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The CSMD1 genome-wide associated schizophrenia risk variant rs10503253 affects general cognitive ability and executive function in healthy males

Erasmia Koiliari [a,1], Panos Roussos [b,c,d,1], Emmanouil Pasparakis [a], Todd Lencz [e,f], Anil Malhotra [e,f], Larry J. Siever [b,d], Stella G. Giakoumaki [g], Panos Bitsios [a,*]

[a] Department of Psychiatry, Faculty of Medicine, University of Crete, Heraklion, Crete, Greece
[b] Department of Psychiatry, Mount Sinai, New York, NY, USA
[c] Department of Genetics and Genomic Science, Institute for Multiscale Biology, Mount Sinai, New York, NY, USA
[d] Mental Illness Research, Education, and Clinical Center (VISN 3), James J. Peters VA Medical Center, Bronx, NY, USA
[e] Division of Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, NY, USA
[f] Feinstein Institute for Medical Research, Manhasset, NY, USA
[g] Department of Psychology, University of Crete, Rethymno, Crete, Greece

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ABSTRACT

Background: The single-nucleotide polymorphism (SNP) rs10503253, located within the CUB and Sushi multiple domains-1 (CSMD1) gene on 8p23.2, has reached genome-wide support as a risk factor for schizophrenia. There is initial but inconclusive evidence for a role of this variant in aspects of cognition.
Methods: We investigated the neurocognitive effects of the CSMD1 rs10503253 (C/A) polymorphism in a large, demographically homogeneous sample of young, healthy Greek Caucasian males (n = 1149) phenotyped for a wide range of neuropsychological measures, most of which have been shown to be reliable endophenotypes for schizophrenia.
Results: The risk 'A' allele was associated with poorer performance on measures of general cognitive ability, strategy formation, spatial and visual working memory, set shifting, target detection and planning for problem solving but not for emotional decision making. Most of these effects were dependent on risk "A" allele dose, with AA and CC homozygotes being the worse and the best respectively, while CA individuals were intermediate. Potential genotype effects in Stroop and verbal memory performance were also suggested by our dataset.
Discussion: These results underline the relevance of the risk "A" allele to neurocognitive functioning and suggest that its detrimental effects on cognition, may be part of the mechanism by which the *CSMD1* mediates risk for schizophrenia.

1. Introduction

General cognitive dysfunction is a core stable trait-like feature of the schizophrenia (SCZ) syndrome, that follows the pattern required of an endophenotype (Gottesman and Gould, 2003): it is observed in SCZ patients prior to illness onset, is largely independent of clinical state and medication status, and is familial in nature (Keshavan et al., 2010; Lewandowski et al., 2011; Keefe and Harvey, 2012). Extensive family and twin data support the role of shared additive genetic factors underpinning both SCZ and cognitive deficits (e.g., Toulopoulou et al., 2010). Also, it has been recently demonstrated, that SCZ polygenic risk scores can predict the total brain and white matter volume (Terwisscha van Scheltinga et al., 2013) and general cognitive ability in the general population (McIntosh et al., 2013), suggesting that, general cognitive ability shares genetic risk with the disease, and may be part of the neural mechanism by which risk is mediated. Several genetic variants that are associated with SCZ have emerged from genome-wide association studies (GWAS) (Ripke et al., 2011; Smoller et al., 2013), but their role in illness pathophysiology remains unclear. One important direction of research effort in the post-GWAS era is the characterisation of the functional effects of novel and poorly understood risk variants on critical 'intermediate' phenotypes such as general cognitive ability. Healthy subjects drawn from the general population are a good model to study the effects of SCZ risk variants on the central nervous system, as they are devoid of confounds related to illness process and state. This research effort has already provided important insights into the neural mechanisms by

* Corresponding author. Tel.: +30 2810 394610.
E-mail address: pbitsios@med.uoc.gr (P. Bitsios).
[1] Erasmia Koiliari and Panos Roussos contributed equally in this article.

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which these variants increase risk for disease (Meyer-Lindenberg, 2010; Roussos et al., 2011a, 2011c, 2012a, 2013a).

From the five novel variants identified in the largest SCZ GWAS to date (Ripke et al., 2011), the SNP rs10503253 located within the CUB and Sushi multiple domains-1 (CSMD1) gene on 8p23.2, seems important given previous evidence of its association with risk for multiple neurodevelopmental disorders (Shimizu et al., 2003; Glancy et al., 2009; Håvik et al., 2011). Furthermore, a recent, joint analysis in five major psychiatric illnesses (autism, ADHD, MDD, BD and SCZ) reported a disorder-specific effect for the rs10503253 and SCZ (Smoller et al., 2013). These results support a "central" role of the rs10503253 as a risk factor of SCZ. In a recent study, the CSMD1 SCZ risk 'A' allele at rs10503253 was associated with poorer performance on neuropsychological measures of general cognitive ability (IQ) and memory function but not attentional control (Donohoe et al., 2013) in two independent case-controlled cohorts. However, the effects of the risk "A" allele were subtle and varied between samples in a non-task specific manner, raising the likelihood of false positives due to small sample sizes.

This interesting but inconclusive first evidence motivated us to investigate the effects of rs10503253 on neuropsychological function in a large, demographically homogeneous sample of young, healthy Greek Caucasian males from the LOGOS study (Roussos et al., 2011a, 2011b, 2011c; Jutras-Aswad et al., 2012; Roussos et al., 2012a; Giakoumaki et al., 2013; Roussos et al., 2013a, 2013b). We tested the hypothesis that the risk allele would be associated with reduced IQ and executive function/memory performance.

2. Methods

2.1. Study participants

Subjects were recruited from the first wave of the LOGOS (Learning On Genetics Of Schizophrenia Spectrum) study. The LOGOS project examined 1149 randomly selected young male conscripts from the Greek Army (mean age 21.95 ± 3.5; range: 18–29), who met the inclusion/exclusion criteria (see below) between June 2008 and December 2010. The study took place between 9 am and 3 pm in the medical quarters of the Military Training Camp of Candidate, Supply Army Officers (SEAP) in Heraklion, Crete. For this purpose, two adjacent rooms in the medical quarters were converted into testing rooms. Following public presentation of the study's methods and goals in each consecutive series of new conscripts, all participants willing to volunteer, had a detailed information sheet and gave written informed consent before screening. All subjects were tested on one single occasion at some point during their two month military training in this establishment. The study was approved by the Ethics Committee of the University of Crete, the Executive Army Bureau and the Bureau for the Protection of Personal and Sensitive Data of the Greek State, and was carried out in accordance with The Declaration of Helsinki. All subjects had been recently screened for current physical and mental health status by the army medical authorities and were physically healthy and free from serious mental illnesses. However, they all underwent a review of their medical history, Mini-International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 1998), urine toxicology and IQ testing with the Raven's Progressive Matrices. Inclusion criteria were (i) healthy male recent conscripts; (ii) right handed; and (iii) informed consent (met by 1254 subjects). Exclusion criteria were (i) personal history of head trauma and medical or neurological conditions; (ii) current use of prescribed drugs or a positive recreational drug screen; and (iii) personal history of DSM-IV Axis I disorders. Based on these criteria, 105 subjects were excluded [38 subjects (3.3%) with a history of head trauma and medical or neurological conditions and 67 subjects (5.34%) with a history or presence of an Axis-I disorder (4.3% with recent history of substance/alcohol abuse and 1.04% with panic, anxiety, depression, insomnia)].

2.2. Genotyping

DNA was extracted from blood or cheek swab samples, using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). For N = 833 subjects, the rs10503253 genotype was extracted from available genome-wide genotyping SNP profiling with the Illumina HumanOmniExpress BeadChip (San Diego, CA, USA) (Roussos et al., 2013a). The genotype for another 316 subjects was determined by direct sequencing on the Applied Biosystems (ABI) 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Primers and conditions for polymerase chain reaction (PCR) amplification are described in Supplement. Genotyping was performed blind to phenotype measures. Genotyping quality control was performed in 50 randomly selected samples (N = 40 included in the genome-wide profiling; N = 10 included in the direct genotyping) by duplicate checking (rate of concordance in duplicates 100%). The call rate across all samples was 95.7% (N = 1099/1149). All subjects were of Caucasian ancestry on the basis of self-report, which was confirmed for the subset of our cohort with genome-wide profiling based on EIGENSOFT analysis (Patterson et al., 2006; Price et al., 2006). Based on these data, the self-report identification of the Caucasian ancestry is highly reliable in our cohort, which makes genetic inhomogeneity of the tested population unlikely.

2.3. Neurocognitive assessment

Subjects underwent cognitive testing using the Cambridge Neuropsychological Test Automated Battery (CANTAB), which includes nonverbal tests administered with the aid of a high-resolution touch-sensitive screen (Advantech) and /or a response key to all subjects in the same order. Working memory and strategy formation were assessed with the Spatial Working Memory task (SWM) (Owen et al., 1990), planning for problem solving was assessed with the Stockings of Cambridge (SoC) (Owen et al., 1990) and sustained attention and vigilance were assessed with the Rapid Visual Information Processing task (RVIP) (Park et al., 1994). We also assessed Visual working memory with the N-Back Sequential Letter Task (Braver et al., 1997), selection of appropriate response and the effects of interference with the Stroop Color/Word Interference Test (Golden, 1978) and set-shifting/rule learning abilities with a computerized version of the Wisconsin Card Sorting Test (WCST) (Birkett et al., 2008). Verbal learning and memory was assessed with the Word Lists (WL) subtest of the Wechsler Memory Scale-Revised (Wechsler, 1997). Finally, all subjects were administered the Iowa Gambling Task (IGT) (Bechara et al., 1994) to assess planning based on emotional processing and integration of incentive information for decision-making. For a detailed description of the tests see Supplementary data.

2.4. Situational mood and feelings

On arrival to the testing room, following acclimatization and instructions about the study, subjects self-rated their moods and feelings on a 16-item visual analog scale (VAS) originally developed for measuring drug-induced changes in mood and alertness. Subsequently, these scales were found to be very sensitive to momentary changes in psychological states caused by verbal instructions and experimental manipulations (Bitsios et al., 1996, 1998a, 1998b).

2.5. Group statistical analyses

For the sake of data reduction and variable classification we submitted the outcome variables from the neuropsychological tasks to principal component analysis (PCA). For PCA, the varimax rotation method was used and components with eigenvalues >1 and factor loadings >0.5 were accepted. QTPHASE (https://sites.google.com/site/fdudbridge/software/), from the UNPHASED package version