psychosis phenotypes was observed in the relatives of patients, whereas in the controls no association was observed. Jumping to conclusion bias was similarly only associated with PRS in the sibling group. Cognitive ability was weakly negatively and non-significantly associated with PRS in both the sibling and the control group.

CONCLUSIONS

The degree of endophenotypic expression of schizophrenia polygenic risk depends on having a sibling with psychotic disorder, suggestive of underlying gene-environment interaction. Cognitive biases may better index genetic risk of disorder than traditional measures of neurocognition, which instead may reflect the population distribution of cognitive ability impacting the prognosis of psychotic disorder.

Introduction

Although there is a growing number of studies on the impact of schizophrenia-associated genetic variation on intermediate phenotypes of schizophrenia (Hatzimanolis *et al.*, 2018, Hatzimanolis *et al.*, 2015, Jones *et al.*, 2016, Mistry *et al.*, 2017, Nivard *et al.*, 2017, Power *et al.*, 2015, Riglin *et al.*, 2017, van Os *et al.*, 2017), more work is required, particularly in order to obtain exact rather than approximate replication (Kapur *et al.*, 2012). In a previous publication, we showed that genetic variation underlying psychosis spectrum disorder was associated with suggested intermediate phenotypes of psychopathology and cognition in a sample of first degree relatives of patients, who have a higher than average genetic and environmental liability, and healthy comparison participants with average genetic liability (van Os *et al.*, 2017). Although the earlier study had adequate sample size, any study in this area is crucially dependent on exact replication with similar measures and study design. The aim of this publication, therefore, was to produce an exact replication study, repeating the previous discovery analysis, comparing similar groups and using the same self-report and interview measures of psychopathology and cognition to analyse associations with the schizophrenia polygenic risk score (PRS), using the latest available GWAS summary statistics from the Psychiatric Genomics Consortium wave-2 SCZ group as a training set. As our previous study indicated differential patterns of association between relatives and healthy comparison participants, which is in line with the concept that relatives can be expected to show stronger endophenotypic associations than healthy comparison participants, we tested for interactions with relative/control status. The replication sample was derived from Workpackage 6 (GxE Vulnerability & Severity) of the international EUGEI study (European Network of National Networks studying Gene-Environment Interactions in Schizophrenia *et al.*, 2014), consisting of 1525 healthy comparison participants and 1282 siblings of patients.

Cognitive vulnerabilities underlying psychosis include cognitive biases such as jumping to conclusion bias, in addition to traditional measures of neurocognition (McLean *et al.*, 2017). Given that jumping

to conclusion bias may be more prevalent in first-degree relatives of patients (Van Dael *et al.*, 2006), and no previous study has tested for molecular genetic association with these cognitive biases, we examined, in the replication sample, for association between PRS and a measure of jumping to conclusion bias.

Thus, in the current paper, we analyse associations between PRS on the one hand and measures of psychopathology, neurocognition and jumping to conclusion bias in siblings of patients with a psychotic disorder and healthy comparison subjects. As described above, two samples were used. The first sample, referred to as the 'GROUP discovery sample' is a re-analysis of our previously published results in the GROUP sample (van Os *et al.*, 2017). The second sample, referred to as the 'EUGEI replication sample' is the analysis of the more recent EUGEI sample.

Method

Samples

EUGEI replication sample

The EUGEI project is a 25-centre, 15-country, EU-funded collaborative network studying the impact of genetic and environmental factors on the onset, course and neurobiology of psychosis spectrum disorder (European Network of National Networks studying Gene-Environment Interactions in Schizophrenia *et al.*, 2014). Workpackage 6, entitled 'Vulnerability and Severity', focussed on the psychometric expression of genetic and environmental liability in the siblings of patients, who are at higher than average genetic and environmental risk compared to well healthy comparison participants. The sample in Workpackage 6 was collected in Spain (5 centres), Turkey (3 centres) and Serbia (1 centre) and consisted of 1525 healthy comparison participants, 1261 patients with a diagnosis of psychosis spectrum disorder (average duration of illness since age of first contact with mental health services: 9.9 years) and 1282 siblings of these patients. Exclusion criteria for all

participants were diagnosis of psychotic disorders due to another medical condition, history of head injury with loss of consciousness, and intelligence quotient < 70. The current analyses were restricted to the group of healthy comparison participants and the siblings. Individuals of non-white ethnic group (n=30) were excluded, as were individuals with missing GWAS information (n=463), leaving 2314 participants (1208 healthy comparison participants and 1106 relatives) for the current analysis.

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 (European Network of National Networks studying Gene-Environment Interactions in Schizophrenia *et al.*, 2014). Both on-site and online training sessions were repeated annually to maintain high inter-rater reliability throughout the study enrollment period (for details see: https://cordis.europa.eu/result/rcn/175696_en.html).

The EUGEI project was 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 and, in the case of minors, such consent was also obtained from parents or legal guardian.

GROUP discovery sample

The current paper also present a re-analysis of the discovery sample, presented in detail in a previous publication, pertaining to the GROUP study, an ongoing multicentre study in the Netherlands of patients diagnosed with schizophrenia and related disorder, as well as their siblings, parents and healthy participants (Korver *et al.*, 2012, van Os *et al.*, 2017). The current analysis in the GROUP sample focussed on 336 healthy comparison participants and 649 siblings of patients, who were assessed three times over a period of six years, yielding 2416 observations.

Allowing for true replication from GROUP discovery to EUGEI replication sample

In order to allow for true replication in the original discovery sample of the Dutch GROUP study, differences between the previous GROUP study and the current EUGEI study that were under our control were dissolved. Thus, one possible reason for discrepancy between studies using polygenic risk for schizophrenia is difference in the platform for molecular genetic analysis used, as well as difference in the version of the GWAS summary statistics from the training sample (Psychiatric Genomics Consortium wave-2 SCZ group). In order to neutralise these factors, GWAS was repeated in the GROUP sample (data version 7.0), using the same genetic analysis platform as used in the current (and later) EUGEI study. Similarly, quality control and calculation of polygenic scores was done in the same fashion as the EUGEI study. In addition, a standardized cognitive score was calculated in the GROUP sample, based on the same variables as in the EUGEI sample. In addition, the same comparison between healthy comparison participants and healthy siblings of patients was conducted. Previous publications in the GROUP sample have shown significant differences between healthy comparison participants and siblings in the measures of CAPE and SIS-R psychosis proneness used in the current analysis, with siblings displaying higher values (Genetic Risk and Outcome in Psychosis Investigators, 2011).

Measures

The GROUP discovery and EUGEI replication samples used the same instruments as listed below, with the exception of the beads task, which was used only in the EUGEI replication sample.

Interview-based schizotypy: SIS-R
The SIS-R was administered to healthy comparison participants, parents and siblings. The SIS-R is a semi-structured interview containing 20 schizotypal symptoms and 11 schizotypal signs rated on a 4-point scale (Kendler *et al.*, 1989, Vollema and Ormel, 2000). Symptoms are defined as verbal

responses to standardized questions concerning, for example, magical ideation, illusions, and referential thinking. Signs refer to behaviours that are rated by the interviewer such as goal-directedness of thinking and flatness of affect. Questions and rating procedures are standardized. Guided by previous research, 33 item scores were reduced *a priori* to 2 dimensional scores, representing the means of 7 *positive* schizotypy items (covering the areas of referential thinking, psychotic phenomena, derealisation, magical ideation, illusions, and suspiciousness) and 8 *negative-disorganized* schizotypy items (covering the areas of social isolation, sensitivity, introversion, restricted affect, disturbances in associative and goal-directed thinking, poverty of speech, and eccentric behaviour).

Self-reported psychotic experiences: CAPE

The Community Assessment of Psychic Experiences (CAPE; www.cape42.homestead.com) was developed to rate self-reports of lifetime psychotic experiences (Konings *et al.*, 2006). Items are modelled on patient experiences as contained in the Present State Examination, 9th version (Wing *et al.*, 1974), schedules assessing negative symptoms such as the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982) and the Subjective Experience of Negative Symptoms (SENS) (Selten *et al.*, 1993), and scales assessing depressive symptoms such as the Calgary Depression Scale (Addington *et al.*, 1993). Items are scored on a 4-point scale. In the current analyses, CAPE dimensions of frequency of positive experiences (20 items), negative experiences (14 items) and depressive experiences (8 items) were included (measured at baseline and 3-year follow-up), representing the person's perceived psychosis load over the lifetime (at baseline) or in the past three years (follow-up). A total score representing the mean of all items was calculated for each dimension.

Cognitive score

Cognition ability was estimated based on a short version of the WAIS-III short form: the Digit Symbol Coding subtest, uneven items of the Arithmetic subtest, uneven items of the Block Design subtest, every third item of the Information subtest (Blyler *et al.*, 2000, Velthorst *et al.*, 2013, Wechsler, 1997). For each test, the Z-score was calculated separately for each country and sex. The cognition score was the mean of the Z-scores of the different tests, expressed as a T-score (cognition score shifted and scaled to have a mean of 50 and a standard deviation of 10). The measure will be referred hereafter as 'cognitive score'.

Beads task (EUGEI replication sample only)

The beads task (Phillips and Edwards, 1966) is an experimental test designed to measure individuals' reasoning style under ambiguous conditions. It was administered only in the replication sample (EUGEI study). A computerised version of the beads task was completed to assess the presence or absence of the Jumping to Conclusions (JTC) bias. Participants were shown two jars containing red and blue coloured beads in opposite ratios. In this study, the ratio of 60 to 40 beads was chosen, resulting in 100 beads in each jar. The jars as well as all instructions were presented on a computer screen. After both jars were shown and a training session was completed, participants were instructed that all beads are drawn consecutively from one jar and, once presented, were returned to the same jar. After each draw, participants were asked whether they wanted to see another bead or make a decision on which jar the beads were drawn, with the possibility to see up to 20 beads before a decision had to be made. The order of presented beads was predetermined and the dominant colour presented in the training session selected at random. The number of beads drawn at was considered to represent individuals' reasoning style, and used as dependent variable in the analyses.

Genotyping, imputation and polygenic risk scores

Samples of all individuals, i.e. pertaining to both the discovery and the replication sample, 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.

Genotype quality control – variants

Quality control was conducted in PLINK v1.07 (Purcell *et al.*, 2007) 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 < 1e-6 in any of these three regions were excluded from the dataset. After QC, 559505 variants remained.

Genotype quality control – samples

Samples with call rate < 98% were excluded from the dataset. A linkage disequilibrium pruned set of variants was calculated using the --indep-pairwise command in PLINK (maximum r2 = 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 (IBD) values were calculated for the sample in PLINK. Samples with 1 or more siblings among the genotyped samples according to the database but no identified genotypic siblings (defined as PI-HAT > 0.35 and < 0.65) were excluded. After these were removed from consideration, samples with 2 or more database 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 5 or more sample exclusions due to heterozygosity or call rate (out of 12 possible samples) were excluded. All samples on chips with 4 or more sample exclusions due to sex or relative checks were also excluded, unless their identity was corroborated by concordance between database and genotype relatedness data with a sample on another chip.

Principal component analysis

Principal components (PCs) were calculated in PLINK using LD pruned variants after combining the dataset with the Thousand Genomes reference dataset. 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.

Imputation

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(Das *et al.*, 2016, Loh *et al.*, 2016). After imputation, variants with an imputation $r^2 > 0.6$, MAF > 0.1% and call rate > 99% were retained (8277535 variants). Best-guess genotypes were generated from genotype probabilities using PLINK.

Polygenic risk score calculation

PRS-SCZ was constructed using summary statistics from the PGC2 genome-wide association study, excluding samples present in the GROUP data (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). 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 INFO score = 0.7), and variants within regions of long-range LD around the genome (including the MHC)

excluded (Price *et al.*, 2008). PRS-SCZ were then constructed from best-guess genotypes using PLINK at 10 different p-value thresholds (P_T=1, 0.5, 0.3, 0.2, 0.1, 0.05, 0.01, 1×10^{-4}, 1×10^{-6}, 5×10^{-8}). We used P_T=0.05 for our primary analysis, as this threshold explained the most variation in the phenotype in the PGC2 analysis(Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

In the EUGEI WP6 dataset, the number of siblings and controls for whom a polygenic risk score could be thus constructed, after QC and imputation, and after exclusion of non-white ethnic groups was: 1106 and 1208, respectively. In the GROUP dataset, a polygenic risk score could be similarly computed for 649 siblings and 336 healthy comparison participants, after exclusion of non-white groups.

Analyses

EUGEI WP6 database version 1.0 And GROUP database version 7.0 were used for the analyses. Bivariate scatterplots were constructed for SIS-R scores, CAPE scores, cognitive score and beads task on the one hand, and PRS on the other. Random intercept multilevel regression models (taking into account clustering of participants within families as well as within countries and, in the re-analysis of the discovery sample, clustering of repeated measures within participants) with SIS-R scores, CAPE scores, cognitive score and beads task as dependent variables were fitted using the MIXED routine in the Stata program, version 15 (StataCorp, 2017). Independent variables were polygenic risk score, *a priori* corrected for age and sex, while all models including PRS were additionally adjusted for ancestry, using the first 10 PCs.

In order to examine robustness of findings with regard to assumptions of normality, log-transformed outcomes of SIS-R scores, CAPE scores, cognitive score and beads task were additionally examined, using the Stata LNSKEW0 routine. LNSKEW0 creates newvar = ln(+/-exp - k), choosing k and the sign of exp so that the skewness of newvar is zero.

In order to examine to what degree the size of any association between PRS and measures of psychopathology/cognition would be stronger for siblings than for healthy comparison subjects, interactions were fitted between group (healthy comparison participant/relative status) and continuous PRS. Interaction analyses were followed by calculation of polygenic risk score effect sizes, stratified by group (i.e. calculating associations between PRS and measures of psychopathology/cognition separately for siblings and healthy comparison subjects), derived from the model with the interaction, using the STATA MARGINS command.

In line with Rothman (Rothman, 1990), corrections for multiple testing were not applied. Instead, we relied on a discussion of coherence and possible unifying pattern of results.

Results

EUGEI replication sample description

Relatives and healthy comparison participants showed similar demographic characteristics and cognitive scores. These variables also showed comparable distributions across the different countries, although participants tended to be younger in Serbia and the proportion of women was smaller in Spain (Table 1). Polygenic risk score scores were higher in Turkey, similar across the sexes and higher in the siblings (Table 2). Siblings had a higher schizophrenia polygenic risk score than healthy participants (standardized effect size: 0.06, p=0.006). Scores of SIS-R and CAPE were similar for men and women, with the exception of the CAPE depressive dimension, which was higher for women. Siblings had significantly higher scores on CAPE and SIS-R measures, with the exception of the CAPE positive dimension, probably due to defensive answering (data not shown) (for descriptives see Tables 3 and 4).

Association between polygenic risk score and group in the EUGEI replication sample

Scatter plots of CAPE, SIS-R and cognitive dimensions on the one hand and polygenic risk score on the other generally showed that (i) the direction of association differed between siblings and healthy comparison subjects and (ii) siblings displayed stronger associations, with the exception of cognitive score (Fig. 1). Accordingly, all multilevel regression models of (log-transformed) SIS-R, CAPE and cognitive scores showed significant interaction between polygenic risk score and group, with the exception of CAPE positive dimension and cognitive score (Table 5). Stratified analyses revealed significant or suggestive positive associations between PRS and SIS-R and CAPE measures (with the exception of the CAPE positive dimension), and a negative association with beads task number of beads in the siblings. In the healthy comparison participants, there was either no association (CAPE measures) or a weak, directionally inverse association (SIS-R measures, beads task)(Table 6). Cognitive scores showed a different pattern, with weak negative associations with polygenic risk in both the sibling and the control groups.

Re-analysis of original GROUP discovery sample

Re-analysis of the GROUP discovery sample, using repeated measures of CAPE, SIS-R and cognitive score (calculated so as to match the cognitive score in the EUGEI study) in 985 healthy participants and siblings of patients (n=336 healthy comparison participants and n=649 siblings), interviewed three times in 6 years, yielding 2416 observations, revealed results that were similar, in terms of direction, effect size and pattern of associations, to the current EUGEI study (Tables 7 and 8). Thus, again with the exception of cognitive score, there were significant interactions for the majority of psychopathological measures indicating no, or weakly negative association in the healthy participant group and significant associations in the sibling group (Tables 7 and 8). There was no association between cognitive score and PRS, and only in the sibling group was the association with cognitive score in the same (negative) direction as in the EUGEI replication sample (Tables 7 and 8).

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Discussion

In a replication study, using similar sampling and methodological strategies across samples, we found that polygenic risk for schizophrenia was associated with intermediate phenotype measures of psychopathology in non-ill participants. However, the pattern of association was differential for groups at average and higher than average genetic risk, showing stronger and directionally dissimilar associations in the sibling group compared to the healthy comparison group. A similar pattern of differential association was present for a measure of cognitive bias (jumping to conclusions). For a measure indexing cognitive ability, the EUGEI replication sample but not the GROUP discovery sample showed non-significant associations in both the sibling and the healthy comparison group, with no suggestion of interaction.

The EUGEI replication findings thus match the results in the GROUP discovery sample (van Os *et al.*, 2017), re-analysed to match the methods of genetic examination in the EUGEI replication sample. We conclude that when using similar instruments and genetic measures, associations between polygenic risk score and psychosis intermediate phenotypes can be reliably replicated from one sample to another, in that associations are readily detectable in the group at higher than average genetic risk, but not, or inversely, in the group at average genetic risk. Overall, the findings suggest, with the exception of the measure of neurocognition, a pattern of qualitative interaction: associations between PRS and psychosis intermediate phenotypes tend to be positive in relatives of patients (PRS predicting poorer outcome) and negative in healthy comparison participants (PRS predicting better outcome).

Polygenic risk and measures of psychopathology and cognition

As far as we are aware, there have been no previous studies differentially examining polygenic risk in individuals at higher than average genetic risk in relation to psychopathology and cognition

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intermediate phenotypes. Studies in healthy participants have shown inconsistent results (Hatzimanolis *et al.*, 2018, Hatzimanolis *et al.*, 2015, Jones *et al.*, 2016, Mistry *et al.*, 2017, Nivard *et al.*, 2017, Riglin *et al.*, 2017), one study notably reporting negative associations between polygenic risk and schizotypy, similar to the current results (Hatzimanolis *et al.*, 2018). Another recent general population study found a protective effect of PRS in that PRS predicted greater levels of positive affect in daily life (Pries *et al.*, 2019). It is likely that crucial variation may be occasioned by platform for genetic analysis and calculation of polygenic risk score. Other factors may include quality checking of GWAS and variation in sampling, instruments and analysis. The results suggest that linking intermediate phenotypes to molecular measures of risk can be reliably achieved in moderately large samples of individuals at higher than average genetic risk. According to the liability-threshold model, a person with a number of risk variants lower than or equal to the critical threshold would not develop schizophrenia, whereas a person with more risk variants would (McGue *et al.*, 1983). Given genetic enrichment in the relatives, this model may explain why polygenic risk was associated with psychometric and cognitive bias intermediate phenotypes in the relatives, but not in the healthy comparison participants. The current findings also indicate that self-report measures function less well than interview-based measures in uncovering associations between psychometric measures and polygenic risk for schizophrenia. Indeed, the findings suggest that siblings in the EUGEI replication sample showed a degree of defensive answering on the CAPE positive dimensions, scoring lower than the healthy comparison participants (Table 3), which was not the case in the discovery sample (van Os *et al.*, 2017).

The positive association between polygenic risk and the jumping to conclusion bias confirms previous results of familial clustering with psychosis (Van Dael *et al.*, 2006). This result suggests that genetic variation may impact psychopathology by moderating underlying styles of thinking which are thought to interact with underling biological mechanisms in creating clinically relevant psychotic symptoms (Howes and Murray, 2014).

The results suggest that the degree of endophenotypic expression of polygenic molecular genetic risk crucially depends on having a first-degree relative with psychotic disorder, i.e. of having higher than average genetic risk for psychotic disorder. In the absence of a sibling with psychotic disorder, expression of polygenic risk may even be protective against expression of psychosis proneness, as also reported by other groups (Hatzimanolis *et al.*, 2018, Pries *et al.*, 2019). The notion of schizophrenia genetic risk having advantage may explain why all humans are carriers of a rich variety of schizophrenia genetic risk variants (Kendler, 2015) that, in addition, in the rare instances where it has been examined, may for example contribute to abilities required for a creative profession (Power *et al.*, 2015). If polygenic risk for schizophrenia reduces the likelihood of schizophrenia-related intermediate phenotypes in non-ill people, siblings should display even lower levels of expression of these intermediate phenotypes, which, however, was not the case. One factor that may explain the differential association as a function of familial presence of psychotic disorder is the environment. Thus, the siblings growing up with a brother or sister diagnosed with psychotic disorder have a higher rate of exposure to known proxy environmental risk factors such as urban environment and ethnic group – which are almost always shared between siblings, but also to risk factors like cannabis use and childhood adversity (Heins *et al.*, 2011, Smith *et al.*, 2008). Although presence of family history is generally taken as an indicator of increased genetic risk, research suggests that molecular measures of polygenic risk explain only around a fifth of the effect of family history, suggesting a substantial part may be explained by epistasis and/or environmental effects and the interactions between genes and environment (Agerbo *et al.*, 2015). Schizophrenia may be in part dependent on gene-environment interplay, amongst others in the form of differential sensitivity to environmental risks (van Os *et al.*, 2010), as indeed shown in a recent analysis of the same EUGEI sample (Guloksuz *et al.*, 2019). Thus, siblings may have higher rates of expression of psychosis intermediate phenotypes not because of higher levels of genetic risk, but because of genetic risk interacting with higher rates of exposure to environmental risks in this group. Another possibility,

not mutually exclusive with gene-environment interplay, is that siblings share gene-gene interactions with their ill relatives that are uncommon in healthy controls, producing a qualitatively different pattern of association with intermediate phenotypes.

<u>Cognitive ability and lack of association and qualitative interaction</u>

Cognitive ability was the only measure where there was no clear pattern of association or qualitative interaction in both samples. Results in the largest sample showed weak and directionally similar negative associations between schizophrenia polygenic risk and cognition in both the healthy comparison and the sibling group. These findings are in line with previous population-based work showing similar weak negative associations between schizophrenia polygenic risk and measures of cognition, indicative of shared common genetic factors between schizophrenia and cognitive ability (Hubbard *et al.*, 2016, Lencz *et al.*, 2014). One explanation for the observed contrast in qualitative interaction between cognitive and non-cognitive intermediate phenotypes is prognostic confounding, or the fact that variation in cognition as observed in schizophrenia may represent a 'prognostic' rather than a 'disease' factor. It has been pointed out that schizophrenia represents the poor outcome fraction of a much broader psychosis phenotype (Guloksuz and van Os, 2018). One of the factors driving prognostic variation in the psychosis spectrum is the degree of comorbid cognitive alterations. If the poor outcome end of the spectrum is defined as a separate disease, of which cognitive alteration is considered a key characteristic, prognostic confounding will arise. In other words, psychosis spectrum disorder may have a poorer outcome when it occurs in people who are at the lower end of the distribution of cognitive ability in the general population. This will occasion an association between schizophrenia genetic risk and cognition if the disease phenotype is conflated with the prognostic characteristic. As a result, schizophrenia genetic risk will predict lower cognitive ability in the general population – but to a similar degree in controls and siblins of patients. This interpretation is in line with recent work indicating that cognition in patients with schizophrenia

is more strongly associated with polygenic risk that indexes cognitive traits in the general population than polygenic risk from mental disorders (Richards *et al.*, 2019).

<u>Methodological issues</u>

The strength of the current study is twofold: within-report true replication and unique large samples of individuals at higher-than average risk. We controlled for potential weaknesses such as the use of a multi-country sample with possible residual underlying population stratification. Future research may include more objective physiological intermediate phenotypes such as EEG or MRI-based measures. A limitation is that the current study lacked statistical power to conduct genome-wide analyses of association with endophenotypic measures, which would have allowed for GWAS stratified by sibling status as a confirmatory strategy.

Table 1. Sample demographics and cognitive scores, by group and country

Group	Age		Sex	Educational level		Cognitive score		Beads task number drawn		N
	mean	Sd	% female	mean	sd	Mean	sd	Mean	Sd	
Healthy comparison participants	34.17	10.44	0.48	3.68	1.17	50.10	7.21	5.76	3.58	1,208
Siblings	34.26	9.46	0.53	3.75	1.19	50.44	7.92	4.32	4.71	1,106
Total	34.21	9.98	0.51	3.72	1.18	50.26	7.59	5.09	4.20	2,314*
Country										
Turkey	33.81	10.45	0.54	3.75	1.18	50.31	7.89	5.25	4.01	1,424
Spain	35.56	9.18	0.44	3.66	1.19	50.24	7.00	4.93	4.45	802
Serbia	29.28	6.81	0.57	3.59	1.07	49.59	7.27	4.23	4.52	100
Total	34.21	9.98	0.51	3.72	1.18	50.26	7.56	5.09	4.20	2,326*

sd = standard deviation

N = number of observations

* 12 individuals had unknown group status

Table 2. Polygenic scores by country, sex and group

Group		Country and sex					
		Turkey		Spain		Serbia	
		Men	Women	Men	Women	Men	Women
Healthy comparison participants	Mean	-16.53	-16.4	-19.73	-20.25	-18.83	-19.26
	sd	2.47	2.42	2.67	2.51	2.02	2.3
	N	361	435	241	123	20	26
Siblings	Mean	-15.52	-15.84	-19.29	-19.52	-19.03	-18.41
	sd	2.45	2.45	2.54	2.55	2.64	2.23
	N	299	329	196	226	23	31

sd = standard deviation

N = number of observations

Table 3. CAPE scores by group and sex

	CAPE positive score			CAPE negative core			CAPE depressive score			CAPE total score		
	mean	sd	N	mean	Sd	N	mean	sd	N	mean	sd	N
Group												
Healthy comparison participants	0.26	0.29	1186	0.46	0.39	1186	0.52	0.40	1189	0.41	0.32	1186
Siblings	0.23	0.21	1001	0.53	0.38	1002	0.59	0.39	1003	0.45	0.29	1001
Total	0.24	0.26	2187	0.49	0.38	2188	0.56	0.40	2192	0.43	0.30	2187
Sex												
Men	0.25	0.25	1070	0.49	0.37	1070	0.50	0.35	1071	0.41	0.28	1070
Women	0.24	0.26	1114	0.49	0.40	1115	0.61	0.43	1118	0.45	0.32	1114
Total	0.24	0.26	2184	0.49	0.38	2185	0.56	0.40	2189	0.43	0.30	2184

sd = standard deviation

N = number of observations

Table 4. SIS-R scores by group and sex

	SIS-R positive score			SIS-R negative score			SIS-R total score		
	mean	sd	N	mean	sd	N	mean	sd	N
Group									
Healthy comparison participants	0.22	0.3	1201	0.22	0.24	1196	0.22	0.23	1199
Siblings	0.42	0.42	1074	0.45	0.37	1013	0.43	0.35	1021
Total	0.31	0.38	2275	0.33	0.33	2209	0.32	0.31	2220
Sex									
Men	0.31	0.37	1121	0.33	0.34	1084	0.32	0.31	1093
Women	0.31	0.38	1151	0.33	0.32	1122	0.32	0.31	1124
Total	0.31	0.38	2272	0.33	0.33	2206	0.32	0.31	2217

sd = standard deviation

N = number of observations

Table 5. Associations between polygenic risk score and CAPE / SIS-R / Cognitive (bias) scores, and interaction with healthy comparison-sibling status

Psychopathology measure	polygenic risk score x healthy comparison-sibling status interaction				
	B (95% CI)			p	N
CAPE positive	-0.0007	-0.0079	0.0064	0.8442	2184
ln_CAPE positive*	0.0177	-0.0039	0.0392	0.1083	2184
CAPE negative	0.0161	0.0053	0.0268	0.0034	2185
ln_CAPE negative*	0.0218	0.0098	0.0339	0.0004	2185
CAPE depressive	0.0091	-0.0019	0.0202	0.1057	2189
ln_CAPE depressive*	0.0125	0.0001	0.0249	0.0474	2189
CAPE total	0.0081	-0.0005	0.0166	0.0646	2184
ln_CAPE total*	0.0163	0.0039	0.0288	0.0103	2184
SIS-R positive	0.0308	0.0210	0.0407	0.0000	2272
ln_SIS-R positive*	0.0881	0.0602	0.1160	0.0000	2272
SIS-R negative	0.0235	0.0148	0.0321	0.0000	2206
ln_SIS-R negative*	0.0544	0.0355	0.0732	0.0000	2206
SIS-R total	0.0270	0.0189	0.0350	0.0000	2217
ln_SIS-R total*	0.0637	0.0452	0.0823	0.0000	2217
Cognitive score	0.0249	-0.1836	0.2335	0.8147	2160
ln_Cognitive score*	0.0002	-0.0018	0.0021	0.8761	2160
Beads task score	-0.3810	-0.4956	-0.2664	0.0000	2219
ln_Beads task score	-0.1218	-0.1516	-0.0919	0.0000	2219

* = log-transformed measure

B=regression coefficient from multilevel model

95% CI = 95% confidence interval

P = p-value

N = number of observations

Table 6. Associations between polygenic risk score and measures of psychopathology, by group*

Psychopathology measure	Stratified association of psychopathology measure with polygenic risk score					Association of log-transformed psychopathology measure with polygenic risk score				
	Group	B (95% CI)			p	N	B (95% CI)			p	N
CAPE positive	Healthy comparison	0.0021	-0.0036	0.0078	0.4671	2184	-0.0039	-0.0209	0.0131	0.6543	2184
	Sibling	0.0014	-0.0046	0.0074	0.6514	2184	0.0138	-0.0042	0.0318	0.1340	2184
CAPE negative	Healthy comparison	-0.0032	-0.0117	0.0053	0.4568	2185	-0.0062	-0.0157	0.0033	0.1999	2185
	Sibling	0.0128	0.0039	0.0218	0.0050	2185	0.0156	0.0056	0.0257	0.0023	2185
CAPE depressive	Healthy comparison	-0.0005	-0.0092	0.0083	0.9183	2189	-0.0027	-0.0124	0.0071	0.5937	2189
	Sibling	0.0087	-0.0006	0.0179	0.0659	2189	0.0099	-0.0005	0.0202	0.0611	2189
CAPE total	Healthy comparison	-0.0004	-0.0071	0.0064	0.9123	2184	-0.0032	-0.0130	0.0066	0.5250	2184
	Sibling	0.0077	0.0005	0.0148	0.0351	2184	0.0131	0.0027	0.0235	0.0133	2184
SIS-R positive	Healthy comparison	-0.0089	-0.0168	-0.0010	0.0273	2272	-0.0296	-0.0520	-0.0073	0.0094	2272
	Sibling	0.0219	0.0138	0.0300	0.0000	2272	0.0585	0.0355	0.0815	0.0000	2272
SIS-R negative	Healthy comparison	-0.0063	-0.0131	0.0005	0.0711	2206	-0.0155	-0.0304	-0.0005	0.0426	2206
	Sibling	0.0172	0.0100	0.0243	0.0000	2206	0.0389	0.0232	0.0546	0.0000	2206
SIS-R total	Healthy comparison	-0.0069	-0.0133	-0.0005	0.0355	2217	-0.0203	-0.0350	-0.0056	0.0067	2217
	Sibling	0.0201	0.0134	0.0268	0.0000	2217	0.0434	0.0280	0.0588	0.0000	2217
Cognitive score	Healthy comparison	-0.1939	-0.3605	-0.0273	0.0225	2160	-0.0018	-0.0034	-0.0002	0.0262	2160
	Sibling	-0.1690	-0.3401	0.0022	0.0531	2160	-0.0016	-0.0032	0.0000	0.0484	2160
Beads task	Healthy comparison	0.0806	-0.0102	0.1714	0.0818	2219	0.0270	0.0034	0.0506	0.0250	2219
	Sibling	-0.3004	-0.3957	-0.2051	0.0000	2219	-0.0948	-0.1196	-0.0700	0.0000	2219

* as derived from linear combination of terms in the model with the interaction

B=regression coefficient from multilevel model

95% CI = 95% confidence interval

P = p-value

N = number of observations

Table 7. Genetic Risk and Outcome of Psychosis (GROUP) sample: Associations between polygenic risk score and CAPE / SIS-R / Cognitive scores, and interaction with Healthy comparison-sibling status

Psychopathology measure	polygenic risk score x healthy comparison-sibling status interaction				
	B (95% CI)			p	N#
CAPE positive	0.0125	0.0051	0.0200	0.0010	2324
ln_CAPE positive*	0.0831	0.0369	0.1293	0.0004	2324
CAPE negative	0.0196	0.0016	0.0375	0.0324	2324
ln_CAPE negative*	0.0235	0.0014	0.0456	0.0374	2324
CAPE depressive	0.0277	0.0095	0.0459	0.0029	2328
ln_CAPE depressive*	0.0297	0.0096	0.0497	0.0037	2328
CAPE total	0.0206	0.0074	0.0337	0.0021	2328
ln_CAPE total*	0.0306	0.0097	0.0515	0.0042	2328
SIS-R positive	0.0294	0.0142	0.0445	0.0001	2416
ln_SIS-R positive*	0.0593	0.0246	0.0940	0.0008	2416
SIS-R negative	0.0089	-0.0015	0.0193	0.0936	2415
ln_SIS-R negative*	0.0197	-0.0029	0.0423	0.0880	2415
SIS-R total	0.0188	0.0077	0.0300	0.0009	2416
ln_SIS-R total*	0.0372	0.0133	0.0610	0.0022	2416
Cognitive score	-0.1465	-0.5360	0.2431	0.4612	2357
ln_Cognitive score*	0.0041	-0.0065	0.0147	0.4474	2357

* = log-transformed measure

B=regression coefficient from multilevel model

95% CI = 95% confidence interval

P = p-value

#N = number of observations in 985 individuals interviewed three times in 6 years

Table 8. Genetic Risk and Outcome of Psychosis (GROUP) sample: Associations between polygenic risk score and measures of psychopathology, by group*

Psychopathology measure	Stratified association of psychopathology measure with polygenic risk score					Association of log-transformed psychopathology measure with polygenic risk score				
	Group	B (95% CI)			p	N	B (95% CI)			p	N#
CAPE positive	Healthy comparison	-0.0067	-0.0128	-0.0006	0.0312	2324	-0.0446	-0.0822	-0.0070	0.0201	2324
	Sibling	0.0058	0.0013	0.0104	0.0120	2324	0.0385	0.0104	0.0666	0.0072	2324
CAPE negative	Healthy comparison	-0.0072	-0.0218	0.0075	0.3362	2324	-0.0105	-0.0285	0.0076	0.2551	2324
	Sibling	0.0124	0.0015	0.0233	0.0257	2324	0.0130	-0.0004	0.0265	0.0577	2324
CAPE depressive	Healthy comparison	-0.0056	-0.0205	0.0092	0.4588	2328	-0.0051	-0.0215	0.0112	0.5377	2328
	Sibling	0.0221	0.0110	0.0332	0.0001	2328	0.0245	0.0123	0.0367	0.0001	2328
CAPE total	Healthy comparison	-0.0068	-0.0175	0.0038	0.2094	2328	-0.0106	-0.0277	0.0064	0.2219	2328
	Sibling	0.0137	0.0057	0.0217	0.0007	2328	0.0200	0.0072	0.0327	0.0021	2328
SIS-R positive	Healthy comparison	-0.0097	-0.0221	0.0026	0.1229	2416	-0.0179	-0.0462	0.0105	0.2165	2416
	Sibling	0.0196	0.0103	0.0289	0.0000	2416	0.0414	0.0202	0.0626	0.0001	2416
SIS-R negative	Healthy comparison	-0.0014	-0.0099	0.0071	0.7476	2415	-0.0050	-0.0235	0.0135	0.5946	2415
	Sibling	0.0075	0.0011	0.0139	0.0211	2415	0.0147	0.0008	0.0285	0.0381	2415
SIS-R total	Healthy comparison	-0.0055	-0.0146	0.0036	0.2356	2416	-0.0109	-0.0303	0.0086	0.2744	2416
	Sibling	0.0133	0.0065	0.0202	0.0001	2416	0.0263	0.0117	0.0409	0.0004	2416
Cognitive score	Healthy comparison	0.0344	-0.2845	0.3534	0.8324	2357	-0.0011	-0.0098	0.0076	0.8081	2357
	Sibling	-0.1120	-0.3505	0.1265	0.3573	2357	0.0030	-0.0035	0.0095	0.3599	2357

* as derived from linear combination of terms in the model with the interaction

B=regression coefficient from multilevel model

95% CI = 95% confidence interval

P = p-value

#N = number of observations in 985 individuals interviewed three times in 6 years

Fig. 1. PRS scatterplots with regression line for CAPE (Fig. 1a), SIS-R and (Fig. 1b) and cognition (Fig. 1c) outcomes

Fig. 1a

[PLACE HERE THE 4 FIGURES FOR FIGURE 1A: FIG_1A_CAPEPOS, FIG_1A_CAPENEG, FIG_1A_CAPEDEP, FIG_1A_CAPETOT]

Fig. 1b

[PLACE HERE THE 3 FIGURES FOR FIGURE_1B: FIG_1B_SISPOS, FIG_1B_SISNEG, FIG_1B_SISTOT]

Fig. 1c

[PLACE HERE THE 2 FIGURES FOR FIGURE_1C: FIG_1C_TCOG, FIG_1C_BEADS]

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