Journal of Affective Disorders 151 (2013) 702–708

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

The retinoid-related orphan receptor alpha (*RORA*) gene and fear-related psychopathology

Mark W. Miller [a,b,*], Erika J. Wolf [a,b], Mark W. Logue [c,d], Clinton T. Baldwin [c]

[a] National Center for PTSD at VA Boston Healthcare System, Boston, MA, USA
[b] Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA
[c] Biomedical Genetics, Boston University School of Medicine, Boston, MA, USA
[d] Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA

ARTICLE INFO

Article history:
Received 26 March 2013
Received in revised form
3 July 2013
Accepted 31 July 2013
Available online 17 August 2013

Keywords:
Retinoid-related orphan receptor alpha
RORA
Gene
PTSD internalizing
Externalizing
Fear

ABSTRACT

Background: This study followed on findings from a recent genome-wide association study of PTSD that implicated the retinoid-related orphan receptor alpha (*RORA*) gene (Logue et al., 2012) by examining its relationship to broader array of disorders.
Methods: Using data from the same cohort ($N=540$), we analyzed patterns of association between 606 single nucleotide polymorphisms (SNPs) spanning the *RORA* gene and comorbidity factors termed fear, distress (i.e., internalizing factors) and externalizing.
Results: Results showed that rs17303244 was associated with the fear component of internalizing (i.e., defined by symptoms of panic, agoraphobia, specific phobia, and obsessive–compulsive disorder) at a level of significance that withstood correction for gene-wide multiple testing.
Limitations: The primary limitations were the modest size of the cohort and the absence of a replication sample.
Conclusions: Results add to a growing literature implicating the *RORA* gene in a wide range of neuropsychiatric disorders and offer new insight into possible molecular mechanisms of the effects of traumatic stress on the brain and the role of genetic factors in those processes.

Published by Elsevier B.V.

1. Introduction

A growing number of genome-wide association studies (GWAS) have identified the retinoid-related orphan receptor alpha (*RORA*) gene as a psychiatric risk locus. Investigators have linked the gene to attention-deficit hyperactivity disorder (Neale et al., 2008), bipolar disorder (Le-Niculescu et al., 2009), major depression (Garriock et al., 2010; Terracciano et al., 2010), autism (Sarachana et al., 2011) and, most recently, posttraumatic stress disorder (PTSD) (Logue et al., 2012). In the latter GWAS, we found an association between a lifetime diagnosis of PTSD and a SNP in the *RORA* gene (rs8042149) that met both genome-wide and Bonferroni-corrected levels of significance in a Caucasian sample. Five other *RORA* SNPs showed suggestive evidence of association with PTSD ($p < 10^{-5}$) in that sample and nominally significant associations between other *RORA* SNPs and PTSD were also observed in an African American subsample from the same study and a second independent African American cohort. The association between rs8042149 and PTSD has since been replicated by an independent team of investigators (Amstadter et al., 2013).

RORA is an interesting new candidate gene for PTSD because of the role that it plays in neuroprotection. The RORA protein is widely expressed in psychiatrically-relevant regions of the brain including the cerebral cortex, thalamus, hypothalamus (Ino, 2004) and has been shown to protect neurons and glial cells from the degenerative effects of oxidative stress (Boukhtouche et al., 2006; Jolly et al., 2012)—a process that has been identified as a mechanism of neurodegenerative effects of traumatic stress (Oosthuizen et al., 2005; Pall, 2001; Richards et al., 2011; Schiavone et al., 2013). Based on this, we hypothesized that individuals with the functional *RORA* risk variant may be less capable of mounting a neuroprotective response to the neurotoxic elements of oxidative stress which contributes to the functional and structural brain alterations that putatively underlie PTSD (c.f., Logue et al., 2012).

Given that *RORA* has been linked to a broad array of psychiatric disorders, it seems untenable to conceptualize variants in this gene as posing risk for PTSD specifically. It is more likely that polymorphisms of the gene confer a general (i.e., non-disorder-specific) risk for the development of neurobehavioral conditions broadly, or that variants in different regions of this very large gene (which spans 741,010 base pairs and includes 11 exons) are

* Corresponding author at: National Center for PTSD at VA Boston Healthcare System, 150 South Huntington Avenue, Boston, MA 02130, USA.
Tel.: +1 857 364 5733.
E-mail address: mark.miller5@va.gov (M.W. Miller).

0165-0327/$ - see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jad.2013.07.022

associated with risk for different forms of psychopathology. In this study, we aimed to examine these alternatives by analyzing patterns of association between 606 SNPs spanning the *RORA* gene and three broad classes of psychiatric illness modeled as latent dimensions of comorbidity.

One of the fundamental challenges of psychiatric genetics research is the problem of heterogeneity within the phenotypes of interest, a primary manifestation of which is diagnostic comorbidity (i.e., the presence of two or more diagnoses in the same individual). Co-occurring disorders are the rule, rather than the exception, in psychiatric samples, and PTSD shows considerable overlap and covariation with neighboring disorders. One implication of this is that multiple overlapping psychiatric phenotypes may be present in any given sample of individuals with PTSD which can potentially obscure the search for genetic risk factors.

One solution to this problem is for theoretical conceptualizations and data analytic approaches to move towards the use of hierarchical structural models in which groups of symptoms are classified at varying levels of specificity with each syndrome containing common (i.e., higher-order) and unique components. Along these lines, factor analytic studies of the structure of comorbidity have shown that the covariation of the most common mental disorders can be accounted for primarily by two broad dimensions: *externalizing* and *internalizing*. Externalizing has been defined as the latent dimension of psychopathology that explains the covariation observed between substance-related and antisocial personality disorders in adults (e.g., Krueger, 1999; Kendler et al., 2011) and the co-occurrence of conduct disorder, oppositional defiant disorder, and ADHD in children (e.g., Dick et al., 2005). Internalizing is the dimension that underlies the co-occurrence of the anxiety and unipolar mood disorders (Krueger, 1999). In many studies it has been subdivided into conceptually distinct, yet correlated, factors termed "distress" (defined by major depression, dysthymia, generalized anxiety disorder) and "fear" (comprised of panic and phobic disorders; Cox et al., 2002; Krueger, 1999; Slade and Watson, 2006).

Twin studies have shown these dimensions to have substantial heritabilities (Krueger et al., 2002; Young et al., 2000; Wolf et al., 2010) with estimates generally higher for these traits than for the individual *DSM* disorders that define them. For example, while estimates of the heritability of individual externalizing disorders have generally fallen in the .18–.66 range (Kendler et al., 2003), published heritability estimates for externalizing are in the .43–.84 range (Krueger et al., 2002; Wolf et al., 2010; Young et al., 2000; Young et al., 2009). Furthermore, studies that have directly compared estimates of the heritability of internalizing versus externalizing have found stronger genetic contributions to externalizing and, conversely, higher estimates of non-shared environmental influences on internalizing (e.g., Kendler et al., 2003; Wolf et al., 2010).

Internalizing and externalizing can be conceptualized as endophenotypic traits which, in theory, are expected to map more directly and completely onto their underlying genetic substrate compared to individual *DSM* disorders. Building on this, the primary objective of this study was to apply this model in a genetic association analysis of *RORA*. Given *RORA*'s established role in neuroprotection, along with evidence for a greater contribution of non-shared environmental influences (e.g., adverse life events) to internalizing, we hypothesized that *RORA* (as a moderator of the molecular stress response) would be more strongly associated with measures of internalizing than with externalizing. Though the extant literature did not suggest differential predictions for the relationship of *RORA* to the fear versus distress components of internalizing, we modeled them separately on the basis of research and theory pointing to their distinct etiologies and mechanisms (e.g., McTeague and Lang, 2012; Patrick et al., in press; Vaidyanathan et al., 2009; Watson, 2005).

2. Methods and materials

2.1. Participants

Eight hundred fifty-two participants enrolled in one of two VA studies. The first enrolled trauma-exposed military veterans who screened positive for PTSD; the second included military veterans with trauma histories and their cohabitating partners. Both studies involved comprehensive psychiatric diagnostic assessments and blood sample collection. See additional details in Logue et al. (2012). The statistical program STRUCTURE (Falush et al., 2003; Pritchard et al., 2000) was used to identify a subgroup of 540 White, non-Hispanic participants on the basis of a Bayesian cluster analysis of 10,000 randomly chosen SNPs with minor allele frequency (MAF) $>.05$ from the full sample. The Caucasian sample was comprised of 375 veterans and 165 partners. The majority was male (60.4%) and the mean age was 51.88 years (range: 21–75, *SD*: 11.09). Participants reported exposure to a wide variety of traumatic events on the Traumatic Life Events Questionnaire (Kubany et al., 2000) with most participants endorsing exposure to mulitple events over the course of their lifespans. The events most frequently endorsed by male participants were sudden death of a loved one (56.8%), combat (52.9%), and accidents other than those involving motor vehicles (44%). For women, the most frequently endorsed events were sudden death of a loved one (61.2%), a life threatening incident involving a loved one (45.2%), and childhood and/or adult sexual assault (44.9%).

2.2. Measures

2.2.1. Structured clinical interview for DSM-IV (SCID-IV; First et al., 1994)

Lifetime Axis I disorders were assessed with the SCID-IV and dimensional scores for each diagnosis by summing scores across symptoms within a module. All interviews were videotaped for the purposes of evaluating diagnostic reliability.

2.2.2. Adult antisocial behavior

Adult antisocial behavior was assessed in the veteran-only study using the International Personality Disorder Examination (IPDE) (Loranger, 1999). In the couples study, it was assessed using the SCID-II (SCID-II; First et al., 1995). To create a single adult antisocial scale across the two measures, the summary scores from matching items on each measure were standardized and then combined. In the subset of participants who completed the IPDE ($n=181$), this variable correlated .99 with the full adult antisocial behavior severity score on the IPDE and .71 with total antisocial personality disorder symptom severity (i.e., adult symptoms +child conduct disorder symptoms).

2.2.3. The clinician administered PTSD scale (CAPS; Blake et Al., 1990)

The CAPS is a 30-item structured diagnostic interview that assesses the frequency and severity of the 17 *DSM-IV* PTSD symptoms, 5 associated features, and functional impairment. Dimensional lifetime severity scores were calculated by summing the frequency and intensity ratings (each range from 0–4) for each of the 17 items (possible range: 0–136; Weathers et al., 1999).

2.3. Procedure

This research was approved and reviewed annually by the appropriate human subjects and institutional review boards. Participants were recruited through medical databases, flyers, clinician referrals, and a database of veterans who had previously