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Genome-wide association study implicates a novel RNA gene, the lincRNA *AC068718.1*, as a risk factor for post-traumatic stress disorder in women

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Abstract

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Contributors

Drs. Guffanti and Yan undertook the statistical analysis. Drs. Roberts and Solovieff contributed with quality control analysis and phenotypic variables preparation for the two datasets involved DNHS and NHSII. Dr. Wildman performed the genotyping in DNHS and Dr. Ranu the genotyping in NHSII. Dr. Koenen supervised the overall project. Drs. Guffanti and Koenen wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest statement

All authors declare that they have no conflicts of interest.

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Posttraumatic stress disorder (PTSD) is a common and debilitating mental disorder with a particularly high burden for women. Emerging evidence suggests PTSD may be more heritable among women and evidence from animal models and human correlational studies suggest connections between sex-linked biology and PTSD vulnerability, which may extend to the disorder's genetic architecture. We conducted a genome-wide association study (GWAS) of PTSD in a primarily African American sample of women from the Detroit Neighborhood Health Study (DNHS) and tested for replication in an independent cohort of primarily European American women from the Nurses Health Study II (NHSII).

We genotyped 413 DNHS women - 94 PTSD cases and 319 controls exposed to at least one traumatic event - on the Illumina HumanOmniExpress BeadChip for > 700,000 markers and tested 578 PTSD cases and 1963 controls from NHSII for replication. We performed a network-based analysis integrating data from GWAS-derived independent regions of association and the Reactome database of functional interactions.

We found genome-wide significant association for one marker mapping to a novel RNA gene, lincRNA *AC068718.1*, for which we found suggestive evidence of replication in NHSII. Our network-based analysis indicates that our top GWAS results were enriched for pathways related to telomere maintenance and immune function. Our findings implicate a novel RNA gene, lincRNA *AC068718.1*, as risk factor for PTSD in women and add to emerging evidence that non-coding RNA genes may play a crucial role in shaping the landscape of gene regulation with putative pathological effects that lead to phenotypic differences.

Keywords

PTSD; GWAS; lincRNA; telomere maintenance; immune system

1. Introduction

Posttraumatic stress disorder (PTSD) is pervasive and debilitating mental disorder that develops in some persons following exposure to a traumatic event. The lifetime prevalence of PTSD is 7.6% in the United States (Kessler et al., 1995). The majority of persons who are exposed to even a severe traumatic event do not develop PTSD (Kessler et al., 1995; Breslau et al., 1999) Why some individuals are vulnerable and others are resilient remains an open question; genetic factors are hypothesized to play an important role (Cornelis et al., 2010). However, a recent review of the biology of PTSD concluded that the candidate gene studies design had not produced robust results (Pitman et al., 2012). To date, only two genome-wide association studies (GWASs) of PTSD have been published. The first identified the retinoid-related orphan receptor alpha (*RORA*) gene as a novel risk locus and was conducted in a primarily male (59.7%) European American sample of military veterans and their partners (Logue et al., 2012). The second was conducted in a European American sample of men and women and found a genome-wide significant hit at rs406001 with the second strongest association in the Tolloid-Like 1 gene (*TLL1*). The *TLL1* association was replicated in an independent sample of European Americans (Xie et al., 2013).

The burden of PTSD is particularly high for women. Women in the general population are at twice the lifetime risk of PTSD as men: 1 in 9 women versus 1 in 20 men in the US will have met criteria for the diagnosis at some point in their lifetime (Resnick et al., 1993; Kessler et al., 1995; Breslau et al., 1998). PTSD in women is more likely to follow a chronic course (Breslau and Davis, 1992; Kessler et al., 1995; Breslau et al., 1998) and may be associated with greater functional impairment (McLean et al., 2011). The traumatic events associated with PTSD in women differ from those in men; women are more likely to be exposed to traumatic events such as sexual abuse, sexual assault, and rape that have high

conditional risks of PTSD (Kessler et al., 1995; Roberts et al., 2012). PTSD may also be more heritable in women; the heritability of PTSD was recently estimated at 72% in a community-based sample of young adult female twins (Sartor et al., 2011), more than twice that of the 30% heritability observed in a male sample of Vietnam era veterans. Recently, animal models and human genetic studies have suggested connections between sex-linked biology and vulnerability to PTSD (Lebron-Milad and Milad, 2012). Mechanisms underlying sex-linked biology may be determined by sex-specific genetic risk factors for the disorder. For example, Ressler and colleagues reported a sex-specific association of SNP rs2267735 mapping to a putative estrogen response element within the gene PACAPR1 (encoded by *ADCYAP1R1*) with PTSD in females only (Ressler et al., 2011) which has been replicated in several studies (Almli et al., 2013; Uddin et al., 2013; Wang et al., 2013). However, Chang and colleagues were unable to replicate the association between rs2267735 and PTSD in either AA or EA females in these two large independent samples (Chang et al., 2012).

Taken together, these findings support the importance of investigating the genetic risk factors that specifically increase vulnerability in women. In order to identify novel risk genes for PTSD in women, we conducted a GWAS in a primarily African American sample of women from the Detroit Neighborhood Health Study (DNHS) (Goldmann et al., 2011), an epidemiologic study of adults in urban Detroit and replicated our top finding in a primarily European American sample from the Nurses Health Study II (NHSII) (Koenen et al., 2009). To better characterize the biological pathways implicated by our GWAS, we conducted an exploratory pathway enrichment analysis using a functional interaction network-based approach of top hits from our initial GWAS.

2. Materials and Methods

2.1 DNHS Sample

Participants were recruited from the DNHS (N = 1,547), a longitudinal cohort of predominately African American men and women adults (18+) living in Detroit, Michigan and screened for lifetime trauma exposure using procedures described in more detail elsewhere (Uddin et al., 2010; Goldmann et al., 2011). The analytic sample for this paper is 413 women, who signed consent to provide DNA samples that were viable for analysis and were exposed to at least one traumatic event. The self-reported ethnicity of these women is: 342 African Americans, 45 European Americans, 26 individuals of other ethnicity. Overall, 406 women identified themselves as non-Hispanic and 7 as Hispanic. 94 had a lifetime diagnosis of PTSD and 319 were trauma-exposed controls. PTSD diagnosis was assessed via structured interview administered via telephone (Breslau et al., 1998). PTSD symptoms were assessed in reference to both the traumatic event the participant regarded as their worst and one randomly selected traumatic event from the remaining traumas the participant experienced. Lifetime PTSD cases met all six DSM-IV criteria in reference to either the worst or random traumatic event. The diagnostic interview showed good validity against the Clinician Administered PTSD Scale (CAPS) (Blake et al., 1995) as described elsewhere (Uddin et al., 2010). The Institutional Review Board of the University of Michigan reviewed and approved the study protocol.

2.2 GWAS Genotyping

DNA was isolated from whole blood or saliva. Whole-blood derived genomic DNA was isolated from 400µl of whole blood using either the DNA Mini Kit (Qiagen, Valencia, CA) or the QuickGene DNA Blood Kit using the Quickgene Mini80 system (Fujifilm, Tokyo, Japan) following the manufacturer's recommended protocol. Saliva-derived genomic DNA was isolated from 4ml of saliva, which was then processed using the Oragene DNA OG-500

kit (Orasure Technologies, Bethlehem, PA) following the manufacturer's recommended protocol. 0.25 µg of each DNA sample was sent to the Applied Genomics Technology Facility (Wayne State University, Detroit, MI) for genotyping using the HumanOmniExpress BeadChips (Illumina Inc, San Diego, CA). The BeadChips were run on an Illumina iScan system using a combination of the Infinium HD Assay Super Manual Protocol and the Infinium HD Assay Super Automated Protocol, switching protocols from manual to automated at the XStain step on Day 3. The GenomeStudio Genotyping (GT) Module (Illumina Inc, San Diego, CA) was used for data normalization and genotype calling.

2.3 Genome-wide association analysis

We genotyped 730,525 SNPs. Genotyping was performed on 471 women. A total of 4 women had samples with call rate < 95% and were removed from the study. Other samples were removed due to duplicate issues, missing phenotype data or because they were not exposed to at least one traumatic event with a remaining sample of 413 women. A total of 688,890 SNPs passed quality control filters (call rate > 95%, minor allele frequency > 0.01, Hardy-Weinberg disequilibrium $p > 1 \times 10^{-6}$). Genotype reports generated using Illumina GenomeStudioGT v. 1.8.4 software were used to generate PLINK input files (i.e. lgen, map and fam files). GWAS was conducted using logistic regression for genotypes coded additively (0,1,2 copies of the minor allele) and PTSD case status. Two Multidimensional Scaling (MDS) components, described in detail in the next section, were used in the regression model as covariates to adjust for population stratification. All analyses were performed using PLINK software package (v 1.07, October 2009) (Purcell et al., 2007). The study had limited power to detect a disease-associated allele at the genome-wide threshold. Simulations showed that in a sample of 94 cases with a control/case ratio of 3.4 like ours, we would have 48% statistical power to detect an associated allele with a population frequency of 0.20 and an odds ratio of 2.5 (at $P = 5 \times 10^{-8}$ under a multiplicative genetic model).

2.4 Population stratification

We used the MDS analysis of genome-wide identity-by-state data implemented in PLINK to determine ancestry in the whole sample. The analysis was conducted using the 688,890 SNPs that passed quality control filters previously described. The first two components from the MDS analysis identified clearly separated clusters that correlate with self-report ethnicity identification. The first two MDS components distinguished African American from European American participants and others and the second component distinguished Hispanic and non-Hispanic subjects. Both components were used to adjust for population stratification in the association analysis.

2.5 Nurses Health Study II sample

We used data from the PTSD diagnostic subsample (N=3,013) of the NHSII. The ascertainment of the subsample has been described in detail previously (Roberts et al., 2012) and the protocol has been published elsewhere (Koenen et al., 2009). See the Supplementary Material for further demographic information.

2.6 Diagnostic Interviews

Lifetime PTSD diagnosis was assessed in relation to participant's worst event via structured telephone interview similar to that used for the DNHS (Roberts et al., 2012).

2.7 Replication genotyping

All samples were genotyped using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format or using the TaqMan

OpenArray™ SNP Genotyping Platform (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. TaqMan® assays were ordered using either Assays-on-Demand or using the ABI Assays-By-Design service.

2.8 Statistical analysis of rs10170218 in the NHSII replication sample

We attempted to replicate our top GWAS finding from the primary PTSD analysis in the female DNHS subsample in an independent cohort of 578 PTSD cases and 1963 trauma-exposed controls from the NHSII. To avoid population stratification biases, we confined the analysis to the subsample of European American females who self-reported as non-Hispanic. After checking for Hardy Weinberg Equilibrium, the SNP with genome-wide significant p-value in the primary analysis was tested for evidence of association in the replication sample using a logistic regression model for genotypes coded additively (0,1,2 copies of the minor allele) and PTSD case status. In consideration of potential differences in the allelic distribution between the primary and the replication samples due to different ancestry-related genetic background, we also tested the association of the SNP with PTSD status using different genetic models, namely the genotypic (2df), dominant and recessive models (1df) using PLINK.

2.9 *In-silico* analysis

To further characterize genes and their potential predicted function, we conducted an *in-silico* computational analysis by integrating the current genome annotations available at UCSC Genome Browser (Rosenbloom et al., 2012). We used the ENCODE/GENCODE (version 7, May 2011) annotation through Ensembl to annotate the region that harbors our top significant SNPs. The potential transposable elements and interspersed repeats overlapping with the DNA sequence of the SNPs/gene found associated were searched for by using the annotation created with Repeatmasker (Smit et al., 1996–2010) program on the reference genome sequence, assembly February 2009, GRCh37/hg19.

2.10 Pathway analysis

To better characterize the top findings of our GWAS, we set out to identify independent regions of association using a clumping algorithm and test pathway enrichment analysis of the genes mapping to these regions through a network-based approach. First, for each SNP with p-value $< 5 \times 10^{-4}$, we looked for any SNPs in linkage disequilibrium (at least above $r^2=0.2$) and associated with PTSD at p-value < 0.05 within 250 Kb distance from the index SNP. Then, we used the Reactome Functional Interaction (FI) network to reconstruct a FI sub-network based on the set of genes mapping on the non-desert genomic regions of association derived from the GWAS. The FI sub-network mines the Reactome manually curated pathway-based protein functional interaction network, which covers close to 50% of human proteins. To reconstruct the network, the algorithm used genes from our list and “linker” genes necessary to identify patterns of functional interactions. We ran a network-clustering algorithm based on spectral partition (Newman, 2006). Pathway enrichment analysis was performed for each module using only genes provided in the original list without considering “linker” genes in the enrichment evaluation. Reactome FI uses the False Discovery Rate approach to determine the adjusted p-values for each enriched pathway. We considered a pathway to be significantly enriched at $FDR < 1 \times 10^{-3}$.

3. Results

3.1 PTSD GWAS in the DNHS

We performed a GWAS in DNHS women. As DNHS is ethnically diverse, all our association analysis used principal components of genetic variation inferred from the whole genome data as covariates to account for ethnicity. The q-q plot of the results shows no

departures of the overall distribution of the observed p-values from the expected distribution, with the only exception of the extreme tail of the distribution of the test statistics (Figure 1). The genomic inflation factor (λ) of 0.99 further confirms that population stratification is not biasing association results. The Manhattan plot illustrates the results of the genome-wide association analysis with PTSD, with 201 SNPs (0.03%) showing evidence of association at p -value $< 5 \times 10^{-4}$ (Figure 1). Table 2 lists the 7 SNPs that yielded p -values $< 1 \times 10^{-5}$ (Table 2). The strongest signal was at SNP rs10170218 with a genome-wide significant p -value (p -value = 5.09×10^{-8} ; OR = 2.88, 95% CI = 1.97–4.23). The Bonferroni corrected p -value is 0.035. SNP rs10170218 is located within *AC068718.1*, a novel long intergenic non-coding RNA (lincRNA) gene located at 2q32.1. The total length of the gene is 578.8 Kb. The region containing *AC068718.1* showed a very mild degree of linkage disequilibrium (LD) (Figure 2). Of the 92 SNPs mapping to *AC068718.1*, 11 additional SNPs were significant with p -value < 0.05 . Among these, the strongest signals were at SNPs rs10181512 and rs10497691, located ~9Kb downstream of SNP rs10170218, and rs6713963, located in the same 28-Kb LD block as rs10170218, with p -values at 10^{-3} threshold of significance.

The next strongest signals were at SNP rs1611133 (p -value = 1.18×10^{-6} ; OR = 3.56, 95% CI = 2.04–6.22) located in a region of high LD on chromosome 6, 10-Kb from the gene *HLA-G*, which encodes for the major histocompatibility complex, class I, G and SNP rs2413187 maps to an intron of *LARGE*, a gene that encodes acetyl-glucosaminyl-transferase-like protein. Of note, SNP rs1611133 was associated with rheumatoid arthritis with p -value = 4×10^{-6} (OR = 0.73) in the opposite direction (Plenge et al., 2007). Both SNP rs7302717 and rs10502692 map to an intergenic region. The first of these two regions is gene desert, while the second harbors the gene *TMCC3*, which encodes the transmembrane and coiled-coil domain family 3, and is located ~75-Kb from the associated SNP. SNP rs11767398 maps to the 3' UTR of gene *C7orf53*, which encodes a coiled-coil domain-containing transmembrane protein. SNP rs4701170 maps in an intergenic region, and is located 14-Kb from the gene *RP11-798K23.1*, a putative lincRNA.

3.2 Top hit SNP rs10170218 replication in NHS-II Sample

We tested the association between SNP rs10170218 and PTSD in the NHSII cohort for replication in an independent female sample. SNP rs10170218 was in HWE and the minor allele C frequency (MAF) was 0.27 in the European American females sample from NHSII (MAF of allele C was 0.22 in DNHS). The association test based on the additive genetic model gave a marginally significant p -value for association with PTSD (p -value = 0.07), with stronger evidence for the genotypic model (p -value = 0.03), and the dominant model (p -value = 0.01).

3.3 In-silico analysis of the genome annotations for lincRNA AC068718.1

Given the novelty of lincRNAs and the relative SNP found associated in our study, we decided to further characterize the lincRNA *AC068718.1* performing an *in silico* computational analysis by integrating the current genome annotations available at UCSC Genome Browser (Rosenbloom et al., 2012). The ENCODE/Gencode (version 7, May 2011) annotation defines *AC068718.1* as a novel lincRNA. We found that ~ 52.38% of the DNA sequence contains transposable elements (TEs) as previously reported elsewhere for other lincRNAs (Jendrzewski et al., 2012). TEs are discrete pieces of DNA that can move within genomes, inserting themselves into new genomic sites and generating interspersed repeats of the original TEs, and have been recently implicated in the origin of lincRNAs (Ponting et al., 2009). Consulting the database of TEs, we found that 7.14% of the reference sequence of the lincRNA *AC068718.1* overlaps with short interspersed nuclear elements (SINEs), 26.51% with long interspersed nuclear elements (LINEs), of which 71% are of

Family L1, and 16.23% with long terminal repeat (LTR) transposable elements from the endogenous retrovirus (ERV) family. We found that exons 1, 2, and 3 are located within repetitive elements, SINE (Family Alu) and LTR (Family Erv1), respectively, while exon 4 seems to be a unique sequence in the genome.

3.4 Pathway analysis of functional interactions network reconstructed in silico based on independent genomic regions of association

The clumping algorithm identified 133 independent genomic regions of association harboring genes (Table S1). 98 of these harbored more than one SNP, with region sizes ranging from 0.8 Kb (i.e., 2 SNPs clumped) to 346 Kb (i.e., 49 SNPs clumped). Then, we used the Reactome Functional Interaction (FI) network to reconstruct a FI subnetwork based on the set of genes mapping on the 133 non-desert genomic regions of association derived from the GWAS (Figure 3). The network-clustering algorithm based on spectral partition (Newman, 2006) identified 9 different modules of highly interacting genes (Table S2). Pathway enrichment analysis within each module revealed enrichment of the Telomere Maintenance pathway ($\text{fdr} < 2 \times 10^{-3}$), and Systemic Lupus Erythematosus pathway ($\text{fdr} < 1 \times 10^{-3}$) in module 2 and a set of signaling pathways pertinent to immunity in module 8, including the Antigen processing and presentation pathway, Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell, Type I diabetes mellitus and Autoimmune thyroid disease pathways with $\text{fdr} < 3 \times 10^{-4}$.

4. Discussion

We have completed the largest and most comprehensive GWAS of PTSD to date in a female sample involving more than 400 women using a dense panel of > 700,000 markers. Our results implicate a novel risk locus associated with PTSD (rs10170218) mapping to a novel RNA gene, the lincRNA *AC068718.1* that reached genome-wide significance. We found suggestive evidence for replication in a female European-American sample from the NHSII. Our exploratory pathway enrichment analysis using a functional interaction network-based approach of top hits from our initial GWAS, albeit preliminary, revealed molecular signatures implicating pathways involved in telomerase maintenance and immune functioning. We considered our findings in reference to the two other published PTSD GWASs (Logue et al., 2012; Xie et al., 2013). With regard to the RORA gene, the strongest association was found for rs2433025 ($\text{p-value} = 8 \times 10^{-4}$). This SNP maps ~ 117 Kb upstream from the SNPs found associated in the first GWAS by Logue and colleagues. As for the second, we were not able to evaluate the association of SNP rs6812849 (*TLL1*) as it was not present in our set of available genotyped SNPs; however, our results revealed a marginal association of SNP rs10031332 with PTSD ($\text{p-value} = 5.2 \times 10^{-3}$). This SNP maps ~ 27 Kb downstream from the top hit by Xie and colleagues.

The DNHS and the NHSII have different ancestry-related genetic background, mainly African American for the DNHS and European American for the NHSII. To be consistent with the analysis of the GWAS in the DNHS sample, we tested the association of SNP rs10170218 using the additive genetic model. The frequency of the putative risk allele of SNP rs10170218 differed somewhat in the two samples ($\text{MAF}_{\text{NHSII}} = 0.27$ versus $\text{MAF}_{\text{DNHS}} = 0.22$). This allelic difference should be considered in the context of the potential heterogeneity in the genetic architecture of the disease in the independent cohorts used in this study. It is well known that various forces, including genetic drift, gene flow, mutation, selection, and admixture, shape the population frequencies at any given locus (Adeyemo and Rotimi, 2010). These factors play a major role in determining the evolutionary history of loci associated to the disease and may lead to a non-homogeneous distribution of genetic risk variants across populations with a different ancestry-related background (Marigorta et al., 2011). The genotypic and the dominant model show a

progressive increase in statistical significance, which is consistent with the idea that a more specific genetic model, i.e. the dominant, when it is in fact true, is more powerful than a more generic one, i.e. the genotypic.

Our genome-wide significant hit maps to a novel RNA gene, the lincRNA *AC068718.1*. Our *in silico* analyses using current genome annotations available at UCSC suggest our finding is consistent with what has been reported elsewhere about the possible origin of exons of noncoding RNA genes by retrotransposable elements. SNP rs1070218 maps to intron 3–4, 236 Kb upstream from the start of exon 4, and overlaps with a repeat sequence of class LTR8B (Family Erv1). Emerging literature supports a functional role of transposable elements such as transposons or retrotransposons in gene regulation (Cordaux and Batzer, 2009). The presence of mobile genetic elements in this lincRNA structure seems to be consistent with a noncoding RNA gene with putative regulatory functions (Mariner et al., 2008; Ponicsan et al., 2010). The absence of information about lincRNA function does not allow studying them in the context of any curated known functional pathways, which makes difficult to fully evaluate their role in the etiology of PTSD.

lincRNAs have been classified as RNA molecules longer than 200 nucleotides that are capped, polyadenylated and often spliced, not overlapping with protein-coding genes or other non-coding RNA genes previously identified (Guttman et al., 2011). Emerging literature suggests a regulatory role of lincRNA on other genes in terms of protein expression, DNA binding, and transcriptional function (Guttman and Rinn, 2012). Further experimental analyses are needed to elucidate the biological mechanism underlying lincRNAs such as *AC068718.1*.

We conducted an exploratory analysis to assess whether our list of protein-coding genes in the independent regions of association that harbored hits with $p\text{-value} < 5 \times 10^{-4}$ was significantly enriched for gene ontology categories, and whether these genes were implicated in known functional pathways. We used a network-based approach to reconstruct the overall network of functional interactions between the genes in our list. Our clustering analysis of highly interacting genes within our reconstructed network identified two sets of pathways of interest in two of the nine modules. The molecular signatures revealed by pathway enrichment analysis are consistent with recent developments related to the pathophysiology of PTSD. In module 2, we found association with the Telomerase Maintenance and the Systemic Lupus Erythematosus pathways, which apply to four genes and six genes respectively, encoding for histone proteins mapping on 6p22.1. Telomeres are nucleoprotein structures that protect chromosome ends from degradation. Telomerase maintenance refers to the enzyme telomerase-dependent mechanisms of maintenance of the telomere length. Histone proteins, associated in our GWAS, are included in this pathway because of their central role for DNA repair and chromosomal stability. Oxidative and psychological stress, including PTSD, has been associated with accelerated telomere shortening (Epel et al., 2004; Danese et al., 2009; O'Donovan et al., 2011). Interestingly, one of the emerging themes among many lincRNAs is the formation of RNA-protein interactions between the ncRNAs and proteins, such as the telomerase, used to carry out their function. Apparently the telomerase activity requires a specific telomerase RNA component, i.e., gene *TERC*, for telomeric regulation (Guttman and Rinn, 2012). Specific *TERC* domains seem to interact with the telomerase bringing specific regulatory components into proximity with each other, which results in the formation of a unique functional RNA-protein functional complex ultimately responsible to telomerase repair.

The second set of pathways in module 8 pointed to immunity-related molecular signature with nominal association of antigen processing and presentation, immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell, Type I diabetes mellitus and

autoimmune thyroid disease pathways, which all apply to the same set of genes encoding for the major histocompatibility complex (HLA) class I heavy chain paralogues, i.e. *HLA-A*, *-G* and *-F*, mapping as well on 6p22.1. Both sets of genes in the most enriched biological pathways map in the two largest LD regions identified by the independent regional association analysis. The first LD region contains 33 and the second LD region contains 49 SNPs with p-value < 0.05 in LD with one of the top nominally associated SNP with p-value < 5×10^{-4} .

Evidence from a variety of sources suggests an association between dysfunction of the innate immune inflammatory system in the pathophysiology of PTSD. Previous work by the our group (Uddin et al., 2010) and others (Smith et al., 2011) has shown that immune system functions were significantly overrepresented among the annotations associated with genes uniquely unmethylated among those with PTSD. In a female sample, childhood abuse related PTSD has been associated with enhanced inflammatory system activity and decreased immune cell glucocorticoid sensitivity (Pace et al., 2012). PTSD in veteran samples has been associated with increased risk of Rheumatoid Arthritis (RA) onset and, among RA patients, greater pain, more functional impairment, and less well-being (Mikulski et al., 2012; Pace et al., 2012). This combined evidence suggests a potential role of dysfunction of the innate immune inflammatory system in the pathophysiology of PTSD.

Our findings must be considered in the context of several limitations. Telephone interviews, used to ascertain PTSD status, may result in PTSD misclassification, biasing our results towards the null. We were powered only to find very strong genetic effects. Therefore, the marginally significant results in this cohort should be considered with caution. Results from GWAS of other psychiatric disorders including schizophrenia, bipolar disorder and major depression suggest these disorders are highly polygenic, characterized by potentially hundreds of variants with weak effects (Purcell et al., 2009; Middeldorp et al., 2011). Whether the genetic architecture of PTSD is similar remains to be seen.

Genome-wide association studies have revealed a number of new genetic variants for common diseases (Hindorff et al., 2009). The identification of genetic variants of susceptibility to PTSD is still relatively in its infancy. Although there have been some notable successes, candidate gene association studies have often failed to deliver robust results. Our results suggest the potential role of a novel RNA gene, the lincRNA *AC068718.1*, in the pathophysiology of PTSD. We also found several additional SNPs with highly significant evidence of association (p-value < 1×10^{-5}), none of which have previously been reported in association with PTSD. Moreover, the possibility of sex-specific genetic effects in PTSD merit further investigation in much larger samples that include both men and women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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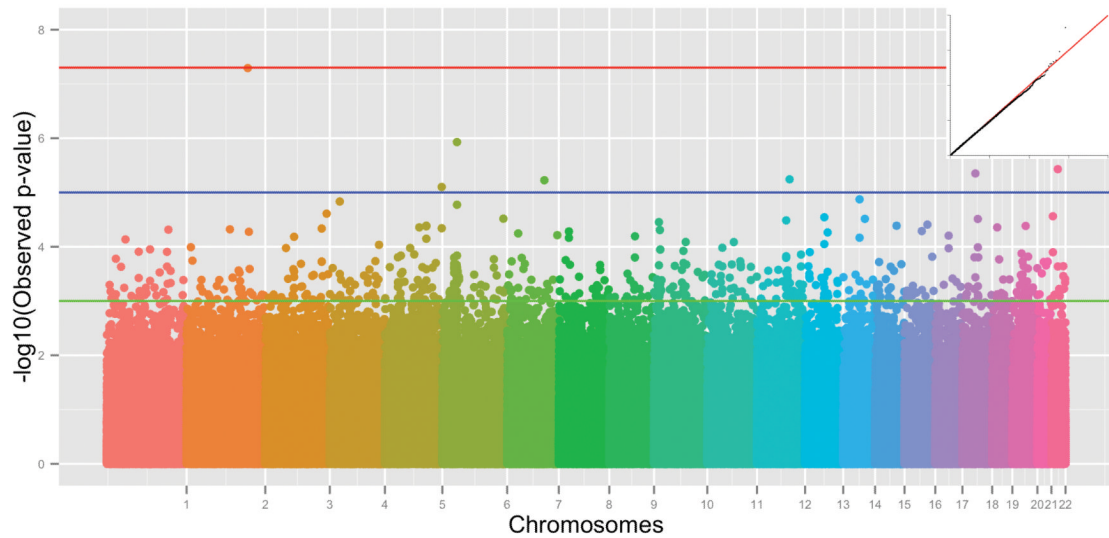


Figure 1.

Manhattan plot and Q–Q plot for the GWAS in the female sample from DNHS. The chromosomes are presented in order and color coded for ease of identification. The individual SNP p-values are represented by circles and are plotted according to $-\log_{10}$ scale. Three lines identify different significance threshold: green for p-value < 0.001 , blue for p-value $< 1 \times 10^{-5}$ and red for p-value $< 5 \times 10^{-8}$ (i.e., genome-wide significance threshold). In the upper right corner, Q–Q plot of GWAS analyses with correction for population stratification (y-axis: observed $-\log_{10}(\text{p-value})$; x-axis: expected $-\log_{10}(\text{p-value})$). $\lambda_{gc} = 0.99$, indicating adequate control of bias. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

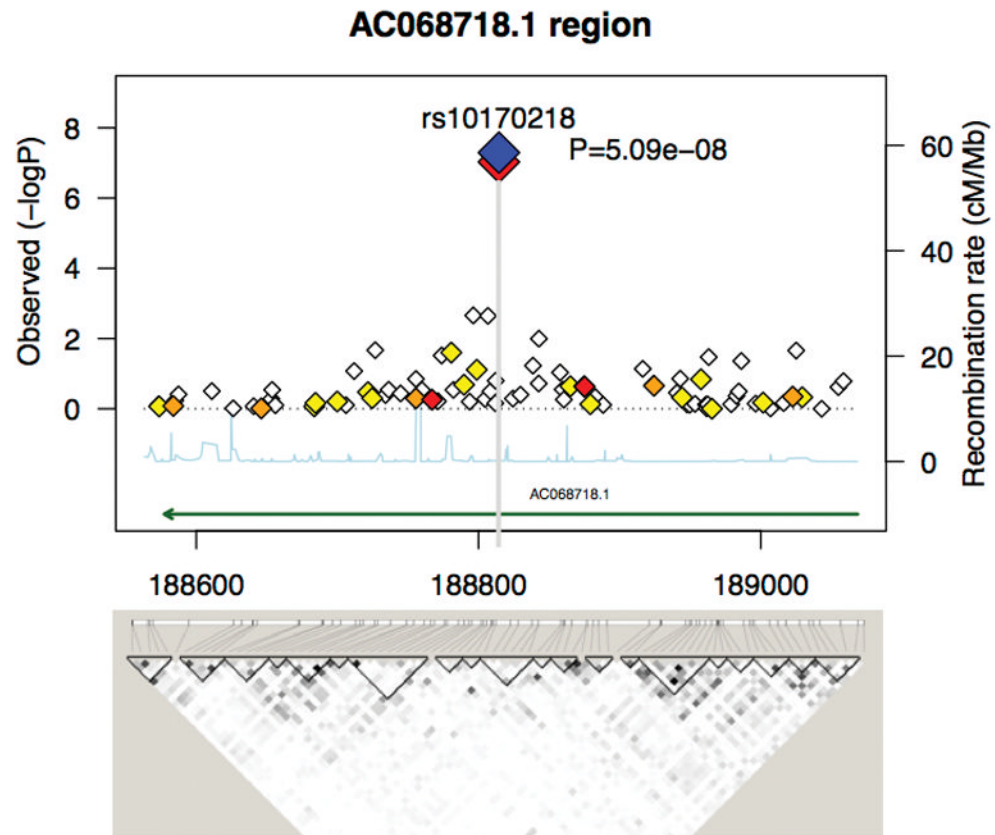


Figure 2.

Case-control association results and linkage disequilibrium (LD) structure at 2q32.1. Results are reported for the most significant SNP rs10170218 and SNPs genotyped across 800 Kb as part of the original GWAS in DNHS. The color of each diamond is based on r^2 (white for $r^2=0$, red for $r^2>0.8$) with SNP rs10170218. The recombination rates are based on YRI HapMap data and are shown in light grey along the x axis. The green arrows indicate gene location. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

Table 1

Demographic characteristics of GWAS subjects

Variable ^b	PTSD Status		Group Comparison P value ^a
	Cases n (%) / Mean (SD)	Controls n (%) / Mean (SD)	
Age	52.2 (13.5)	54.3 (15.9)	0.2325
Race			0.4080
White	10 (10.6%)	35 (11.0%)	.
Black	81 (86.2%)	261 (82.1%)	.
Other	3 (3.2%)	22 (6.9%)	.
Ethnicity			1.0000
not Hispanic	93 (98.9%)	313 (98.4%)	.
Hispanic	1 (1.1%)	5 (1.6%)	.
Education			0.9289
Less than high school	12 (12.8%)	44 (13.8%)	.
High school grad/GED	25 (26.6%)	80 (25.1%)	.
some college	37 (39.4%)	114 (35.7%)	.
college degree	14 (14.9%)	58 (18.2%)	.
grad degree	6 (6.4%)	23 (7.2%)	.
Marital Status			0.3417
married	22 (23.4%)	65 (20.4%)	.
divorced or separated	33 (35.1%)	88 (27.6%)	.
widowed	14 (14.9%)	58 (18.2%)	.
never married	25 (26.6%)	108 (33.9%)	.
Number of traumatic events	9.0 (4.5)	5.5 (3.8)	<.0001

^aP-values for continuous variables are from Wilcoxon exact test, while Monte Carlo estimate for Pearson chi-square exact test for categorical variables

^bCounts may not add up to 449 due to missing values from some variables

Table 2

Loci with strongest evidence of association (p -value $< 1 \times 10^{-5}$) with PTSD in the DNHS.

Chr	SNP	Coordinate annotated	Alleles (Minor/Major)	Minor Allele Frequency	Beta	p-value	Odds Ratio (95% CI)	Gene Annotation			Variant Annotation	
								Gene Symbol	Gene Type	Gene Status	Variant type	Variant distance (bp) to gene
2	rs10170218	188,814,444	C/A	0.22	5.45	5.10E-08	2.89 (1.97–4.23)	AC068718.1	lincRNA	Novel	intronic	0
6	rs1611133	29,809,382	A/G	0.17	4.47	1.18E-06	3.56 (2.04–6.22)	HLA-G3	Protein coding	Known	intergenic	10,480
22	rs2413187	33,864,817	G/A	0.21	4.86	3.71E-06	2.78 (1.84–4.19)	LARGE	Protein coding	Known	intronic	0
18	rs10502692	36,040,352	A/G	0.22	-4.53	4.46E-06	0.35 (0.22–0.55)	Intergenic	NA	NA	intergenic	NA
12	rs7302717	95,119,816	A/G	0.39	-4.54	5.72E-06	0.41 (0.28–0.60)	TMCC3	Protein coding	Known	intergenic	-75,478
7	rs11767398	112,130,009	C/A	0.29	4.59	5.95E-06	2.54 (1.71–3.78)	C7orf53	Protein coding	Known	3 UTR	0
5	rs4701170	178,897,590	A/G	0.09	4.63	7.92E-06	2.41 (1.66–3.51)	RP11-798K23.1	lincRNA	Putative	intergenic	14,494