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Investigating the role of early childhood abuse and HPA axis genes in suicide attempters with bipolar disorder

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Abstract

Objective—Genes and environment both play a major role in risk for attempted suicide, and environments harboring stressors, such as early childhood abuse, have been linked to suicidal behavior. Such environments also disrupt the Hypothalamic-Pituitary-Adrenal (HPA) axis pathway, which has been hypothesized to play a role in suicidal behavior. We investigated whether the risk for attempted suicide was attributable in part to the interaction between childhood physical and/or sexual abuse and genetic variation in nineteen genes ($\pm$ 5kb) integral to the HPA axis pathway.

Materials and Methods—Using the Genetic Association Information Network Bipolar Disorder (GAIN-BP) and Translational Genomics Research Institute (TGEN) cohorts we implemented PLINK's logistic regression-based “interaction” approach to search for evidence of an interaction between 235 genotyped HPA axis single nucleotide polymorphisms (SNPs) and early childhood abuse. Our study included 631 BP suicide attempters and 657 BP non-attempters with information regarding abuse.

Results—After correction for multiple testing, no significant interaction between the 235 HPA axis SNPs and early childhood abuse was found. Our strongest interaction was found with rs2664008 in the corticotropin releasing hormone receptor 1 (*CRHR1*) gene with a nominal interaction P-value of 1.22×10^{-2} and interaction odds ratio of 0.47.

Conclusion—Our findings suggest further work and larger sample sizes are required to elucidate the link between early childhood abuse and the HPA axis in suicidal behavior.

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Keywords

suicidal behavior; bipolar disorder; gene-by-environment; early childhood abuse

Introduction

Suicide is the tenth leading cause of death in the U.S. (Centers for Disease Control and Prevention, 2010) and approximately one million Americans attempt suicide each year (Crosby *et al.*, 2011). Compelling evidence from family, twin and adoption studies (Brent and Mann, 2005, Brent and Melhem, 2008, Brezo *et al.*, 2008a) suggest both a genetic and environmental component to suicidal behavior, which includes both completed and attempted suicide. This gives us cause to investigate not only the genetic variation found in individuals with suicidal behavior, but also interacting environmental factors that may increase the risk of this phenotype, such as a history of early childhood trauma (Brent and Mann, 2005, Enns *et al.*, 2006, Brodsky and Stanley, 2008).

The Hypothalamic-Pituitary-Adrenal (HPA) system, which controls the response to stressors, involves the interaction of numerous hormones, chaperones and receptors and genetic variation in any of these genes may interfere with the functioning of this pathway. Disruption of this system has previously been observed in suicidal behavior (Coryell and Schlessner, 1981, Nemeroff *et al.*, 1988, Kamali *et al.*, 2012) and in individuals with a history of childhood trauma (Heim *et al.*, 2008). A study by Roy *et al.* (2010) suggested that an interaction between genetic variation in *FKBP5*, a negative regulator of the glucocorticoid receptor (GR), and childhood trauma increased the risk of suicidal behavior. Another study noted that a significant interaction between a single nucleotide polymorphism (SNP) in *CRHR1* and childhood sexual abuse affected decision-making in suicide attempters (Guillaume *et al.*, 2013). Further HPA axis studies have identified a sex-specific link between suicidal behavior, *CRHR1*, and physical assault in childhood and adolescence (Ben-Efraim *et al.*, 2011).

The aim of this study was to investigate whether genetic variation in nineteen genes important to the HPA axis interacts with a history of early childhood physical and/or sexual abuse to increase the risk of suicidal behavior in 631 suicide attempters and 657 non-attempters.

Materials and Methods

This study utilized the Genetic Association Information Network Bipolar Disorder (GAIN-BP) and Translational Genomics Research Institute (TGEN) cohorts within our bipolar disorder (BP) attempted suicide genome-wide association study (Smith *et al.*, 2009, Smith *et al.*, 2011, Willour *et al.*, 2012). This provided genotype data from 982 BP suicide attempters and 1,143 BP non-attempters all of whom were unrelated and of European-American ancestry. Subjects were originally ascertained, assessed and consented as part of the NIMH Genetics Initiative for BP (see Supplementary Materials for details). Prior to enrollment, all participants signed IRB-approved written informed consent forms. Subjects were defined as

suicide attempters if they answered “yes” to the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger *et al.*, 1994) question O.1. “Have you ever tried to kill yourself?”

From this, 631 BP suicide attempters and 657 BP non-attempters provided information regarding early childhood abuse (as described in Supplementary Table 1). In total, 297 suicide attempters and 199 non-attempters had a history of physical and/or sexual abuse, as determined by answering the question: “Were you physically abused by any one [between the ages of 3 – 12]?” Respondents were allowed to include sexual abuse in their answers. The abuse question was taken from the Early Life Events Scale (ELES), developed by William Lawson and Elliot Gershon for the NIMH Genetics Initiative (unpublished). The rates of early childhood abuse based on this question were significantly higher ($P < 1 \times 10^{-5}$) in suicide attempters (47.07%) versus non-attempters (30.29%), which is consistent with expectation, indicating the validity of the question.

Both datasets were merged and quality control filters were applied to exclude individuals with missing data rates $\geq 5\%$ and exclude SNPs with a minor allele frequency (MAF) $< 1\%$, with missing data rates $\geq 5\%$ and Hardy-Weinberg equilibrium P-values of $< 1 \times 10^{-6}$.

Nineteen genes from the genome-wide dataset were chosen for study due to their integral role in the HPA axis pathway as determined by a literature search (see Table 1 and Supplementary Table 2). All genotyped SNPs in our merged and filtered dataset that were within 5kb of our chosen genes were considered in the analysis, leaving 235 variants to include in the study.

We implemented PLINK’s v1.07 (Purcell *et al.*, 2007; <http://pngu.mgh.harvard.edu/purcell/plink/>) logistic regression-based “interaction” approach to search for evidence of an interaction between 235 genotyped HPA axis SNPs and a history of early childhood abuse. SNP genotypes were coded assuming an additive model. We controlled for sex, study and the top three principal components (PCs) which allowed us to account for population stratification. The top PCs were determined using EIGENSTRAT in EIGENSOFT 3.0 (Price *et al.*, 2006) and a linkage disequilibrium pruned subset of the genome-wide SNPs. Age was not controlled for as it was not significantly different between suicide attempters and non-attempters with abuse data ($P=0.586$).

Results

We investigated whether the risk for attempted suicide was attributable in part to the interaction between a history of childhood physical and/or sexual abuse and genetic variation in the HPA axis pathway. To do this, we tested for interactions between genotypes at 235 SNPs in nineteen HPA axis pathway genes and a history of early childhood abuse in 631 suicide attempters and 657 non-attempters. A total of five SNPs had an interaction P-value < 0.05 (see Table 2 and Supplementary Table 3). However, none of the interactions were significant after correction for multiple testing using the conservative Bonferroni correction ($P < 2.13 \times 10^{-4}$). As shown in Table 2, the most significant interaction was found with rs2664008 in the first intron of the *CRHR1* gene with an interaction P-value of 1.22×10^{-2} and an interaction odds ratio of 0.47 (MAF in suicide attempters=0.083, MAF in non-

attempters=0.088). The second top SNP, rs6727239, located in the fifteenth intron of the *NCOA1* gene had an interaction P-value of 1.78×10^{-2} and an interaction odds ratio of 2.77 (MAF in suicide attempters=0.040, MAF in non-attempters=0.059).

Discussion

This study sought to identify an interaction between the HPA axis and a history of early childhood abuse in suicide attempters. While we did not detect any study-wide significant results, we did see a number of nominally significant ones. Our strongest association signal was identified in the *CRHR1* gene, one of the main G protein-coupled receptors of CRH, the stress hormone. Genetic studies investigating *CRHR1* and suicide have produced mixed results. In an African-American sample set, a variant in *CRHR1* specific to this ancestry showed evidence of association with suicidal behavior (Roy *et al.*, 2012). No significant associations between *CRHR1* and suicide attempt was found in two European studies (Murphy *et al.*, 2011, Leszczynska-Rodziewicz *et al.*, 2013) and one schizophrenia cohort (De Luca *et al.*, 2010).

CRHR1 studies that include both gene and environment have been just as complex. A family trio study stratifying by stress levels found both an association and linkage with suicide attempt in depressed males with low stress (Wasserman *et al.*, 2008). An expansion of this study linked female suicide attempters with a history of physical abuse (before the age of eighteen) and a genetic variant in *CRHR1* and male suicide attempters with a history of physical abuse (after the age of eighteen) and a genetic variant in *CRHR1* (Ben-Efraim *et al.*, 2011). However, other studies found no gene-by-environment interactions with *CRHR1* (Murphy *et al.*, 2011, Roy *et al.*, 2012). These studies illuminate the complexity of not only suicidal behavior, but also the specific environmental stressors that affect this phenotype.

Our second most significant result was found in *NCOA1*, a co-activator of nuclear receptors, including the GR. This regulator is highly expressed in the brain (Meijer *et al.*, 2000) and mouse knock-out studies have shown the necessity of *NCOA1* (also known as *SRC-1*) for HPA axis regulation (Winnay *et al.*, 2006). To the best of our knowledge this is the only study to test for an association between *NCOA1*, early childhood abuse and suicidal behavior.

FKBP5 is one of the most extensively studied HPA axis genes in relation to suicide. It is a component of the inactivating complex, which decreases cortisol binding and prevents GR translocation to the nucleus. Previous haplotype studies have found suggestive evidence of an association with *FKBP5* and suicidal behavior in European (Leszczynska-Rodziewicz *et al.*, 2014) and Japanese populations (Supriyanto *et al.*, 2011). Genetic studies also identified an association between *FKBP5* and suicidal events (which includes suicidal ideation and attempt) (Brent *et al.*, 2010). *FKBP5* studies that include gene and environment have also shown promise. Most notably, Roy *et al.* identified an interaction with *FKBP5*, childhood trauma and African-American suicide attempters (Roy *et al.*, 2010, Roy *et al.*, 2012).

Other genetic studies have investigated HPA axis related genes such as *CRH*, *CRHBP*, *CRHR2*, *MC2R* and *NR3C1* (De Luca *et al.*, 2007, Wasserman *et al.*, 2008, De Luca *et al.*,

2010, Roy *et al.*, 2012, Leszczynska-Rodziewicz *et al.*, 2013). Positive findings include *NR3C1* and *CRHBP* which showed an association with attempted suicide (De Luca *et al.*, 2007, De Luca *et al.*, 2010). Variants in *CRHBP* have also been found to interact with childhood trauma to increase the risk of suicidal behavior (Roy *et al.*, 2012). Finally, *CRHR2* was linked with severity of suicidal behavior in subjects with bipolar disorder (De Luca *et al.*, 2007).

There were certain limitations to our study. We had an estimated 80% power (calculated using Quanto v1.2.4; <http://biostats.usc.edu/Quanto.html>) to detect a locus with an interaction effect size ranging from 2.11 – 3.29 (depending on MAF) assuming a study-wide significance of 2.13×10^{-4} . While this effect size has been described in other gene-by-environment studies (Ben-Efraim *et al.*, 2011, Ben-Efraim *et al.*, 2013) it suggests that more samples may be needed to help determine if we have false negative results.

Another limitation was the broad definition of a history of early childhood abuse, which did not separate physical and sexual abuse. These samples were collected over a five year period at eleven sites using the Early Life Events Scale (William Lawson and Elliot Gershon; unpublished) to assess childhood trauma. Statistical testing of our abuse data shows that a history of early childhood abuse is significantly associated with attempted suicide ($P < 1 \times 10^{-5}$). The effect of child abuse on suicidal behavior has been shown to vary depending on the type of abuse (Brodsky and Stanley, 2008) and our study suggests that future investigations integrating a history of early childhood abuse data should separate physical and sexual abuse when possible or quantify abuse as a quantitative trait, as others have noted (Brezo *et al.*, 2008b).

Other factors that may contribute to the differing results found in this study include sex and age. Some childhood trauma studies have indicated a sex-specific component to the interaction between physical and/or sexual abuse and suicidal behavior (Wasserman *et al.*, 2009, Ben-Efraim *et al.*, 2011), but we did not examine this due to sample size limitations. Our age range of between 3–12 years old does not match other more commonly used questionnaires, such as the Childhood Trauma Questionnaire (CTQ), where teenage years are taken into consideration (Bernstein, 1998).

A role for epigenetics in the relationship between HPA axis and suicidal behavior has been suggested, with increased DNA methylation within the *NR3C1* promoter region correlating to decreasing expression levels in suicide completers with early childhood abuse (McGowan *et al.*, 2009). In future studies, investigating both genetic and epigenetic features of the HPA axis may provide more significant interactions.

To date, this is one of the largest genetic assessments of early childhood abuse and the HPA axis in attempted suicide. No results reached study-wide significance. Future analyses using even larger-scale sample sets might have power to reveal more modest interactions than could be detected in our data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

The selected nineteen HPA axis genes and their role in the HPA axis.

Gene	Gene Symbol	Function	No. of SNPs analyzed
BCL2-associated athanogene	BAG1	Negative regulator of Glucocorticoid Receptor (GR) (Grad and Picard, 2007, Bourke <i>et al.</i> , 2013).	5
Corticotropin Releasing Hormone	CRH	Stimulates adrenocorticotrophic hormone (ACTH) secretion (Vale <i>et al.</i> , 1981).	5
Corticotropin Releasing Hormone Binding Protein	CRHBP	Regulates activation of the HPA axis by sequestering CRH (Orth and Mount, 1987).	4
Corticotropin Releasing Hormone Receptor 1	CRHR1	Binds to CRH following stress and stimulates the release of ACTH (Smith <i>et al.</i> , 1998, Reul and Holsboer, 2002).	71
Corticotropin Releasing Hormone Receptor 2	CRHR2	Involved in the negative feedback control of the HPA axis (Coste <i>et al.</i> , 2000, Reul and Holsboer, 2002).	18
FK506 Binding Protein 4, 59kDa	FKBP4	Replaces FKBP5 in the GR/HSP90 complex causing receptor activation and translocation (Davies <i>et al.</i> , 2002).	1
FK506 Binding Protein 5	FKBP5	Co-chaperone which inactivates GR by decreasing cortisol affinity and translocation (Binder, 2009).	18
Heat Shock Protein 90kDa Alpha (Cytosolic), Class A Member 1	HSP90AA1	Chaperone and positive regulator of GR ligand binding and translocation to the nucleus (Grad and Picard, 2007).	11
Heat Shock Protein 90kDa Alpha (Cytosolic), Class B Member 1	HSP90AB1		5
Heat Shock Protein 90kDa Beta (Grp94), Member 1	HSP90B1		8
Heat Shock 70kDa Protein 1B	HSPA1B	Chaperone and negative regulator of GR ligand binding (Grad and Picard, 2007).	1
Heat Shock 70kDa Protein 1-Like	HSPA1L		1
Melanocortin 2 Receptor (Adrenocorticotrophic hormone)	MC2R	Binds to circulating ACTH in the adrenal cortex leading to secretion of cortisol (Dores <i>et al.</i> , 2014).	19
Melanocortin 2 Receptor Accessory Protein	MRAP	Involved in the translocation of MC2R to the cell surface, MC2R signaling and ligand binding (Metherell <i>et al.</i> , 2005, Hinkle and Sebag, 2009).	10
Nuclear Receptor Co-Activator 1	NCOA1	Facilitates DNA binding and transcriptional regulation by GR (Onate <i>et al.</i> , 1995, Winnay <i>et al.</i> , 2006).	28
Nuclear Receptor Subfamily 3, Group C, Member 1	NR3C1	NR3C1 encodes for GR protein. Cortisol binds to GR causing activation and translocation to the nucleus where it binds to glucocorticoid response elements as a transcriptional regulator (Chandler <i>et al.</i> , 1983, Mangelsdorf <i>et al.</i> , 1995).	17
Proopiomelanocortin	POMC	ACTH is synthesized from posttranslational modifications of POMC (Nakanishi <i>et al.</i> , 1979).	7
peptidylprolyl isomerase D	PPID	Co-chaperone assisting GR translocation into the nucleus (Kumar <i>et al.</i> , 2001, Bourke <i>et al.</i> , 2013).	2
Prostaglandin E Synthase 3 (cytosolic)	PTGES3	Co-chaperone linked with HSP90 which helps stabilize the complex (Dittmar <i>et al.</i> , 1997, Grad and Picard, 2007).	4

SNP and gene information collated from RefSeq genes and dbSNP Build 130 using NCBI Build 36/hg18 from the University of California Santa Cruz (UCSC) Genome Browser.

Gene regions include ± 5 kb.

Table 2

The ten most associated SNPs from the interaction analysis.

SNP	Chr.	Nearest Gene	Location	Minor Allele	MAF in Attempters ^(a)	MAF in Non-attempters ^(a)	Interaction Odds Ratio ^(b)	Interaction P-value
rs2664008	17q21.31	CRHR1	Intronic	A	0.083	0.088	0.47	1.22×10^{-02}
rs6727239	2p23.3	NCOA1	Intronic	C	0.040	0.059	2.77	1.78×10^{-02}
rs6724282	2p23.3	NCOA1	Downstream	G	0.040	0.059	2.54	2.94×10^{-02}
rs10495750	2p23.3	NCOA1	Downstream	G	0.040	0.060	2.47	3.08×10^{-02}
rs1724425	17q21.31	CRHR1	Intronic	T	0.448	0.440	1.42	4.08×10^{-02}
rs1526123	17q21.31	CRHR1	Intronic	C	0.455	0.455	1.39	5.36×10^{-02}
rs6503447	17q21.31	CRHR1	Intronic	T	0.309	0.303	1.42	6.06×10^{-02}
rs11655764	17q21.31	CRHR1	Intronic	A	0.306	0.294	1.41	6.62×10^{-02}
rs2267716	7p15.1	CRHR2	Intronic	C	0.246	0.242	0.72	9.18×10^{-02}
rs6719226	2p23.3	POMC	Upstream	G	0.046	0.048	2.01	1.04×10^{-01}

Abbreviations: SNP denotes Single Nucleotide Polymorphism; Chr. denotes Chromosome Position; MAF denotes Minor Allele Frequencies.

SNP and gene information collated from RefSeq genes and dbSNP Build 130 using NCBI Build 36/hg18 from the University of California Santa Cruz (UCSC) Genome Browser.

^(a)MAF calculated only for subjects with abuse data.

^(b)The odds ratio is determined for the minor allele.