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A genome-wide association study of posttraumatic stress disorder identifies the retinoid-related orphan receptor alpha (*RORA*) gene as a significant risk locus

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Abstract

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We describe the results of the first genome-wide association study of PTSD performed using trauma-exposed white non-Hispanic participants from a cohort of veterans and their intimate partners (295 cases and 196 controls). Several SNPs yielded evidence of association. One SNP (rs8042149), located in the retinoid-related orphan receptor alpha gene (*RORA*), reached genome-wide significance. Nominally significant associations were observed for other *RORA* SNPs in two African American replication samples—one from the veteran cohort (43 cases and 41 controls) and another independent cohort (100 cases and 421 controls). However, only the associated SNP from the veteran African American replication sample survived gene-level multiple testing correction. *RORA* has been implicated in prior GWAS studies of psychiatric disorders and is known to play an important role in neuroprotection and other behaviorally-relevant processes. This study represents an important step towards identifying the genetic underpinnings of PTSD.

Keywords

Posttraumatic stress disorder; Genome-wide association; *RORA*

Introduction

Posttraumatic Stress Disorder (PTSD) is a psychiatric disorder defined by profound disturbances in cognitive, emotional, behavioral and physiological functioning that occurs in response to a psychologically traumatic event. The DSM-IV¹ specifies that the diagnosis applies to individuals who develop a constellation of symptoms after exposure to an event involving perceived or threatened loss of life, serious injury, or loss of physical integrity. The symptoms of PTSD in DSM-IV are organized under three clusters: 1) re-experiencing (e.g., intrusive thoughts, nightmares, flashbacks, and reactivity to reminders of the trauma); 2) avoidance and emotional numbing (e.g., avoiding stimuli associated with the trauma and anhedonia); and 3) hyperarousal (e.g., anger, hypervigilance, exaggerated startle response, and sleep disruption).

Epidemiological studies have shown that the majority of individuals in the general population experiences a traumatic event meeting the PTSD stressor criterion at some point during their lifetime though only a minority go on to develop PTSD². After exposure, the probability of developing PTSD is estimated to be approximately 10% in the general population, though higher rates (i.e., closer to 25%) have been observed in samples of individuals exposed to severe traumas involving violence and/or life threat (e.g., combat or rape). The traumatic event is, by definition, necessary for the development of PTSD, but the fact that not everyone exposed to trauma goes on to develop PTSD indicates that individual difference factors, including genes, likely play a significant role in the etiology of the disorder^{3, 4}.

Twin and heritability studies suggest that 30–70% of variation in PTSD risk is determined by genetic factors^{5–7} and recently there has been an increase in the number of studies examining the molecular genetics of PTSD (for review see ³). Most of these studies have been limited to candidate genes commonly examined in psychiatric disorders such as those involved with the regulation of dopamine, serotonin, and glucocorticoid metabolism. Results of those studies have been inconsistent and the heritability of PTSD remains largely unexplained.

In this article, we report the first positive results from a genome-wide scan for genetic risk factors for PTSD. We genotyped 2.5 million SNPs covering the entire genome and found an association at genome-wide levels of significance between PTSD and the Retinoic Acid Orphan Receptor A (*RORA*) gene in a U.S. Department of Veterans Affairs (VA) sample of

trauma-exposed white non-Hispanic men and women. Nominally significant associations between RORA SNPs and PTSD were also observed in a replication sample of individuals of African American descent from the VA sample and in a cohort of trauma-exposed African Americans from the Detroit Neighborhood Health study⁸.

Materials and Methods

Participants

The VA sample was comprised of U.S. military veterans and a subset of their intimate partners (N=852) who were enrolled in one of two studies conducted at two U.S. Department of Veterans Affairs (VA) Healthcare facilities. Both studies were reviewed and approved by the appropriate human subjects and local institutional review boards. Data were not analyzed for 42 cases who did not complete the protocol, 25 cases who enrolled in both studies (in which case, only data from the first study was included) and DNA was not available for 24 participants due to problems with the blood draw. This yielded a subsample of (n=761) with complete data, of whom 729 had been exposed to a traumatic event meeting the DSM-IV PTSD Criterion A definition as determined by a clinician using the Clinician-Administered PTSD Scale (CAPS⁹). Of these events, the most common type was combat trauma, endorsed by 33.6% of the sample (54.7% of men; 2.4% of women). The trauma-exposed subsample was predominantly male (n=435, 59.7%) with a mean age of 51.5 (SD=10.9). 496 (68%) were veterans and the remaining 233 were their spouses or intimate partners. 409 participants (53.7%) met criteria for a lifetime diagnosis of PTSD based on the CAPS.

Ancestry was determined using 10,000 randomly chosen markers with MAF>.05 by the program STRUCTURE^{10, 11} which performs a Bayesian clustering analysis to assign subjects to ancestry groups (see additional details below). From this analysis, 491 individuals with a history of trauma exposure and valid genotype data were identified as having primarily white non-Hispanic ancestry, 84 were identified as having African American ancestry, and 105 were of “other” ancestry (including individuals self-described as having Hispanic, Native American, or Asian ancestry). We found no evidence of PTSD-associated population substructure within the VA-sample ancestry groups (see supplementary materials for details).

Instruments

The Clinician Administered PTSD Scale (CAPS)⁹—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. VA study participants were administered the CAPS to assess current and lifetime PTSD symptoms. All interviews were video-recorded and approximately one-quarter (n=197) of them were viewed and scored by an independent rater for purposes of quality control and evaluating inter-rater reliability. Inter-rater reliability for lifetime PTSD diagnosis, based on comparison of the primary interviewer and secondary raters scores was excellent (kappa=.87).

Traumatic Life Events Questionnaire (TLEQ)¹²—The TLEQ is a self-report measure that assesses exposure to 22 types of potentially traumatic events that meet the DSM-IV PTSD Criterion A1 definition for a traumatic event. For each traumatic event that is endorsed, the respondent is subsequently asked to provide (1) the number of times the event was experienced using a 7-point scale ranging from “never” to “more than five times” and (2) whether he or she experienced intense fear, helplessness, or horror in response to the event (i.e., to assess DSM-IV PTSD Criterion A2). The TLEQ has shown good test-retest reliability over a two-week interval (mean kappa=.63, mean percent agreement=86%),

excellent convergent validity with interview-based measures of trauma exposure (mean percent agreement=92%), and good predictive validity for PTSD status¹².

Genotyping

In the VA sample, DNA was isolated from peripheral blood samples on a Qiagen AutoPure instrument with Qiagen reagents and samples normalized using PicoGreen assays (Invitrogen). Each DNA sample was run on an Illumina OMNI 2.5–8 array and scanned using an Illumina HiScan System according to the manufacturer's protocol. All available DNA samples from the two studies were genotyped (n = 810). Seven samples had an overall call rate of < 95% and were eliminated from analysis. In the remaining 803 samples, the call rate was 99.34%. Of those, one was excluded due to a typographical error in the ID key, which prevented linking their data to the phenotypic database. There were 36 duplicates from subjects who participated in both studies (the 24 mentioned earlier, plus additional duplicates from subjects who did not complete one protocol or the other, but which provided genetic samples), in which case the second sample was removed from analysis. In addition, subjects were checked for consistency between their self-reported sex and the sex indicated by X-chromosome homozygosity using PLINK. Ten mismatches were detected. Seven of these were determined to be sample swaps that could be resolved based on prior genotyping. Two of the mismatches could not be resolved and were excluded from further analysis. One of the apparent mismatches was explained due to the subject's transgendered status, this subject was also removed to prevent bias in the estimation of sex-specific effects. This left 763 genotyped cases whose genotype data were analyzed with STRUCTURE.

SNPs were removed from the analysis if they yielded greater than 5% missing genotypes or if they were rare (< 5% minor allele frequency). After these filters were applied, 1,199,491 autosomal SNPs remained for analysis in the non-Hispanic white group. None of the reported SNPs failed Hardy-Weinberg Equilibrium tests in Controls.

Statistical Methods

Because PTSD is conditional on trauma exposure, we restricted our analyses to subjects who reported experiencing a qualifying trauma as assessed by a clinician during the CAPS interview. Association between the autosomal SNPs and PTSD was evaluated using an allelic test of association and performed in PLINK (v 1.07, October 2009)¹³. Significance was determined using Fisher's Exact Test. The white non-Hispanic group was analyzed separately as the discovery sample. The African American group was analyzed as a replication sample. (We will not be presenting the results for the third [Hispanic, Native American, and Asian] group due to the small number of controls [n=35] and admixed nature of the sample.) Association between all common SNPs (MAF>.05) was assessed in the white non-Hispanics. We used established levels of significance for genome-wide studies and considered $p < 10^{-5}$ as suggestive evidence of association and $p < 5 \times 10^{-8}$ as genome-wide significant. In the VA African American replication sample, all SNPs passing quality control and MAF filters within 5 kb of implicated genes were examined. The max(T) permutation procedure in PLINK was used to correct for the testing of multiple SNPs within a gene in the replication sets. Haploview¹⁴ was used to compute LD information. Plots of association in genes of interest were created with LocusZoom¹⁵. Subsequent exploratory analysis examining the possibility of a SNP×trauma-exposure interaction were performed in R¹⁶.

Detroit Neighborhood Health Study (DNHS)

Subsequent to our analysis of the VA discovery and replication samples, we examined data from the Detroit neighborhood Health study (DNHS), a longitudinal cohort of predominately African American adults (18+) living in Detroit, Michigan and screened for

lifetime trauma exposure. Data collection and genotyping procedures are described in detail in our supplementary material and additional information reported elsewhere^{8, 17}. In particular, association was assessed for SNPs from the Illumina HumanOmniExpress BeadChip in and near implicated genes in a sample of trauma-exposed African Americans (n= 100 cases and 421 controls). As in the VA replication sample, PLINK was used to compute the allelic test of association for all SNPs passing QC with MAF>.05 within 5 kb of implicated genes, and gene-level multiple-testing significance was assessed using the PLINK max(T) permutation procedure.

Results

GWAS Results

The Manhattan Plot showing the results of the genome-wide analysis of lifetime PTSD within trauma-exposed individuals from the discovery sample (295 cases and 196 controls) is shown in Figure 1. Results for all SNPs with $p < 10^{-4}$ are available in supplementary Table 1. A total of 10 SNPs reached the “suggestive” level of significance ($p < 10^{-5}$). These are presented in Table 1 and could be categorized into 4 haplotype blocks: one each on chromosomes 1, 6, 15 and 17. The haplotype blocks on chromosomes 1 and 17 were at considerable distance from any well-characterized genes. SNPs on chromosome 6 and 15 were within haplotype blocks for the brefeldin A-inhibited guanine nucleotide-exchange protein 3 (*BIG3*) gene and the retinoid-related orphan receptor alpha (*RORA*) gene respectively.

One SNP within the *RORA* gene (rs8042149) met genome-wide significance ($p < 5 \times 10^{-8}$) as well as Bonferroni-corrected ($p < 4.16 \times 10^{-8}$ after correcting for 1,199,491 SNPs examined) significance thresholds. An additional 4 SNPs in *RORA* were suggestive of association (Figure 2A). The evidence of association was not specific to individuals of a particular sex. P-values were proportional to sample size for each of the sexes and OR estimates were similar; in n=319 males the $p_{\text{Male}} = 1.35 \times 10^{-5}$ and in n=172 females $p_{\text{Female}} = .00050$; $\text{OR}_{\text{Male}} = 2.13$ and $\text{OR}_{\text{Female}} = 2.19$; $p = .95$ for a test of equality of rs8042149 effect by sex.

To further explore the relationship between rs8042149 and PTSD, we next examined the relationship between its genotype, the probability of PTSD, and the total number of traumatic events (NTE) as measured by the TLEQ¹² as a quantitative measure of environmental adversity. Table 3 lists the percentage of cases with PTSD for the entire sample as a function of Low/Medium/or High levels of traumatic exposure, with the cutoffs determined to approximate the terciles of the NTE distribution. Examining the probability of a PTSD diagnosis by genotype indicated that subjects with relatively low levels of exposure to trauma with the high-risk (GG) genotype had a similar likelihood of developing PTSD as those from the high-exposure group but with the low-risk (AA) genotype (63% and 61% respectively). A logistic regression model indicated that the NTE is strongly associated with the risk of PTSD ($p = 1.5 \times 10^{-11}$). In a logistic regression model that included NTE and rs8042149, the effect of rs8042149 was significant ($p = 4.4 \times 10^{-8}$). A further logistic model indicated that NTE \times SNP interaction was not significant ($p = .4576$).

Replication Analyses

We next analyzed trauma-exposed cases and trauma-exposed controls in the African American subset of the VA cohort (43 cases/41 controls) for association to SNPs implicated in the discovery cohort. The peak SNP in white non-Hispanics, rs8042149, was not associated with PTSD in the African American sample ($p = .87$). However, when we expanded our search, other SNPs in *RORA* were found to be nominally significantly associated with PTSD (Figure 2B and Table 3). The most significant SNP, rs11071588, had

a $p=6.77 \times 10^{-5}$ which remained significant after multiple testing correction ($p_{ADJUSTED}=0.018$).

Subsequent to our analyses of the VA data, we requested association results for a set of SNPs in *RORA* from the DNHS. In trauma-exposed African Americans from the DNHS (100 cases/421 controls), the most significant SNP associated with lifetime PTSD within *RORA* was rs16942660 ($p=.0009$) 633 kb from the peak SNP in African American's from the VA sample and 324 kb away from the peak SNP observed in the discovery sample of white non-Hispanic subjects. The most significant SNP in the VA African American sample, rs11071588, was not significant in the Detroit sample ($p=.43$). However, there was a cluster of nominally significant SNPs flanking this SNP; there were 5 SNPs with $p<0.05$ within 50 kb of rs11071588 compared to 7 nominally significant SNPs observed anywhere else in the gene region which spans 760 kb. The most significant of these was rs893290 ($p=.0052$) approximately 50 kb from rs11071588 followed by rs782928 ($p=.018$) which is 26 kb from rs11071588 in the opposite direction. While the number of nominally significant SNPs clustering around the peak African American SNP from the VA sample is interesting, none of the *RORA* SNPs in the DNHS survive correction for multiple testing at the gene-based level (Table 3).

Discussion

This report describes the results of a high-density GWAS of PTSD using diagnostic information based on clinical interview and genomic data from a 2.5 million SNP array. Analyses of data from a white non-Hispanic cohort of adult men and women with histories of exposure to trauma revealed a significant association between a lifetime diagnosis of PTSD and a SNP in the *RORA* gene (rs8042149) that met genome-wide and Bonferroni-corrected levels of significance. We observed a notably high (>60%) incidence of the disorder among those with limited histories of trauma exposure who carried the high-risk allele, though the prevalence of PTSD was elevated for individuals with the high-risk genotype across levels of exposure. In our analysis, which only included trauma-exposed individuals, no significant interaction between level of exposure and genotype was observed. Nominally significant associations between other *RORA* SNPs and PTSD were found in an African American subsample from the same study, and in a second much larger independent African American cohort, although only the most significant SNP from the VA-African American cohort survived gene-level multiple testing correction. To our knowledge, this is the first report of findings from a GWAS of PTSD and the first discovery of a significant risk marker for the disorder using this approach.

This is not, however, the first GWAS study to implicate *RORA* as a psychiatric risk factor. Prior studies have found *RORA* to be associated with attention-deficit hyperactivity disorder¹⁸, bipolar disorder¹⁹, and autism^{20, 21}. In addition, perhaps the most extensive evidence for the role of *RORA* in psychiatric illness comes from research on the genetics of major depression. Depression is particularly relevant because of its high rates of comorbidity with PTSD and twin studies that have shown the two disorders to be influenced by a common genetic factor (e.g. ^{22, 23}). Evidence for a significant association between *RORA* and depression comes from several sources including a GWAS of anti-depressant response in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study that found the third most significant SNP to be located in *RORA* ($p=8.19 \times 10^{-6}$ at rs809736)²⁴. This SNP (at 61.3 Mb) was also associated with depression remission ($p=7.64 \times 10^{-5}$). In a GWAS of trait depression, as indexed by a self-report measure of a facet of neuroticism, the single most significant SNP was also in *RORA* (rs12912233 $p=6.3 \times 10^{-7}$ at rs12912233 at 61.3 Mb)²⁵. Similarly, a candidate gene study examining circadian rhythm genes involved in depression showed evidence of association with a SNP in Intron 2 of *RORA* (rs2028122 at

60.8 Mb multiple-testing corrected $p=.03$; Lavebratt et al. 2010)²⁶. Taken together, prior GWAS studies suggest that polymorphisms of the *RORA* gene likely confer a general (i.e., non-disorder-specific) risk for the development of psychopathology, possibly via its relationship to a common vulnerability factor such as trait negative emotionality (i.e., neuroticism). This dimension of individual differences has long been conceptualized as the primary temperamental risk factor for disorders of the internalizing spectrum (i.e., anxiety and unipolar mood disorders^{27, 28}) though more recent evidence suggests that it plays a more ubiquitous role in a broader array of psychopathologies including externalizing disorders and PTSD^{29, 30}.

RORA belongs to the NR1 subfamily of nuclear hormone receptors. Other members of this family include the glucocorticoid, estrogen, progesterone, and androgen receptors, all of which have established roles in brain development and function. The function of the *RORA* gene is complex and the protein encoded by it involved in an intriguing variety of processes including brain development, neuroprotection, and the regulation of circadian rhythms and steroid hormones. Though research on the possible mechanism(s) by which polymorphisms of the *RORA* gene might confer risk for PTSD is in its infancy, we suggest a model in which alterations in *RORA* levels, due to inherited genetic variation, may reduce the capacity of neurons to respond to the biochemical stressors (oxidative stress, steroid hormone elevations, and inflammation) induced by traumatic stress. *RORA* is widely expressed in neurons of cortical and subcortical structures³¹ and serves several neuroprotective functions. It protects cortical neurons against oxidative stress-induced apoptosis by increasing the expression of the antioxidant proteins³². In glial cells, its expression is upregulated by the presence of destructive pro-inflammatory cytokines, and during hypoxia, its expression is upregulated in neurons and astrocytes³³. Oxidative stress and inflammation have been implicated as possible mechanisms of the deleterious effects of traumatic stress on the brain³⁴ and these neurodegenerative processes have been linked to functional and structural abnormalities in PTSD-relevant brain structures including the hippocampus³⁵, dorsolateral and ventromedial prefrontal cortex^{36, 37}, and anterior cingulate cortex^{38, 39} (for a review see ⁴⁰). *RORA*'s role in this pathology is further supported by evidence that polymorphisms of the gene are linked to thickness of cortical gray matter and fractional anisotropy of cerebral white matter in humans⁴¹. In that study, *RORA* SNP rs341401, which was the 10th most strongly associated with PTSD in this study ($p=6.20 \times 10^{-5}$), was one of the strongest predictors of a composite index of structural brain imaging parameters.

In addition to *RORA*, we found other regions that showed suggestive evidence of association (Table 3). However, none of the SNPs were in or near genes that at this point would conceivably be related to PTSD pathogenesis (e.g., *BIG-3* is involved in cartilage development, and the function of *C17orf97* is unknown). We also failed to find significant or suggestive evidence of association with genes previously studied in PTSD including those involved in dopamine and serotonin metabolism. Thus, a limitation of this study, as in all GWAS studies, is the likelihood of false positive associations due to chance. At the same time, the stringent P-value thresholds applied to GWAS studies can limit the ability to detect true associations with modest levels of association.

The primary strength of this study was the carefully assessed trauma-exposed discovery sample that was characterized by a high prevalence of lifetime PTSD. The primary weaknesses were the relatively modest sizes of the available samples. While the size of our discovery sample was not in the range of GWASs published by major consortia for disorders such as bipolar disorder or schizophrenia (e.g. ^{42, 43}), it was within the range of samples used in other published GWAS studies of psychiatric disorders. For example, in a 2011 review of GWAS studies of schizophrenia and related phenotypes, there were 17 case/control studies identified, 5 of which had a smaller number of cases than in our discovery

sample and 3 were published with a similar number or fewer controls than our discovery sample⁴⁴. That said, given our modest sample sizes (especially in our replication samples) and lack of a white non-Hispanic replication sample, findings from this study should be considered provisional until further evidence of replication is obtained. As with any complex disease, much larger samples will be necessary to fully characterize the impact of *RORA* on PTSD susceptibility and to confirm our “suggestive” level associations on chromosomes 1, 6, and 17.

Based on these findings, we believe that it would be useful for future investigations to evaluate the hypothesis that *RORA* moderates the neurotoxic effects of traumatic stress through studies examining genetic associations with structural and functional neural imaging parameters in trauma exposed individuals with and without PTSD. In addition, research using methods such as whole genome sequencing and testing for association in other cohorts will be needed to identify the particular functional variant within *RORA* that is responsible for the association with PTSD. Our observation that different *RORA* SNPs are associated with PTSD in African Americans suggests that humans harbor multiple variants affecting protein function or gene expression of *RORA*. This variability may also be driven by differences in the pattern of linkage disequilibrium between the populations. In sum, the association of PTSD with *RORA* offers new insight into possible biological and neurological underpinnings of the psychopathological response to traumatic stress and we hope that these findings will stimulate the development of new models of gene-environment interactions in PTSD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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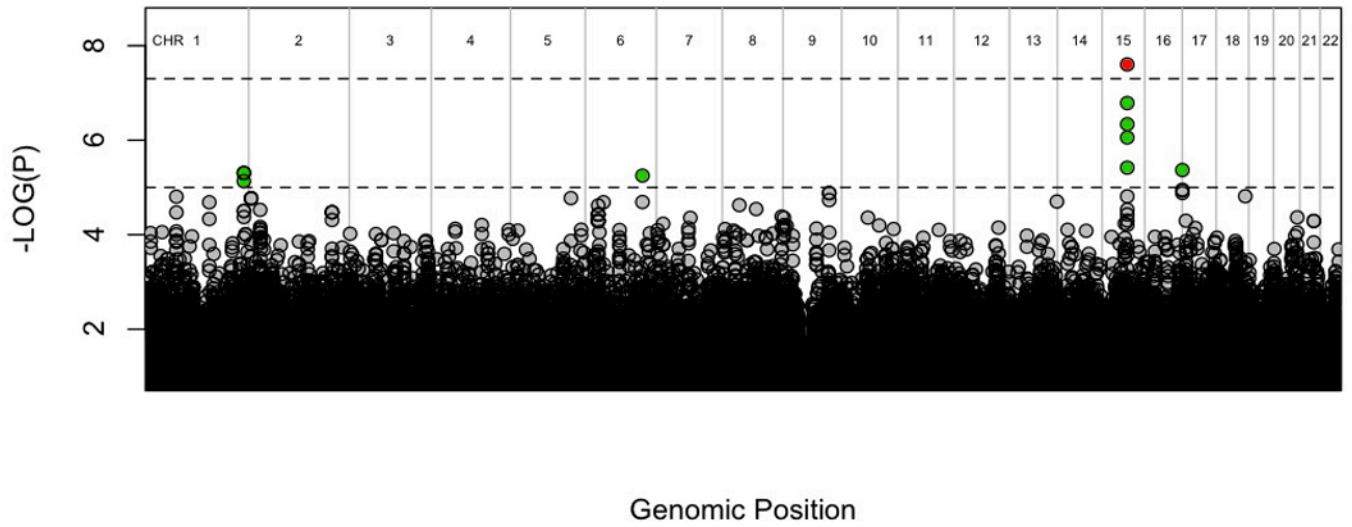


Figure 1. Manhattan plot of genome-wide association results in discovery group. Dashed lines and colors represent suggestive and genome-wide significance respectively, with green= $p < 10^{-5}$ and red= $p < 5 \times 10^{-8}$.

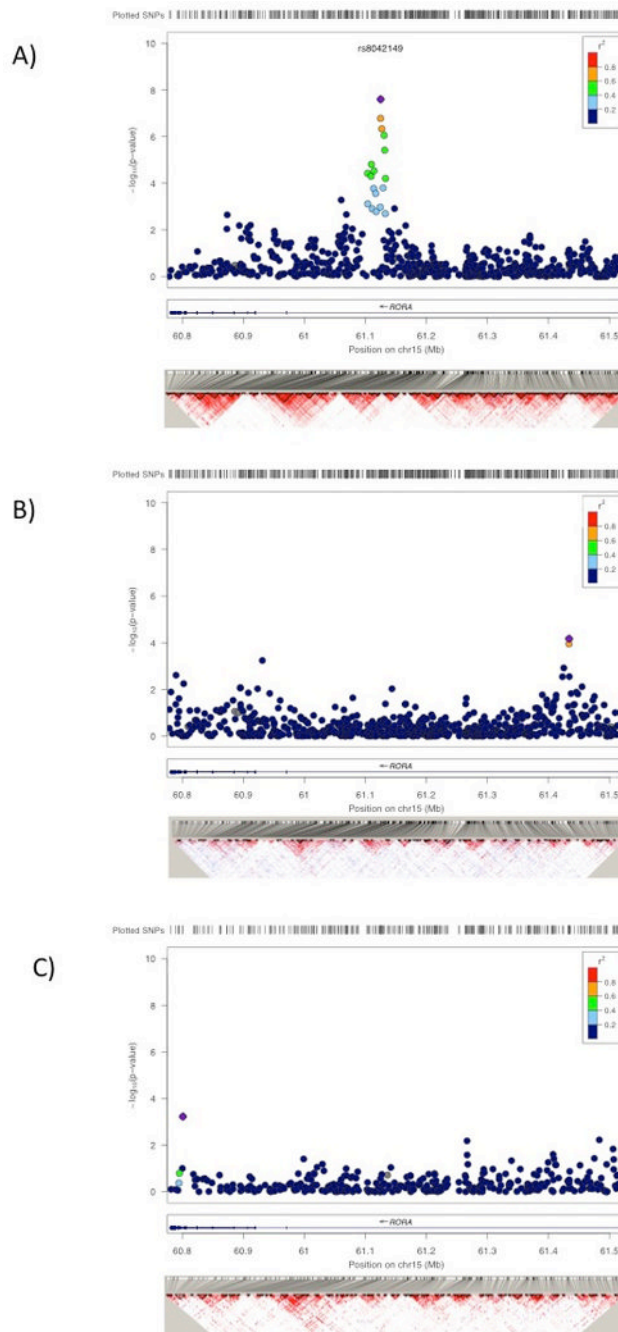


Figure 2. Association results in the *RORA* gene for VA Discovery sample of white non-Hispanics (A), the VA African American replication sample (B), and the Detroit Neighborhood Health Study African American replication sample (C).

Table 1

SNPs with $P < 10^{-5}$ from the discovery GWAS of PTSD in trauma-exposed white non-Hispanic subjects (n=295 cases and 196 controls).

SNP	CHR	Position	Gene	Risk Allele	AF Cases	AF Controls	OR	P
rs1021356		238,573,691		G	76%	62%	1.9	4.9×10^{-6}
rs12118091	1	238,576,614	--	C	76%	62%	1.9	4.9×10^{-6}
rs113508478		238,576,715		A	75%	61%	1.9	7.4×10^{-6}
rs72986828	6	138,484,125	<i>BIG3</i>	C	12%	3.6%	3.5	5.6×10^{-6}
rs8041061		61,124,838	<i>RORA</i>	A	58%	41%	2.0	1.6×10^{-7}
rs8042149		61,124,953		C	61%	43%	2.1	2.5×10^{-8}
rs4775301	15	61,126,859		G	60%	43%	2.	4.6×10^{-7}
rs8024133		61,130,639		A	61%	45%	1.9	8.8×10^{-7}
rs11071561		61,131,683		T	64%	49%	1.8	3.8×10^{-6}
rs71355256	17	259,248	<i>C17orf97</i>	G	97%	90%	3.7	4.3×10^{-6}

Table 2

Percentage of participants with PTSD by trauma exposure and rs8042149 genotype in the white non-Hispanic discovery sample.

	Trauma Exposure	Low (1 to 17)	Medium (18 to 31)	High (>31)
% PTSD for ALL Subjects:		43% (n=159)	59% (n=166)	78% (n=166)
% PTSD for subjects by genotype of rs8042149	AA	22% (n=36)	46% (n=37)	61% (n=36)
	AC	40% (n=75)	56% (n=84)	77% (n=74)
	CC	63% (n=48)	76% (n=45)	89% (n=56)

Table 3

Most significant *RORA* SNPs from case/control analysis of trauma-exposed subjects from the African American replication samples

Group	SNP	Position	Risk Allele	AF Cases	AF Controls	OR	Nominal P	Adjusted P
VA-African Americans (n=43 cases and 41 Controls)	rs11071587	61,433,543	A	45%	17%	4.03	.00011	.029
	rs11071588	61,433,578	C	53%	23%	3.81	6.77×10 ⁻⁵	.018
African Americans from the DNHS (n=100 cases and 421 controls)	rs16942660	60,801,066	G	94%	86%	2.65	.00090	.202
	rs893290	61,483,157	C	18%	11%	1.85	.0052	.698