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The Relationship of Common Risk Variants and Polygenic Risk for Schizophrenia to Sensorimotor Gating

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ABSTRACT

BACKGROUND: Prepulse inhibition (PPI) of the startle reflex has been suggested as a candidate endophenotype for schizophrenia research, as it shows high heritability and has been found deficient in schizophrenia spectrum disorders. The objectives of the study were to 1) identify common genetic variants associated with baseline startle and PPI; 2) estimate the single nucleotide polymorphism heritability; and 3) examine the relationship of polygenic score for schizophrenia with baseline startle and PPI.

METHODS: A cohort of healthy young male subjects ($n = 1493$) originating from the Learning on Genetics of Schizophrenia Spectrum project was assessed for baseline startle and PPI. The most recent genome-wide association study in schizophrenia from the Psychiatric Genomics Consortium 2 was used to calculate polygenic scores.

RESULTS: Eleven loci showed suggestive association ($p < 10^{-6}$) with baseline startle and PPI in the discovery cohort. Additional genotyping in a replication cohort identified genome-wide significant association at two loci (rs61810702 and rs4718984). These loci were co-localized with expression quantitative trait loci associated with gene expression of nerve growth factor (*NGF*) and calneuron 1 (*CALN1*) genes. Estimation of the genetic and environmental contributions to baseline startle and PPI showed a substantial single nucleotide polymorphism heritability for 120-ms PPI stimuli. Increased polygenic risk score for schizophrenia was associated with reduced PPI.

CONCLUSIONS: Common genetic variation has an important role in the etiology of schizophrenia and PPI impairments. Overall, these data support the idea that PPI is a valid endophenotype that can be used to explore the genetic architecture of schizophrenia.

Keywords: Baseline startle, Endophenotypes, eQTL, GWAS, Heritability, Prepulse inhibition

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Schizophrenia is an idiopathic mental disorder in which genetic factors account for approximately 80% of the total variation in liability. Multiple genome-wide association studies (GWASs) in schizophrenia support the notion that the heritability of the disorder is explained by a polygenic component, consisting of multiple common single nucleotide polymorphisms (SNPs), with each exerting a small effect (1–3). Schizophrenia is frequently associated with abnormalities in the prepulse inhibition (PPI) of the acoustic startle reflex (4). Because in the PPI paradigm, a sensory event (the prepulse) inhibits a motor response (the startle reflex), the process underlying the PPI phenomenon has been termed sensorimotor gating, which is thought to reflect a form of central nervous system inhibition wherein irrelevant sensory information is filtered out during the early stages of processing so that attention can be focused on more salient features of the environment (5). PPI has been suggested as an endophenotype for schizophrenia research based on 1) PPI heritability studies (6–8), and 2) PPI abnormalities, which are observed in patients with schizophrenia (5), their first-degree relatives (9,10), and individuals with schizophrenia spectrum disorders (9,11). If this hypothesis is true, then part of the phenotypic variation in PPI, and the risk for schizophrenia, could be attributed to common genetic factors.

Over the last decade, multiple candidate-gene studies have provided evidence for genetic association of schizophrenia risk variants (e.g., *COMT, NRG1, DAO, DRD3, PRODH, CHRNA3, TCF4, HTR2A*) with PPI abnormalities (12–24). These studies provided initial evidence supporting PPI as an endophenotype for schizophrenia. More direct evidence is expected to emerge by examining molecular genetic variants associated with both PPI deficits and schizophrenia at a genome-wide level. The relationship of the underlying genetic architecture between schizophrenia and PPI deficits can be

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examined using polygenic score analysis, in which large numbers of alleles demonstrating subtle levels of association with schizophrenia are used to generate a single quantitative index of genetic risk profile (1). The association of the genetic risk profiles with variation in PPI could then be used to estimate the degree of genetic overlap between the two phenotypes.

In the present study, we examined the contribution of common genetic variants on baseline startle and PPI in a general population sample of healthy young male individuals. Loci with suggestive genome-wide significance were further examined in an additional sample. The possible functional impact of noncoding variants was assessed using expression quantitative trait loci derived from human brain tissue. The genetic and environmental contributions to baseline startle and PPI were examined based on genome-wide SNP data in unrelated individuals. Finally, risk profile scores based on the Psychiatric Genome Consortium 2 (PGC2) data set (3), the largest available genome-wide association study to date, was applied to test the polygenic effect on baseline startle and PPI.

METHODS AND MATERIALS

Participants and Screening Methods

A total of $n = 1493$ individuals (age mean ± SD: 22.05 ± 3.45, range: 18–30 years), who were recruited for the Learning on Genetics of Schizophrenia (LOGOS) study, underwent PPI assessment and consented to providing DNA. In addition, a review of the participants' medical history was taken and the Mini-International Neuropsychiatric Interview (25), urine toxicology, hearing test (cutoff threshold of 1 kHz >20 dB), and IQ testing with the Raven's Progressive Matrices (26) were performed. The study was conducted according to the principles expressed in the Declaration of Helsinki and was approved by the Ethics Committee of the University of Crete, the Executive Army Bureau, and the Bureau for the Protection of Personal Data of the Greek State. Following presentation of the study's methods, all participants received a detailed information sheet and gave written informed consent before participation. After quality control, we had both genome-wide genotype data and baseline startle or PPI for 792 and 686 subjects, respectively (discovery cohort). As a replication cohort, an additional targeting genotyping group of 405 or 306 subjects was available for baseline startle or PPI (Supplemental Figure S1 and Supplemental Table S1). The replication cohort, also part of the LOGOS study, was recruited and tested with identical procedures, applying the same inclusion and exclusion criteria as the discovery cohort.

Acoustic Startle and PPI

A commercially available electromyographic startle system (EMG SR-LAB; San Diego Instruments, San Diego, California) was used to examine the eye-blink component of the acoustic startle response from the right orbicularis oculi muscle. Pulses consisted of 40-ms, 115-dB white noise bursts, and prepulses consisted of 20-ms, 75- and 85-dB white noise bursts over 70-dB background noise. Three lead intervals were used (30, 60, 120 ms). The recording period comprised 12 pulse-alone trials and 36 prepulse–pulse trials. Subjects with hearing threshold lower than 40 dB at 1 kHz were excluded. Only subjects with high-quality PPI data were included for further analyses (see Supplement 1 for details).

Genotyping, Quality Control, and Imputation

A subset of our cohort ($n = 871$) were successfully genotyped on the Illumina OmniExpress (San Diego, California) (~770 K SNPs). The preprocessing of SNP data was performed using PLINK (v1.07; http://pngu.mgh.harvard.edu/~purcell/plink/) (27). The initial number of SNPs was 731,442, and after quality control, we had 633,213 SNPs that were imputed using the 1000 Genomes reference panel (phase I; www.1000genomes.org). Prephasing and imputation of genotyping data were carried out with SHAPEIT (v2; https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) (28) and Impute2 (v2.3.0; https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) (29), respectively. After imputation and quality control (see the Supplement for details), we had genome-wide profiling for 4,064,734 SNPs in 833 individuals. Targeting genotyping was performed, blind to phenotype measures, in 426 individuals with a competitive allele-specific polymerase chain reaction system (LGC Genomics, Herts, England).

Expression Quantitative Trait Loci Data Sets

Brain expression quantitative trait loci (eQTLs) were generated using the gene expression and genotyping data of Caucasian samples included in the BrainCloud (30) (Gene Expression Omnibus [GEO] accession number: GSE30272), National Institute on Aging/National Institutes of Health (31) (GEO accession number: GSE15745), and Harvard Brain Tissue Resource Center (32) (GEO accession number: GSE44772) data sets, as described previously (33). Known (age, sex, postmortem interval, pH, RNA integrity number, and batch) and hidden confounders were removed using a Bayesian framework (34) for joint modeling of diverse sources of phenotypic variability. We define a significant *cis* interaction as any SNP that lies within 1 megabase (Mb) upstream or downstream from a gene. For multiple testing corrections, we applied a false discovery rate at 5%.

Statistical Analysis

Genome-wide Association Analysis. Genome-wide association analysis of baseline startle was performed using linear regression (additive model) in PLINK (v1.07) (27), adjusted for age, smoking status, and population stratification using the first two multidimensional scaling (MDS) components. For PPI, we combined results from all PPI trials, using a meta-analysis approach developed earlier for conducing multiregional analysis of eQTLs (35) (see the Supplement for details). The two challenges in conducting meta-analyses using different PPI trials are 1) intercorrelations of PPI trials that violate the independency of data subjected to meta-analysis and cause false-positives (Supplemental Figure S7); and 2) heterogeneity where the effect of variants differs on PPI trials. In this study, we apply a linear mixed model to account for the correlation of outcome variables between PPI trials and correct the effect sizes before performing the meta-analysis.