The ankyrin-3 gene is associated with posttraumatic stress disorder and externalizing comorbidity

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Summary

Background: The ankyrin 3 gene (*ANK3*) produces the ankyrin G protein that plays an integral role in regulating neuronal activity. Previous studies have linked *ANK3* to bipolar disorder and schizophrenia. A recent mouse study suggests that *ANK3* may regulate behavioral disinhibition and stress reactivity. This led us to hypothesize that *ANK3* might also be associated with stress-related psychopathology such as posttraumatic stress disorder (PTSD), as well as disorders of the externalizing spectrum such as antisocial personality disorder and substance-related disorders that are etiologically linked to impulsivity and temperamental disinhibition.

Methods: We examined the possibility of association between ANK3 SNPs and both PTSD and externalizing (defined by a factor score representing a composite of adult antisociality and substance abuse) in a cohort of white non-Hispanic combat veterans and their intimate partners

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(*n* = 554). Initially, we focused on rs9804190—a SNP previously reported to be associated with bipolar disorder, schizophrenia, and ankyrin G expression in brain. Then we examined 358 additional ANK3 SNPs utilizing a multiple-testing correction.

Results: rs9804190 was associated with both externalizing and PTSD (*p* = 0.028 and *p* = 0.042 respectively). Analysis of other *ANK3* SNPs identified several that were more strongly associated with either trait. The most significant association with externalizing was observed at rs1049862 (*p* = 0.00040, $p_{corrected}$ = 0.60). The most significant association with PTSD (*p* = 0.00060, $p_{corrected}$ = 0.045) was found with three SNPs in complete linkage disequilibrium (LD)—rs28932171, rs11599164, and rs17208576.

Conclusions: These findings support a role of *ANK3* in risk of stress-related and externalizing disorders, beyond its previous associations with bipolar disorder and schizophrenia.

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1. Introduction

The ankyrin 3 gene (*ANK3*) encodes the ankyrin G protein, a key scaffolding protein that anchors integral membrane proteins to the spectrin/actin cytoskeleton. Amongst other functions, ankyrin G has fundamental roles in neurotransmission by clustering sodium and potassium channels at the neuron axon initial segments and Nodes of Ranvier (Zhou et al., 1998) that initiate and propagate action potentials, as well as in formation and maintenance of neuron axodendritic structure, and regulating neuronal excitability (Leussis et al., 2012b). In recent years, *ANK3* has attracted the interest of psychiatric geneticists after being associated with bipolar disorder in multiple genome-wide association studies (GWAS), GWAS meta-analyses, and targeted replication studies. To date, the SNPs most reliably associated with bipolar disorder are rs9804190 at the 3′ end of *ANK3* (Schulze et al., 2009; Sklar et al., 2011; Tesli et al., 2011) and a series of 5′ SNPs, including rs10994336, rs10994397, and rs1938526 (Ferreira et al., 2008; Schulze et al., 2009; Scott et al., 2009; Sklar et al., 2011; Tesli et al., 2011) that are in linkage disequilibrium (LD) and represent a single association signal (Sklar et al., 2011). The observed associations with rs9804190 in the 3′ region and the 5′ region SNPs appear to represent two independent risk loci (Schulze et al., 2009). In addition to the bipolar disorder associations, both rs9804190 (3′ region) and rs1938526 (5′ region) have been associated with altered expression of *ANK3* transcripts in human brain (Roussos et al., 2012; Rueckert et al., 2012). Specifically, the rs9804190 major allele C has been associated with lower ankyrin G expression and increased risk of bipolar disorder (Baum et al., 2008; Roussos et al., 2012; Schulze et al., 2009; Sklar et al., 2011), while the rs1938526 minor allele C was associated with lower expression of specific ankyrin G transcripts and increased risk of bipolar disorder (Ferreira et al., 2008; Rueckert et al., 2012; Smith et al., 2009). *ANK3* has also been associated with schizophrenia, anhedonia, behavioral activation, and novelty-seeking (Athanasiu et al., 2010; Ripke et al., 2011; Roussos et al., 2011, 2012), suggesting a broader role in psychiatric illness and traits.

Other evidence relevant to *ANK3*'s possible involvement in psychiatric traits comes from a recent mouse model study by Leussis et al. (2012a) that examined the behavioral effects of perturbed expression of the mouse ortholog of the *ANK3* gene (*Ank3*). Mice with 40% lower expression of ankyrin G brain-specific isoforms exhibited significantly altered behaviors in explicit domains. Specifically, compared to wild-type *Ank3*+/+ mice with normal ankyrin G expression, *Ank3*+/− mice with reduced expression rapidly entered aversive environments in several tasks that present a conflict between exploration motivated by natural rewards (e.g., finding food, mates) and avoidance behavior in the presence of threat cues, and they also displayed increased consumption of a food reward in a safe environment. One possible interpretation of these findings is an increase in behavioral disinhibition associated with *Ank3* suppression in brain. Interestingly, when exposed to chronic stress, the behavioral changes of *Ank3*+/− mice were reversed and depression-related features emerged (increased immobility in the forced swim test). In contrast, wild-type *Ank3*+/+ mice were unaffected by the stress exposure, suggesting that reduced expression of ankyrin G in brain is associated with heightened reactivity to stressors. Persistent elevation in plasma levels of the stress hormone corticosterone at baseline and after stress exposure in *Ank3*+/− mice provided further evidence for a link between ankyrin G and the stress response.

These findings led us to hypothesize that *ANK3* expression might be linked to the prototypical human stress-response disorder, posttraumatic stress disorder (PTSD), which is defined, in part, by anhedonia and diminished interest following traumatic stress exposure. Additionally, we decided to investigate the relationship of ANK3 to disorders of the externalizing spectrum (e.g., antisocial personality and substance-related disorders) that have been linked to impulsivity, excessive reward-seeking, deficits in the ability to inhibit pre-potent behavioral responses and problems mediating conflicting action tendencies—traits that share similarities with behaviors observed in *Ank3*+/− mice (Krueger and South, 2009; Patrick et al., in press). We focused our analyses of externalizing on a latent variable representing a composite of disorders in the externalizing spectrum on the basis of prior evidence suggesting that this representation yields higher heritability estimates than any of the individual disorders within that spectrum, presumably because of the closer correspondence between the latent phenotype and its biological substrates (Wolf et al., 2010). Furthermore, numerous studies have found a high degree of comorbidity between PTSD and externalizing disorders. There is also evidence for both an externalizing subtype of PTSD (Miller et al., 2004) and a common genetic factor underlying PTSD and externalizing (Wolf et al., 2010). Thus, the primary aim of this study was to examine possible associations between *ANK3* SNPs—and in particular, expression-associated SNPs in ANK3—and both PTSD and externalizing psychopathology

using a sample of U.S. military veterans and their intimate partners with a high prevalence of PTSD.

2. Materials and methods

2.1. Sample

Sample ascertainment, characterization, genotyping, and data cleaning methods have been described elsewhere in detail (Logue et al., 2012) and diagrammed in Supplementary Fig. 1. Briefly, we examined a subset of a cohort of military veterans and their intimate partners ascertained from two studies performed at U.S. Department of Veterans Affairs (VA) medical centers. Both studies were reviewed and approved by the appropriate institutional review boards. In this study, we focused on the subset of this cohort determined to be of white non-Hispanic ancestry (*n* = 554) based on STRUCTURE (Falush et al., 2003; Pritchard et al., 2000) analysis. Within these study participants, there were 339 males and 215 females. There were 334 participants in couple pairs (60%, 167 couples). In this sample, 15 PTSD cases met criteria for a bipolar 1 diagnosis and two met for a bipolar 2 diagnosis. The presence of schizophrenia was not assessed.

The subsample of participants included in analyses of the externalizing phenotype consisted of 531 white non-Hispanic veterans and their intimate partners (60.3% male) with a mean age of 51.68 years (range: 21—75). Participants were included in this analysis whether or not they had experienced a trauma. The subsample of participants included in analyses of lifetime PTSD diagnosis were 491 white non-Hispanic veterans and their intimate partners (65% men, mean age: 51.93, range: 21—75), all of whom had been exposed to a *DSM-IV* defined PTSD Criterion A traumatic event (*n* = 295 cases and *n* = 196 controls). The vast majority of the participants (90.7%) were included in both subsamples.

All study participants were assessed using the Clinician-Administered PTSD Scale (CAPS) (Blake et al., 1990, 1995)—a structured diagnostic interview that assesses the frequency and severity of the 17 *DSM-IV* PTSD symptoms, along with associated features of the disorder, and degree of functional impairment. Lifetime PTSD diagnosis inter-rater reliability was excellent (kappa = 0.87).

Externalizing factor scores were computed from dimensional symptom summary scores on the lifetime alcohol abuse and dependence, cannabis abuse and dependence, cocaine abuse and dependence, and adult anti-social personality disorder modules of the Structured Clinical Interview for *DSM-IV* disorders (First et al., 1994) and the SCID-II (First et al., 1995). To obtain dimensional scores for each SCID-assessed diagnosis, we administered all symptoms within a module and anchored them temporally to a consistent time period to ensure that the rated symptoms were defined as co-occuring. In a subset of participants, adult antisociality was assessed using the International Personality Disorders Exam (IPDE) (Loranger, 1999). Overlapping items from the SCID and IPDE measures of antisocial personality disorder were then standardized and combined to form a single adult antisociality symptom score for analysis. Summary scores on adult antisociality, alcohol abuse/dependence, cannabis abuse/dependence, and cocaine abuse/dependence were then sub-

mitted to a confirmatory factor analysis which specified that a latent externalizing factor underlied the variance and covariance of the symptom summary scores for these variables. This model fit the data well per fit indices and all indicators loaded significantly on the latent variable (details available upon request). Dimensional factor scores on the latent externalizing variable were then extracted and used in the genetic analyses.

2.2. Genotyping

Genotyping was performed using the Illumina (San Diego, CA, USA) HumanOmni2.5-8 array containing approximately 2.5 million markers and scanned using an Illumina HiScan System. Samples with a call rate of <95% or whose sex differed from the sex determined by X-chromosome homozygosity were excluded from the analysis. SNPs with a call rate of <95% were excluded from analysis. None of the *ANK3* SNPs had Hardy—Weinberg equilibrium (HWE) test *p*-values <0.00014—the Bonferroni-corrected significance threshold for the 358 SNPs examined within *ANK3*. None of the SNPs reported in Table 1 had HWE test *p*-values <0.01.

2.3. Genetic association analyses

We performed a two-stage analysis that initially focused on the 3′ SNP rs9804190 based on its prior association with both bipolar disorder and ankyrin G expression (Baum et al., 2008; Roussos et al., 2012; Schulze et al., 2009; Sklar et al., 2011), followed by examining each of the 358 genotyped SNPs with MAF > 5% within 5 kb of the chromosome 10 region encompassing all ankyrin G isoforms (NCBI nucleotide ID NM_001204403 from chr10: 61,786,056—62,493,284), including the brain-specific isoforms (Zhou et al., 1998) of particular interest based on the mouse model studies (Leussis et al., 2012a) (NM_020987.3 from chr10: 61,786,056—62,149,634). A 5′ region SNP that has also been associated with both bipolar disorder and ankyrin G expression in the brain, rs1938526 (Ferreira et al., 2008; Rueckert et al., 2012; Smith et al., 2009), was not genotyped in this cohort and therefore not analyzed. SNPs rs10994336 and rs10994397 in the 5′ region that had previously associated with bipolar disorder but not ankyrin G expression were tested in the second stage (Ferreira et al., 2008; Schulze et al., 2009; Scott et al., 2009; Sklar et al., 2011; Tesli et al., 2011). Although we started with the prior hypothesis that the association between rs9804190 would be in the same direction as the observed association with bipolar disorder (the major C allele conferring risk), we report the two-sided association test results for rs9804190 so that the *p*-values are comparable to those reported for other *ANK3* SNPs for which we did not have a strong prior hypothesis.

Association between lifetime PTSD diagnosis and *ANK3* SNP genotypes was assessed using Fisher's Exact test of association as computed by PLINK (v. 1.07, October 2009) (Purcell et al., 2007). A principal components (PC) analysis of 10,000 randomly chosen markers performed in EIGENSTRAT (Price et al., 2006) indicated no PTSD- or externalizing-associated population substructure (*p* > 0.05 for each of the first 10 PC), and hence PC were not included in any models described below. Association between externalizing

Table 1 Association results for rs9804190 and *ANK3* SNPs with nominal $p < 0.01$ (in bold) for either externalizing or post-traumatic stress disorder (PTSD). Statistically significant p in either stage 1 (uncorrected for SNP rs9804190) or stage 2 ($p_{corrected}$ for all other SNPs) are underlined.

| SNP information | | | | Externalizing results | | | PTSD results | | | | |
#	SNP	BP	Annotation	MA	β	p	$p_{corrected}$	MAF_{Cases}	$MAF_{Controls}$	OR	p	$p_{corrected}$
1	rs1049862	61,789,272	3′ UTR	G	−0.45	**0.00040**	0.060	0.43	0.47	0.85	0.23	1
2	rs12357206	61,790,383	Intron	G	−0.44	**0.00060**	0.064	0.43	0.47	0.85	0.24	1
3	rs10821659	61,793,424	Intron	A	−0.42	**0.0018**	0.16	0.35	0.37	0.93	0.64	1
4	rs11813307	61,793,520	Intron	A	−0.44	**0.00080**	0.085	0.42	0.47	0.82	0.15	1
5	rs72818458	61,799,888	Intron	A	−0.33	0.12	1	0.06	0.13	0.47	**0.0020**	0.11
6	rs28932171	61,831,290	Missense[a]	G	−0.25	0.21	1	0.08	0.16	0.48	**0.00060**	<u>0.045</u>
7	rs11599164	61,831,984	Intron	A	−0.25	0.21	1	0.08	0.16	0.48	**0.00060**	<u>0.045</u>
8	rs17208576	61,834,573	cds-synon	A	−0.25	0.21	1	0.08	0.16	0.48	**0.00060**	<u>0.045</u>
9	rs9804190	61,839,831	Intron	T	−0.33	<u>0.028</u>	0.90	0.20	0.26	0.71	<u>0.042</u>	0.93
10	rs7917800	61,840,167	Intron	A	−0.20	0.30	1	0.09	0.16	0.54	**0.0046**	0.22
11	rs7098848	61,841,144	Intron	G	−0.13	0.50	1	0.09	0.15	0.53	**0.0028**	0.20
12	rs11599447	61,869,059	Intron	G	−0.10	0.70	1	0.04	0.08	0.44	**0.0054**	0.34

BP is base-pair position on chromosome 10 based on the hg19 human genome assembly. Annotation from dbSNP v. 135. MA is minor allele, β is the coefficient of the SNP dosage in an additive model of association with externalizing. p and $p_{corrected}$ represent the nominal p-value, and multiple testing corrected p-values. MAF_{cases} and $MAF_{controls}$ represent the observed minor allele frequency in those with and without PTSD, respectively. OR is the estimated odds ratio for the minor allele.

[a] The SNP rs28932171 is an I3117V variant predicted to be benign according to Polyphen2 (http://genetics.bwh.harvard.edu/pph2/).

and *ANK3* SNPs was examined using a regression model including sex and the SNP coded additively as predictors using PLINK's- -linear option. The significance of the association (for both Fisher's test and the regression model) was determined by permutation based on 5000 replicates. Unless otherwise noted, all p-values listed are uncorrected. The gene-level (358 SNP) multiple-testing corrected p-values ($p_{corrected}$) were determined using PLINK's Max(T) procedure. Linkage disequilibrium plots were generated using Haploview (Barrett et al., 2005). Haplotype frequency estimation and haplotype-association analysis was performed using PLINK. The possibility of bias due to non-independence within couple pairs was explored using a Generalized Estimating Equation (GEE) (Liang and Zeger, 1986; Zeger and Liang, 1986) analysis as implemented in the GEE library in the R statistical computing language (R Development Core Team, 2008). Plots were generated using R. Functionality of one missense mutation (presented in the footnote to Table 1) was examined using Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) (Adzhubei et al., 2010). All genomic positions are based on the hg19 (February 2009) human genome assembly.

3. Results

3.1. SNP analyses

To limit multiple testing, we initially focused our attention on the bipolar-associated SNP rs9804190, and found it to be associated with both PTSD and externalizing (Table 1). For externalizing, $\beta = -0.33$ (where β represents the average change per minor T allele) and $p = 0.028$. In the analysis of PTSD, the estimated odds ratio (OR) for the minor T allele was 0.71, and $p = 0.042$.

While association with rs9804190 is sufficient in itself to demonstrate a role of ANK3 in PTSD and externalizing, we expanded our search to the full set of SNPs spanning the *ANK3*

locus, and found several that were more significantly associated with externalizing or PTSD prior to multiple testing correction. Fig. 1 displays association results of all examined *ANK3* SNPs. Table 1 summarizes the results of those SNPs with nominal (uncorrected) p-values of <0.01 for either externalizing or PTSD. In brief, 4 SNPs (Table 1, SNPs 1—4) were associated with externalizing at the $p < 0.01$ level (from
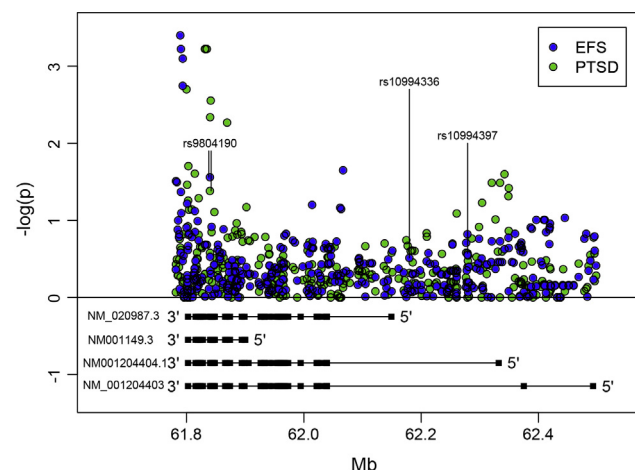

Figure 1 Association results for the externalizing factor score (EFS; blue circles) and for posttraumatic stress disorder (PTSD; green circles), and corresponding positions of the brain-specific (NM_020987.3) and other *ANK3* isoforms. The 3′ SNP rs9804190 was tested in the first stage based on prior evidence for association with both bipolar disorder and ankyrin G expression in brain. SNPs spanning the ANK3 locus encompassing all isoforms were tested in the second stage, including rs10994336 and rs10994397 previously associated with bipolar disorder. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

chr10: 61,789,272 to 61,793,520 bp), just upstream and leading into the 3′ UTR (as *ANK3* is coded on the reverse strand) (Fig. 1). The most significant association observed in this region occurred at rs1049862 in the 3′ UTR ($p = 0.00040$). The simulation-based corrected p-value narrowly missed significance when the 358 SNPs examined within the gene were simultaneously considered ($p_{corrected} = 0.060$). None of the SNPs that were associated with externalizing at the $p < 0.01$ level were associated with PTSD (all $p > 0.15$). When we examined *ANK3* for association with PTSD, the most significant result was observed at three SNPs in perfect LD, all with $p = 0.00060$ (Table 1, SNPs 6—8). These SNPs remained significant after multiple-testing correction ($p_{corrected} = 0.045$). Other SNPs with $p < 0.01$ were observed over a 69 kb interval at chr10:61,799,888—61,869,059 which includes the location of rs9804190. The bipolar disorder-associated SNP rs9804190 was the only SNP in *ANK3* that was nominally significantly associated ($p < 0.05$) with both PTSD and externalizing. No SNPs with $p < 0.01$ were observed outside of the boundaries of the

brain-specific isoforms for either trait. This included the two genotyped bipolar disorder risk SNPs in the 5′ region, rs10994336 and rs10994397 (externalizing $p = 0.31$ and $p = 0.28$ respectively, PTSD $p = 0.48$ and $p = 0.27$ respectively).

Omitting the subjects with bipolar 1 and 2 from the analysis did not change the significance or effect size estimates for rs9804190 or the peak SNPs for either PTSD or externalizing (Supplementary Table 1). Additionally, as 60% of our data occur in couple pairs, we explored whether there might be non-independence of either PTSD or externalizing within couples that could bias results. We found no evidence that PTSD was correlated within couples (estimated OR for lifetime PTSD diagnosis = 0.63, Fisher's exact test $p = 0.24$). However, externalizing was moderately correlated within couple pairs ($r = 0.39$, $p = 2.97 \times 10^{-7}$). Therefore, we additionally examined externalizing using GEE models, explicitly allowing for correlation within couple pairs. GEE results for externalizing-associated SNPS (Table 1, SNPs 1—4) were very similar to those obtained without adjustment for couple
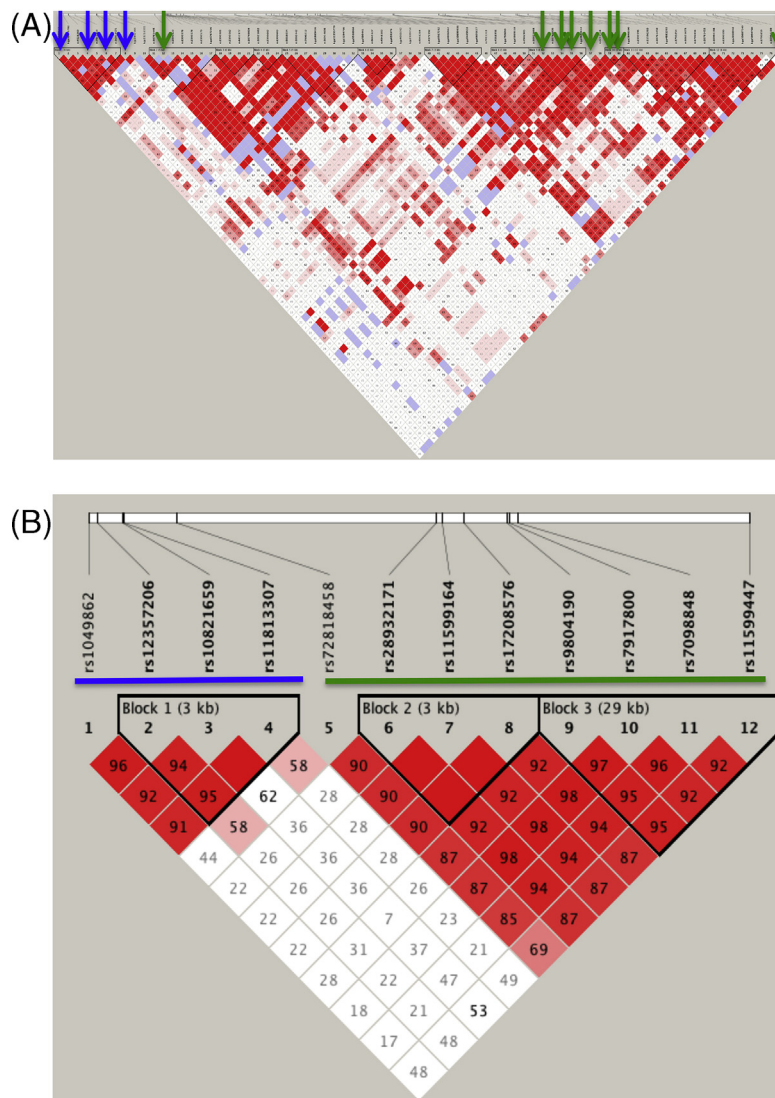

Figure 2 Linkage disequilibrium in the associated region of *ANK3* (from chr 10: 61,789,272 to 61,869,059). Plots contain either (A) all SNPs or (B) only PTSD and externalizing-associated SNPs presented in Table 1. Blue line/arrows indicate externalizing-associated SNPs (Table 1, SNPs 1—4). Green line/arrows indicate PTSD-associated SNPs including rs9804190 (Table 1, SNPs 5—12). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

status (β = -0.39 to -0.43, p = 0.0002 to 0.0006). Hence, it does not appear that the strength of the observed associations was inflated by dependence between subjects.

3.2. Haplotype analyses

Apart from rs9804190, the externalizing- and PTSD-associated regions of *ANK3* appeared to be distinct as, despite their close proximity, none of the PTSD-associated SNPs were nominally associated with externalizing or vice versa. To assess whether these signals were, in fact, independent, we examined the LD structure across the regions. We found moderate-to weak LD across the region, with some pockets of long range LD within the region of PTSD-associated SNPs, indicated by clusters of high LD (red) within an otherwise low LD (white) region (Fig. 2A). When we restricted our attention to the SNPs in Table 1 (Fig. 2B), we found that the associated SNPs clearly fell into two clusters of LD, the externalizing-associated SNPs (SNPs 1—4), and the PTSD-associated SNPs and rs9804190 (SNPs 5—12). We then explored reasons for the observed long range LD within the region containing the PTSD-associated SNPs. When we estimated the haplotype frequency, it became clear that the PTSD-associated SNPs tag a single haplotype. Starting with the 3 SNPs in perfect LD, and adding adjacent SNPs that either increased the association or did not break down association, we identified a core PTSD-associated haplotype spanning 41 kb—from rs72818458 to rs7098848 (chr10: 61,799,888–61,841,144). In particular, the AGAAAAG haplotype of SNPs 5—11 (Table 1) occurred at an estimated 5.1% frequency in cases and 11% in controls (7.4% frequency overall) and was more strongly associated with protection from PTSD than any individual SNP (OR = 0.426, p = 0.00042). The addition of other intervening SNPs to the haplotype did little to change the frequency of the haplotype defined by SNPs 5—11 or alter the evidence of association with PTSD. This reinforces the notion that these SNPs tag a single ancestral haplotype that is likely to harbor a protective allele. Additionally, when 79 subjects predicted to have the protective haplotype with probability $\geq$ 50% were removed from the analysis, SNPs 5—12 in Table 1 were no longer associated with PTSD (all p > .50, OR between 0.8 and 1.1 based on an analysis of 267 cases and 154 controls), suggesting the observed association is driven by this haplotype. When we analyzed this haplotype as a predictor of externalizing, we found marginally significant evidence of association (β = -0.5716, p = 0.022).

We also examined haplotypes consisting of the four externalizing-associated SNPs (Table 1, SNPs 1—4). We found that the haplotype formed by the four SNPs was more significantly associated with externalizing than any individual SNP (β = -0.5063 and p = 0.00025 for the GGAA haplotype). This haplotype was not associated with PTSD (p = 0.60).

4. Discussion

This study examined the relationship of *ANK3*, a gene previously associated with risk of bipolar disorder, schizophrenia, and other psychiatric-related traits, to PTSD and a variable reflecting the common externalizing factor underlying adult antisociality, as well as substance abuse/dependence symptoms. We began by focusing our attention on rs9804190 because it has been associated previously with both bipolar disorder and ankyrin G expression in human brain (Baum et al., 2008; Roussos et al., 2012; Schulze et al., 2009; Sklar et al., 2011) and was available in the genome-wide SNP data from our sample. Specifically, in a sample of schizophrenia postmortem brains, Roussos et al. (2012) found lower ankyrin G expression in superior temporal gyrus was associated with the rs9804190 CC genotype (C is the major allele associated with bipolar disorder risk; Schulze et al., 2009). Further, the rs9804190 C allele was associated with the presence of schizophrenia in the postmortem sample (p = 0.03 in n = 208 cases and n = 64 controls) and with performance on schizophrenia-related tests of cognition and memory in healthy (without schizophrenia) subjects. The authors suggested that variants in *ANK3* affecting ankyrin G expression represent a causal mechanism by which *ANK3* contributes to schizophrenia risk.

This previously established association between rs9804190 and decreased ankyrin G expression led us to hypothesize that facets of psychopathology in humans corresponding to the behavioral phenotype observed in mice with reduced expression of Ank3 brain-specific isoforms (Leussis et al., 2012a) might be associated with rs9804190 genotype. We obtained nominally significant evidence of association between this expression-associated SNP and two characteristics implicated by the mouse study: externalizing and PTSD (p = 0.028 and p = 0.042). The direction of association was as expected based on the prior association between rs9804190 and bipolar disorder, schizophrenia, and ankyrin G expression, and the behavioral changes in Ank3 mice. That is, the minor T allele, which was associated with higher ankyrin G expression and lower risk of bipolar disorder and schizophrenia, was here associated with reduced frequency of PTSD and less externalizing psychopathology. Conversely, the rs9804190 C allele linked to lower ankyrin G expression was associated with higher frequency of PTSD and a greater degree of externalizing behaviors, which corresponds to the heightened reactivity to stressors and increased behavioral disinhibition observed in mice with reduced ankyrin G expression.

The report of reduced *ANK3* mRNA expression in the superior temporal gyrus of subjects with the rs9804190 CC genotype (Roussos et al., 2012) is interesting in light of the role of *ANK3* in neuronal activity. Loss of Ank3 in mice disrupts action potential firing (Zhou et al., 1998), therefore lower expression in the superior temporal gyrus may impair neuronal activity in this region. Although mainly responsible for processing sound and speech, the superior temporal gyrus has also been implicated in emotion processing, specifically emotion recognition in facial expressions and speech (Fruhholz et al., 2012; Park et al., 2010; Robins et al., 2009). A recent study found substantial differences in synchronous neural interactions in the right superior temporal gyrus between veterans with PTSD and resilient veterans, suggesting that modulation of neural networks by trauma, particularly in this region, may be a marker of resilience (James et al., 2013).

When we expanded our attention beyond rs9804190, analyses revealed four *ANK3* SNPs associated with externalizing, the most significant of which was rs1049862 (p = 0.00040). In addition, seven *ANK3* SNPs in LD with rs9804190 were associated with PTSD at the p < 0.01 level. The most significant of these was a cluster of three SNPs in complete LD. By examining patterns of LD between

PTSD-associated SNPs, we identified a haplotype containing the high-expressing rs9801490 T allele that was more significantly associated with a protective effect for PTSD than any single SNP. This haplotype was also nominally associated with externalizing, again with a protective effect. Omitting those with either bipolar 1 or 2 from our analysis did not change our results, hence we do not believe that the presence or absence of bipolar disorder is central to the increased risk of PTSD or externalizing associated with *ANK3* variants. It is more likely that ANK3 variants are not specific to any one disorder, as recent research has indicated that some of the common genetic risk variants (and one ANK3 variant in particular) may be shared across a broad class of psychological disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013).

Consistent with prior evidence of allelic heterogeneity in *ANK3* (Schulze et al., 2009), association was observed in two distinct regions—one more closely associated with externalizing, in the region just upstream and leading into the 3′ UTR, and a neighboring region more closely associated with PTSD. Even though these associations appear to be either PTSD- or externalizing-specific, apart from rs9804190, we caution against over-interpreting the specificity of association given our modest sample size. The observed β and OR were congruent for all associated SNPs (Table 1), with the minor alleles of each SNP having the same direction of effect for lower externalizing and lower risk of PTSD. Further, an analysis of association between externalizing and a core PTSD-associated haplotype was nominally significant. Moreover, while we have only identified associated SNPs in the 3′ region, further investigation of association of PTSD and externalizing to the 5′ region previously implicated in bipolar disorder and schizophrenia is necessary, since SNPs in this region may well demonstrate association in a larger sample. We can only conclude that the observed associations are consistent with allelic heterogeneity—although our results suggest allelic heterogeneity in two distinct 3′ regions, as opposed to the 3′ and 5′ regions detected by others.

There are additional caveats that need to be taken into account when interpreting this study. While our results are consistent with the existence of a protective haplotype defined in part by the T allele of rs9804190 that has been associated with high ankyrin G expression (Roussos et al., 2012), we do not have neuronal tissue from the subjects in this study with which to measure ankyrin G expression. While there is no reason to assume that expression changes based on rs9804190 genotype observed in Roussos et al. (Roussos et al., 2012) do not similarly apply to subjects in this study, we are unable to examine whether the identified protective haplotype is more closely associated with expression than rs9804190 itself. Second, we do not have any reason to believe that rs9804190 is itself causal. Sequencing and bioinformatics analysis will be necessary to identify the putatively causal variant. Another caveat is the apparent behavioral disinhibition and stress sensitivity observed in mice with reduced Ank3 expression (Leussis et al., 2012a), which motivated this study, requires further characterization at the level of behavior and neural circuits to better delineate the phenotype and connect it to human psychiatric behaviors. It will also be important to investigate the association between *ANK3* and behavioral disinhibition in bipolar disorder and schizophrenia, which has not yet been addressed. Other study limitations include the modest size of the cohort and absence of a replication sample.

This study design also has several notable strengths. The focus on genetic association analyses of two related psychiatric phenotypes suggested by findings from an animal model, and latent variable modeling of a broad class of psychopathology (externalizing), is novel. The study also featured a highly traumatized subject sample with a high base-rate of PTSD and other disorders, with diagnoses based on clinician-administered interviews, which is not common in the PTSD genetics field. Another strength is the dense coverage of SNPs spanning the ANK3 gene that enabled more comprehensive analyses than targeted gene association studies that examine only a few SNPs.

To conclude, our data reinforce the results of studies of mouse models and schizophrenia indicating that *ANK3* is not only a risk gene for bipolar disorder, as initially identified in patient GWAS. Rather, *ANK3* may be more broadly associated with a range of processes, including memory (Roussos et al., 2012), reactivity to stress, and impulse control. Identification of the genetic variants in *ANK3* that drive these associations is likely to yield valuable clues for the etiological basis of a range of psychiatric disorders and behavioral traits. Determining whether the different *ANK3* alleles that are implied by allelic heterogeneity observed here and in bipolar disorder genetic studies all share the same causal mechanism, whether it be altered AIS function, neuronal excitability, or other processes involving ankyrin G, may shed light on common neurobiological underpinnings of these disorders.

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Conflict of interest

The authors report no financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2013.04.013.

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