NPY MODERATES THE RELATION BETWEEN HURRICANE EXPOSURE AND GENERALIZED ANXIETY DISORDER IN AN EPIDEMIOLOGIC SAMPLE OF HURRICANE-EXPOSED ADULTS

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Background: Neuropeptide Y (NPY) has been found to be anxiolytic in animals and humans. A recent study found NPY expression to be inversely correlated with trait anxiety. We examined whether rs16147, a functional single nucleotide polymorphism in the promoter region of NPY, moderated the relationship between hurricane exposure and risk for generalized anxiety disorder (GAD) in an epidemiologic sample of adults living in areas affected by the 2004 Florida Hurricanes. Methods: Data from this study comes from 616 adults from the 2004 Florida Hurricanes study who returned buccal DNA samples via mail. Selection of participants occurred via random digit-dial procedures. Participants were interviewed via telephone about hurricane exposure and posthurricane GAD symptoms. The outcome measure was DSM-IV GAD diagnosis, assessed via structured interview. Results: Rs16147 in NPY was associated with increased risk of GAD diagnosis under conditions of high hurricane exposure (P < .01). This gene by environment interaction remained significant after adjustment for sex, ancestry (as determined by Bayesian clustering of genotypes), and age. Conclusions: NPY rs16147 modifies risk of postdisaster GAD under conditions of high stressor (hurricane) exposure. This is the first demonstration of gene–environment interaction for this locus. Depression and Anxiety 27:270–275, 2010.

Key words: generalized anxiety disorder; anxiety; NPY; SNP; association analysis; trauma

INTRODUCTION

Exaggerated and uncontrollable worry or tension about everyday events at a level more severe than warranted by the situation typifies generalized anxiety disorder (GAD), a common and sometimes debilitating condition with a lifetime prevalence of 5.7% and a

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12-month prevalence of 3.1%. Other symptoms of GAD include restlessness, being easily fatigued, having difficulty concentrating, irritability, muscle tension, and sleep disturbance (APA, 1994). Both genetic influences and environmental factors have been shown to be important in the etiology of GAD. A recent meta-analysis of twin studies estimated the heritability of GAD to be 31%. However, to date, progress has been slow in identifying specific genes that increase risk of GAD. Environmental factors associated with increased risk of GAD include natural disasters and stressful life events involving loss and danger. To date, there are no published studies that address gene-by-environment interaction for GAD. This study examines the interaction of a functional neuropeptide Y (NPY) variant and hurricane exposure in the etiology of GAD.

It has been shown that the risk for mood and anxiety disorders, including GAD, increases after exposure to natural disasters, such as hurricanes, in fact, in the present 2004 Florida Hurricanes data, hurricane exposure was a strong predictor of GAD. It is likely that this risk is mediated by the property damage, personal injury, and life threat that accompany such events. In addition to these environmental factors, growing evidence suggests that genetic factors also explain risk for mental health phenotypes postdisaster. In the 2004 Florida Hurricanes data, genetic factors have been identified that explain risk for GAD, and that interact with environmental factors to confer risk for posttraumatic stress disorder and major depressive disorder. With the exception of the present dataset, earlier genetic association studies of GAD have not included stress-exposed samples. Moffitt et al. (2005) proposed the exposed cohort design as an efficient way of testing genotype-environment interaction: “If a good candidate gene is available, such an exposed sample could be used to test the hypothesis that genotype-risk individuals develop psychopathology but genotype-controls do not.” Mounting data support the role of NPY as a candidate gene.

NPY, an endogenous peptide with anxiolytic properties that is released during times of stress, is primarily expressed in the amygdala and hippocampus. NPY has been demonstrated to have a synchronizing effect on EEG pattern that is strikingly similar to effects produced by benzodiazepines and barbiturates. This anxiolytic effect has been demonstrated in a variety of animal models, and NPY Y-1 receptors have been found to mediate this effect. Expression studies in rats indicate that acute stress down-regulates mRNA expression of NPY, whereas prolonged stress has the inverse effect (up-regulation of expression), which has been posited as a neuroadaptive response to cope with chronic stress. We showed previously that a different, less common, functional variant at NPY is associated to alcohol dependence and “suggestively” associated to PTSD.

Recent evidence in humans suggests that NPY expression modulates stress reactions and emotional responding. Specifically, low NPY expression has been associated with higher emotion-induced activation of the amygdala through a threat-related facial expression presentation model. The low NPY expression haplotype was also predictive of negative affect and subjective pain during a painful stressor task. Further, a NPY haplotype was inversely related to self-reported trait anxiety in a healthy control sample. Notably, rs16147, located in the promoter region of NPY, was found to account for a 30% decrease in expression (in vivo).

Given evidence of the anxiolytic effects of NPY, the demonstrated effects on expression, and the earlier report of possible association to PTSD, we hypothesized that rs16147, a single nucleotide polymorphism in the untranslated region of the NPY gene, would be related to GAD in an epidemiologic sample of hurricane-exposed older adults. An exploratory hypothesis, based on how NPY functions during acute vs. prolonged stress in animal models, was that the effects of NPY would be moderated by the level of hurricane exposure.

**MATERIALS AND METHODS**

**DATA COLLECTION AND SAMPLE**

This article focuses on 616 hurricane-exposed participants from the 2004 Florida Hurricane Study. These participants completed structured telephone interviews and returned saliva samples via US mail from which DNA was extracted that yielded valid genotype data for the rs16147 polymorphism. Detailed methodological descriptions regarding the sampling procedure for the Florida Hurricane Study are provided in earlier articles.

As approved by all relevant institutional review boards, all participants provided verbal consent, and they were mailed a letter containing details of their verbal consent that also included contact information for the principal investigator. Participants who completed the diagnostic interview and returned saliva samples were paid $20.

**ASSESSMENT PROCEDURE**

A probability sample of English- and Spanish-speaking adults from telephone households in 38 counties in Florida within six to nine months of the 2004 Florida hurricane season were interviewed via telephone between April 5 and June 12, 2005. Oversampling of older adults (ages 60 and older) was employed to address research questions specific to this age group. Sample selection and telephone interviewing was performed by a large, experienced survey research firm. Households within the sampling frame were located by random digital procedures. Respondents were randomly selected when multiple adults were present within a household. Highly structured assessments were performed by use of a structured interview using computer-assisted telephone interview (CATI) format. The CATI format uses a computer program that projects instructions and questions on a computer screen that are read verbatim by interviewers to participants over the telephone. Participants’ responses were recorded electronically by the interviewers with closed-ended response options. Supervisors randomly monitored interviews in progress, thereby, providing considerable quality control over data collection. Interviews averaged 26.5 min in length.
**DSM-IV criteria using yes/no response options. To obtain a diagnosis of GAD, participants must have reported excessive and poorly controlled anxiety and worry occurring more days than not for a period of 6–9 months (“since the hurricanes”), and they must have endorsed three of six hallmark GAD symptoms (restlessness, fatigue, concentration problems, irritability, tension, and sleep disturbance). High internal consistency was found for this scale in this sample among individuals screening into the module (Cronbach’s α = .85).

Two environmental risk variables, hurricane exposure and social support, were associated with GAD in this sample[30] and included as covariates. Five important indicators identified in earlier research on ancestry.

**RESULTS**

**PREVALENCE OF GAD AND NPY GENOTYPE**

Descriptive statistics for independent variables are provided in Table 1. Posthurricane GAD was prevalent in 41 participants (6.7%). NPY SNP rs16147 genotype frequencies were consistent with Harvey–Weinberg equilibrium expectations. Regarding genotype frequencies, 26.8% (n = 163) had “CC” genotype, 49.4% (n = 304) had “CT” genotype, and 23.9% (n = 147) had “TT” genotype. Self-reported race/ethnicity was not associated with the prevalence of GAD (χ²[3, n = 613] = 7.05, P = .07). However, self-reported race/ethnicity was associated with genotype frequencies (χ²[6, n = 613] = 17.04, P < .01); therefore, correction based on ancestry coefficients was used to ensure that population stratification was not a potential cause of false positive findings.

**GENE–ENVIRONMENT CORRELATION**

To examine if genotype was related to environmental exposure variables, chi-square analyses between rs16147 and these variables were conducted. NPY genotype was not related to hurricane exposure (χ²[1, n = 613] = 1.25, P = .26) or to social support (χ²[1, n = 614] = 0.54, P = .46).

**ASSOCIATION BETWEEN GAD AND NPY GENOTYPE**

The chi-squared linear-by-linear association test revealed no significant association between rs16147 and GAD (χ²[2, n = 616] = 0.41, P = .52). However, a significant association between the interaction of rs16147 and high hurricane exposure and GAD was found (χ²[1, n = 616] = 4.33, P < .05); no significant linear-by-linear interaction was found for rs16147 and social support (χ²[1, n = 615] = 0.54, P = .46), or for the three-way interaction χ²[1, n = 615] = 1.66, P = .20. To test whether the association between GAD and hurricane exposure remained significant after controlling for sex, age, ancestry, and the main effects of hurricane exposure and genotype, logistic and linear regression analyses were conducted (Tables 2 and 3, respectively), revealing the same pattern of results.
TABLE 1. Frequencies for independent variables (N = 616)

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 59 years</td>
<td>140</td>
<td>22.9</td>
</tr>
<tr>
<td>≥ 60 years</td>
<td>471</td>
<td>77.1</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>218</td>
<td>35.4</td>
</tr>
<tr>
<td>Female</td>
<td>398</td>
<td>64.6</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>556</td>
<td>90.7</td>
</tr>
<tr>
<td>African American</td>
<td>23</td>
<td>3.8</td>
</tr>
<tr>
<td>Hispanic</td>
<td>23</td>
<td>3.8</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td>High hurricane exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>337</td>
<td>54.7</td>
</tr>
<tr>
<td>No</td>
<td>279</td>
<td>45.3</td>
</tr>
<tr>
<td>Low social support</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>226</td>
<td>36.8</td>
</tr>
<tr>
<td>No</td>
<td>388</td>
<td>63.2</td>
</tr>
</tbody>
</table>

TABLE 2. Final logistic regression analysis of the association between NPY genotype and generalized anxiety disorder (GAD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>2.7</td>
<td>1.2–6.3</td>
<td>.02</td>
</tr>
<tr>
<td>Age less than 60 years</td>
<td>1.3</td>
<td>0.6–2.7</td>
<td>.56</td>
</tr>
<tr>
<td>Ancestral proportion score</td>
<td>0.4</td>
<td>0.04–2.8</td>
<td>.31</td>
</tr>
<tr>
<td>High hurricane exposure</td>
<td>0.6</td>
<td>0.2–1.7</td>
<td>.31</td>
</tr>
<tr>
<td>Low social support</td>
<td>1.9</td>
<td>1.0–3.6</td>
<td>.06</td>
</tr>
<tr>
<td>NPY rs16147</td>
<td>0.4</td>
<td>0.2–0.91</td>
<td>.03</td>
</tr>
<tr>
<td>NPY x Hurricane exposure</td>
<td>3.6</td>
<td>1.4–9.8</td>
<td>.01</td>
</tr>
</tbody>
</table>

Under conditions of high hurricane exposure, those with the “TT” genotype were 3.6 times more at risk for being diagnosed with GAD than were those with “CC” and low hurricane exposure (see Fig. 1).

When we stratified by level of hurricane exposure, we found that the “TT” genotype of rs16147 predicted decreased risk of GAD among individuals who had low hurricane exposure (OR = 0.42, 95% CI: 0.19–0.91, P = .03), but increased risk in those who experienced high hurricane exposure (OR = 1.52, 95% CI: 0.82–2.79, P = .18). We note that perhaps due to the loss of power in stratified analyses, only the effect of the NPY genotype in the low hurricane exposure group was statistically significant.

A fourth regression was conducted to also control for RGS2 (rs4606), as this SNP was earlier found in this sample to be related to GAD. Results that remained indicated that even when controlling for this genotype, the interaction between NPY and hurricane exposure remained significant in the prediction of GAD.

DISCUSSION

Our results suggest that the NPY rs16147 SNP and hurricane exposure interact to confer risk for GAD. Notably, no gene–environment correlation was found (rs16147 was unrelated to hurricane exposure and social support) and no main effect of this SNP was found without controlling for other variables, but a significant interaction between level of hurricane exposure and genotype was found. Stratified analyses revealed that for those with low hurricane exposure, the “TT” genotype was protective. This is consistent with earlier work which has found the “TT” genotype of rs16147 to have high promoter activity in vitro. For those with high hurricane exposure, this SNP in NPY operates in the opposite direction, in contrast to the results found by Zhou and colleagues. Although our and Zhou et al.’s findings point toward a relevant polymorphism for anxiety-related disorders in the promoter region of the NPY gene, the inconsistency with regard to the risk allele raises questions as to whether rs16147 is the causal variant or if this SNP is in linkage disequilibrium with another functional variant, and the direction of LD differs by population.
Other possibilities include that multiple testing, population stratification, or another confounding variable may explain the differences in observed risk allele. This warrants future research examining this polymorphism and others in the NPY gene, under a variety of stress conditions. Nonetheless, whereas genetic-by-environment interactions have been frequently reported for depression, this is the first demonstration of a GxE interaction for GAD.

NPY is relevant for study of stress-related pathology, as it has been demonstrated in animal[16] and human studies[21] to function as an anxiolytic. NPY expression is high in the amygdala and the hippocampus, areas of the limbic system that are involved in emotional responding. This SNP, rs16147, is of particular interest as it is located in the promoter region, and in a recent report this SNP accounted for the majority of variation in expression in vivo.[21] Given our findings of this SNP moderating risk for GAD under stress conditions, the relevance of this polymorphism in NPY for stress-related phenotypes is underscored, as this variant may help explain individual response to stress.

GAD is common following major stressors, such as natural disasters.[4,6,7] In fact, the 6-month prevalence of GAD in this sample is 6.7%, which is more than twice the 12-month prevalence in the general population (3.1%) and almost twice the lifetime prevalence (3.6%) among adults aged over 60.[2,3] highlighting the need for postdisaster studies of this disorder that identify genetic and environmental risk and resilience variables. A considerable methodological concern, however, is that by its very nature, GAD symptoms may decrease the “objectiveness” by which individuals report on their level of disaster exposure (e.g., those with higher anxiety may over-report disaster-exposure items). Nonetheless, this study and earlier articles from this data[9–12] demonstrate that this is a viable methodological approach for the detection of specific genetic variants and specific environmental conditions, which may increase risk for phenotypes that are prevalent poststressor. The prevalence of GAD (or other phenotypes) is likely to be higher in the months following a disaster, and statistical power may be increased. Research in this area is important, as it will inform poststressor identification of individuals at risk, and for poststressor service allocation.

Although this study used a novel methodological approach that has several advantages, there are also limitations. This cross-sectional data was collected six to nine months following hurricanes and, therefore, retrospective recall bias may exist. DNA samples were returned for less than one half of the sample. However, there were no significant differences between returners and nonreturners of saliva samples. We used a slightly modified version of the SCID-IV, administered by lay interviewers over the telephone rather than from in-person clinical interviews. However, there is substantial evidence supporting the reliability and validity of the SCID-IV[36–39] and the validity of this measure as a lay screener for GAD.[40,41] Moreover, the reliability of this measure in this study was high (.85). We also note that we are limited by having a relatively low number of GAD cases, and the study is limited in that we were unable to control for past psychiatric diagnoses. A further limitation is that we did not have any corroborating information on the disaster exposure questions, and those with anxiety may be more likely to positively endorse disaster exposure items. Although we view our work as exploratory and hypothesis generating, it should be noted that another possible limitation of this study is its lack of statistical control for multiple comparisons from previous articles.[9–12] If a conservative correction was made, such as a Bonferroni correction, the GxE interaction would remain significant but the main effect of NPY would not. We believe that the only way to demonstrate true associations is through replication, and perhaps this data may serve to stimulate replication attempts.

Despite these limitations, this is the first GxE study of GAD, of which we are aware, within a sample that was recently exposed to a significant stressor. This study offers the unique opportunity to examine genetic effects and GxE interactions in an acutely stress-exposed sample. Our findings in conjunction with data from animal models and an emerging literature on NPY and stress-related conditions in humans suggest the need for future studies of this gene that undertakes fine mapping of the promoter region, and are warranted to further our knowledge of its role in GAD and other anxiety disorders (e.g., PTSD, panic disorder).

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