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Bipolar disorder *ANK3* risk variant effect on sustained attention is replicated in a large healthy population

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Abstract

Independent genome-wide association studies have implicated a common single nucleotide polymorphism within the *ANK3* gene (rs10994336) in bipolar disorder (BD) susceptibility, thus establishing rs10994336 marker as a strong candidate predisposing genetic factor for BD. Furthermore, recent findings demonstrate that this variant impacts on cognitive functioning in BD patients, their unaffected relatives and healthy controls, by influencing sustained attention. Herein, we set out to replicate this finding in a large population-based sample of healthy young adults (n=1808). Sustained attention was evaluated with the Continuous Performance Test (CPT) as in the original study and working memory was assessed with the n-back task. Individuals carrying the BD risk T-allele showed significantly reduced sensitivity in target detection, increased errors of commission and atypical response latency variability. Additionally, we confirmed the lack of association between rs10994336 variant and working memory, as well as general intellectual ability, suggesting a specific effect on CPT performance.

Keywords

sustained attention; working memory; cognition; polymorphism; ankyrin-G; *ANK3*

Introduction

Several lines of evidence from family, twin and adoption studies support the existence of a substantial genetic component in bipolar disorder (BD) susceptibility (Gershon *et al.*, 1982;Rice *et al.*, 1987). The largest genome-wide association study (GWAS) in BD by Ferreira *et al.* (Ferreira *et al.*, 2008) has implicated an intronic variant within the *ANK3* gene

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(rs10994336) in disease susceptibility. The association of this common polymorphic marker with BD has been replicated in a subsequent independent GWA study (Scott *et al.*, 2009), thus establishing rs10994336 as a prominent candidate predisposing genetic factor for BD. Notably, different *ANK3* gene variants have also been associated with BD susceptibility in European as well as in Asian populations (Schulze *et al.*, 2009;Smith *et al.*, 2009; Lee *et al.*, 2011;Takata *et al.*, 2011), strengthening the notion that *ANK3* variation may truly represent a strong BD liability genetic factor. *ANK3* gene encodes a cytoskeletal scaffold protein (Ankyrin-G) which is localized at the axonal initial segment and nodes of Ranvier, modulating neuronal excitability (Zhou et al., 1998).

Recently, it has been reported that rs10994336 impacts on cognitive functioning in BD patients, their unaffected relatives and healthy controls regardless of diagnosis (Ruberto *et al.*, 2011). Homozygotes of the minor T-allele which confers risk for BD, exhibited significantly aberrant performance in the Continuous performance task (CPT), a widely used test of cognitive executive function, measuring sustained attention capacity. Of note, no associations could be detected in this study between rs10994336 risk variant and other aspects of cognitive functioning, such as general intellectual ability, episodic memory, decision making and response inhibition, indicating a rather selective effect on the sustained attention phenotype. Sustained attention deficits as measured with different versions of the CPT task have been consistently observed among BD patients and their offspring (Clark *et al.*, 2002; Diwadkar *et al.*, 2011, Burdick *et al.*, 2011) and are likely to constitute a fundamental behavioral feature of BD pathophysiology. In addition, several genetic loci have been associated with CPT performance in clinical and non-clinical populations (Blasi G *et al.*, 2011), suggesting that inter-individual sustained attention variability is at least in part attributable to complex genetic influences.

In this report, we aimed to replicate the *ANK3* rs10994336 association with CPT performance reported by Ruberto *et al.*, in a large Greek general population sample of young male volunteers for whom multiple cognitive assessments were obtained as part of the Athens Study of Psychosis Proneness and Incidence of Schizophrenia (ASPIS).

Materials and Methods

Details on the study population, cognitive assessments, and outcomes have been presented previously (Stefanis *et al.*, 2007). Briefly, the ASPIS study examined 2130 randomly selected young male conscripts aged 18–24 years from the Greek Air Force in their first two weeks of admission to the National Basic Air Force Training Center. Conscripts underwent an extensive interview of computerized neurocognitive abilities, evaluating sustained attention with the Continuous Performance Task-Identical Pairs version (CPT-IP) (Cornblatt *et al.*, 1988), verbal and spatial aspects of working memory with the n-back task (Gevins *et al.*, 1996; Smyrnis *et al.*, 2007) and general intellectual ability with the Raven progressive matrices (Raven, 1982). Phenotypic outcomes included response time for correct responses, variability of response time, sensitivity measures (d′ index), hit rate and false alarms (commission errors) rates for the CPT-IP task. In accordance with our previous work, we a priori excluded data from further analyses, if the central index of performance (d′) on CPT-

IP and 2-backtaskwas < 0 and if there were ≥ 3 unsuccessful trials (of 5) for verbal and spatial 2-back task.

After obtaining written informed consent from all subjects, genomic DNA was extracted from mouthwash samples and genotyping was performed with a commercially available Taqman 5′-exonuclease allelic discrimination assay. Genotype calls were obtained using an ABI 7900HT real-time PCR instrument and the SDS 2.1 software (Applied Biosystems). To evaluate genotyping accuracy, 10% of the samples were re-genotyped and no discrepancies were found. In our association analyses, we included 1808 individuals who were successfully genotyped and had valid data in all cognitive assessments (Raven IQ, CPT, verbal and spatialn-back), as defined previously. Very few minor allele (T/T) homozygotes were identified (n=12) and they were analyzed together with the heterozygotes. CPT-IP and n-back raw scores were log-transformed to reach normality and univariate analysis of covariance (ANCOVA) was utilized to test genotype effects on each cognitive outcome, entering age and education level as potential confounders. All statistical analyses were carried out using the SPSS 18.0 software. This study was approved by the Bioethics and Medical Deontology Committee of the University Mental Health Research Institute.

Results

In total, 1808 unrelated individuals were successfully genotyped for rs10994336 SNP and the genotype frequencies were in Hardy-Weinberg equilibrium (C/C=1612, C/T=184, T/T=12). In our southern European population, rs10994336 minor allele frequency (MAF = 0.058) was very similar with the one observed in the CEU HapMap population (0.067) and the control individuals examined in the original GWAS by Ferreira *et al.* (0.053). As seen in Table 1, rs10994336 genotype was specifically associated with CPT-IP performance. Specifically, in line with the findings reported by Ruberto *et al.*, carriers of the BD risk T-allele (n=196) showed reduced sensitivity in target detection ($F = 5.154$, one-tailed $P = 0.0115$, Cohen's d = 0.17) as measured with the CPT d′ index, as well as significantly more errors of commission ($F = 9.644$, one-tailed $P = 0.001$, Cohen's d = 0.23) compared with C/C major allele homozygotes (n=1612). Hit rate and response time measures were not related to *ANK3* rs10994336 genotype status ($P > 0.19$), however the variability of response time differed significantly between groups ($F = 6.23$, two-tailed $P = 0.006$). In *post-hoc* analyses, we observed that in our population, age and education level were significantly associated with CPT performance ($P < 0.001$) and therefore we repeated our analysis controlling for these two covariates. rs10994336 genotype effects on CPT d′ index and errors of commission remained significant (one-tailed $P = 0.023$ and $P = 0.002$, respectively). Furthermore, we set out to examine whether *ANK3* rs10994336 genotype is associated with working memory ability, assessing both verbal and spatial aspects with the n-back task, which is highly related to CPT-IP task (Pearson's r > 0.4). We could not detect any suggestive associations with either target sensitivity index (d′) nor response time variability (all $P > 0.05$, two-tailed). Similarly, no effect was noted when general intellectual ability (Raven IQ score) was tested ($F = 1.51$, $P = 0.22$).

Discussion

In this report, we replicated previous findings implicating BD linked *ANK3* rs10994336 polymorphism in human cognitive function by demonstrating a specific effect of *ANK3* variability on the central sensitivity index of attention performance as well as on errors of commission. This suggests a specific modulatory effect of the GWAS derived *ANK3* risk T-allele in decreasing signal detection, thus offering at the molecular level a partial explanation for the attentional deficits observed at the clinical level. We provide evidence that the rs10994336 risk T-allele, or a nearby rare variant in strong linkage disequilibrium, impacts on sustained attention performance in an independent and much larger population of young adults, validating with high confidence the results reported initially by Ruberto *et al.* In addition, we were able to confirm the lack of association between rs10994336 and general intellectual ability (IQ) as well as working memory performance, indicative of a relatively specific effect on CPT performance. Thus, it appears likely that the cognitive phenotype associated with *ANK3* variability is sustained attention capability rather than memory consolidation or retrieval processes. The above assumption is further supported by the fact that a closely related cognitive phenotype to sustained attention, namely verbal and spatial working memory, is not influenced by *ANK3* genotype. This also excludes plausible bias in our results due to the evaluation of sustained attention with a slightly different version of the CPT task, the CPT-Identical Pairs version, which requires a minimal working memory effort (Cornblatt et al., 1988). It is worthy to mention that the effect of *ANK3* rs10994336 polymorphism on two discrete but highly related CPT cognitive tasks adds further validity and robustness to the reported association. The results presented herein, together with the observation made by Ruberto *et al.*, demonstrate that a genome-wide BD risk variant influences an established psychosis-related cognitive endophenotype in the general population. Sustained attention deficits are well validated among euthymic and manic BD patients and have been proposed as a neuropsychological core vulnerability marker of BD (Clark *et al.*, 2002; Najt *et al.,* 2005), indicating that it is a suitable disease-related trait which has the potential to be successfully utilized in BD molecular genetic studies.

Collectively, our results highlight the influence of rs10994336 on a specific aspect of cognitive function, that is sustained attention and establish *ANK3* genetic variation as a vulnerability factor for cognitive aberrations among healthy individuals. Certainly, we acknowledge that the evaluation of a single common polymorphism herein could be considered a limitation. Therefore, further more detailed and functional studies are fully encouraged in order to identify the exact neural mechanism by which this variant affects brain function, human cognition and eventually BD disease clinical presentation.

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Table 1

Mean (±SD) cognitive scores and association analysis stratified by *ANK3* genotype

	C/C (n=1612)	C/T + T/T (n=196)	*F*	*P* [a]
Raven IQ score	44.7 (9.0)	43.8 (8.5)	1.508	0.22
CPT-IP task				
Hit rate	0.749 (0.2)	0.741 (0.2)	0.29	0.296
False alarms	0.227 (0.15)	0.262 (0.16)	9.644	**0.001 (0.002[b])**
Response time (msec)	492.41 (60.8)	488.34 (67.6)	0.761	0.192
Variability of response time	0.236 (0.05)	0.246 (0.05)	6.228	**0.003(0.006[b])**
Target sensitivity (d′)	1.716 (0.91)	1.559 (0.90)	5.154	**0.012 (0.023[b])**
Verbal n-back task				
Response time (msec)	936.85 (205.9)	927.25 (200.9)	0.33	0.564
Variability of response time	0.326 (0.06)	0.334 (0.07)	2.6	0.107
Target sensitivity (d′)	2.564 (0.87)	2.435 (0.92)	3.337	0.068
Spatial n-back task				
Response time (msec)	875.95 (200.4)	877.51 (201.6)	0.01	0.922
Variability of response time	0.348 (0.07)	0.351 (0.07)	0.28	0.59
Target sensitivity (d′)	2.682 (1.03)	2.603 (1.08)	0.897	0.344

Abbreviations: CPT-IP, Continuous Performance Test-Identical Pairs version

Significant ($P < 0.05$) values are shown in bold.

[a] one-tailed *P* values are reported for the CPT-IP outcomes given prior evidence of association

[b] adjusted *P* values for age and education level