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Brief report

Validation of candidate anxiety disorder genes using a carbon dioxide challenge task

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ABSTRACT

Few replicable genetic variants have been identified in the etiology of heritable anxiety disorders such as panic disorder. Endophenotypic measures that have reduced heterogeneity may provide more powerful targets for gene identification. We assessed hypersensitivity to carbon dioxide (a reliable endophenotype of panic and anxiety) in 174 Caucasian college students, who were genotyped on 26 polymorphic markers from 11 genes previously associated with panic/anxiety. Individual trajectories of respiratory and subjective anxiety response to carbon dioxide were measured and tested for association with these genetic markers. One marker in the acid-sensing ion channel 1 (*ASIC1*) gene, rs1108923, had a significant association with respiratory rate. No genes had a significant association with subjective anxiety response. Our findings support previously reported associations between *ASIC1* and panic/anxiety, but not other genes previously associated with anxiety disorders. The use of endophenotypic markers is a promising avenue for gene identification in anxiety and other complex disorders.

1. Introduction

Research has begun to achieve some success in identifying genetic variants underlying heritable psychiatric disorders, yet molecular genetic studies of anxiety disorders lag behind. Well-powered genome-wide association studies in this area are still rare, thus most of the existing knowledge is based on candidate gene studies, which have largely been inconsistent and unreplicated (Maron, Hettema, & Shlik, 2010). One reason for this difficulty is that anxiety disorders may be genetically heterogeneous (Smoller & Tsuang, 1998), with genetic determinants that cross diagnostic boundaries (Hettema, Neale, Myers, Prescott, & Kendler, 2006). A possible solution is to utilize endophenotypes, intermediate phenotypes that sit in the biological pathway between genes and psychological outcomes and may be more powerful targets for gene discovery (Gottesman & Gould, 2003; Meyer-Lindenberg & Weinberger, 2006).

The goal of the present study was to replicate previously reported candidate gene associations with anxiety disorders using hypersensitivity to carbon dioxide (CO_2), a well-validated endophenotype of panic disorder (PD) (Coryell, 1997) and other anxiety disorders (Caldirola, Perna, Arancio, Bertani, & Bellodi, 1997; Telch, Rosenfield, & Pai, 2012). We examined the association between CO_2 hypersensitivity and 11 genes using both subjective and physiologic measures (respiratory rate). Subjective anxiety post-CO_2 inhalation has strong support as a heritable, panic-relevant trait marker (Battaglia et al., 2007; Coryell, Fyer, Pine, Martinez, & Arndt, 2001; Schmidt & Szolensky, 2007; Vickers, Jafarpour, Mofidi, Rafat, & Woznica, 2012), and respiratory rate appears to be moderately heritable ($h^2 = 0.51–0.62$; Snieder, Boomsma, Van Doornen, & De Geus, 1997). CO_2 response differentiates anxiety disorder patients and at-risk persons from controls (Griez, de Loof, Pols, Zandbergen, & Lousberg, 1990; Perna, Barbini, Cocchi, Bertani, & Gasperini, 1995; Rassovsky & Kushner, 2003).

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2. Methods

2.1. Sample

Participants were 174 Caucasian students from two large, urban, public universities in Virginia (53.4% female, age: $M = 19.8$, $SD = 2.6$, range = 18–38). Participants were not screened for anxiety disorders, but completed the Anxiety Sensitivity Index (ASI; Reiss, Peterson, Gursky, & McNally, 1986), and were selected to form approximately equal numbers across all quartiles of the score distribution. The university Institutional Review Boards approved this study and participants provided informed consent. Genetic analyses were limited to Caucasian students to control against spurious results from population stratification (Cardon & Palmer, 2003).

2.2. Measures

We used a sustained carbon dioxide (CO_2) breathing task that included three phases: a 5-min baseline of breathing room air,

Table 1
Genetic markers included in the present study.

Gene	Marker/haplotype	Alleles	MAF	Reason for inclusion
Cholecystokinin B receptor (*CCKBR*)	rs906895	T < C	0.50	Tagging SNPs in the gene; gene associated with PD (Kennedy et al., 1999); gene related to stress-induced fear memory and anxiety in mice (Chen et al., 2010); cholecystokinin agonist is a panicogen (Singh, Lewis, Field, Hughes, & Woodruff, 1991)
	rs2941025	A < G	0.29	
	rs1396860	C < T	0.20	
	rs2880898[a]	G < A	0.18	
Solute carrier family 6, member 4 (*SLC6A4*)	rs3813034	A < C	0.5	Associated with PD (Gyawali et al., 2010) and fear extinction memory (Hartley et al., 2012)
	rs140701	T < C	0.49	Associated with PD and PD/social anxiety disorder (Strug et al., 2010)
	rs6354	G < T	0.18	Associated with anxious depression (Wray et al., 2009)
	rs2020936	G < A	0.21	Associated with anxious depression (Wray et al., 2009)
	rs4251417- rs2020934	C-A	–	Haplotype tags the short version of the serotonin transporter promoter (5HTTLPR; Wray et al., 2009), which is associated with fear acquisition (reviewed by Lonsdorf & Kalisch, 2011)
Glutamate decarboxylase 1 (*GAD1*)	rs2241165-rs769407-rs3791851-rs3791850	A-C-G-C	–	Haplotype associated with an anxiety-neuroticism factor in females (Hettema et al., 2006); rs2241165 associated with PD in females (Weber et al., 2012)
Catechol-O-methyltranferase (*COMT*)	rs4680	A < G	0.44	Associated with an anxiety-depression factor score in females (Hettema et al., 2008); increased risk for PD (Rothe et al., 2006); resistance to fear extinction (Lonsdorf et al., 2009)
	rs165599	G < A	0.43	Associated with an anxiety-depression factor score in females (Hettema et al., 2008)
	rs4680-rs165599	G-A	–	Haplotype strongly associated with an anxiety-depression factor score in females (Hettema et al., 2008)
Brain-derived neurotrophic factor (*BDNF*)	rs6265	T < C	0.18	Associated with anxious depression in men (Middledorp et al., 2010); neuroticism (Frustaci, Pozzi, Gianfagna, Manzoli, & Boccia, 2008); fear extinction (Soliman et al., 2010)
Transmembrane protein 132D (*TMEM132D*)	rs7309727	T < C	0.28	Replicated association in a GWAS meta-analysis of PD (Erhardt et al., 2012)
	rs11060369	C < A	0.39	Replicated association in a GWAS meta-analysis of PD (Erhardt et al., 2012)
Acid-sensing (proton-gated) ion channel 1 (*ASIC1*)	rs1108923	T < G	0.11	Gene associated with fear acquisition/conditioning in mice (Wemmie et al., 2004) and fear behavior in response to CO_2 inhalation in mice (Ziemann et al., 2009)
	rs685012	C < T	0.31	
	rs10875995	C < T	0.25	
Acid-sensing (proton-gated) ion channel 1 (*ASIC2*)	rs9915774[a]	A < G	0.16	Significant association in a GWAS of PD (Gregersen et al., 2012)
Corticotropin releasing hormone receptor 1 (*CRHR1*)	rs878886	G < C	0.12	Associated with PD (Keck et al., 2008); fear acquisition (Heitland, Groenink, Bijlsma, Oosting, & Baas, 2013)
Adenylate cyclase activating polypeptide 1 (pituitary) receptor type 1 (*ADCYAP1R1*)	rs2267735	G < C	0.47	Associated with PTSD and impaired startle discrimination (Ressler et al., 2011) and dark-enhanced startle response (Jovanovic et al., 2013)
FK506 binding protein 5 (*FKBP5*)	rs1360780	T < C	0.30	Related to attention bias toward threat (Fani et al., 2013) and threat-related amygdala reactivity (White et al., 2012)

GWAS = genome-wide association study, MAF = minor allele frequency, PD = panic disorder, PTSD = post-traumatic stress disorder.
[a] SNP did not pass quality control in our sample.