The Human Ortholog of Acid-Sensing Ion Channel Gene ASIC1a Is Associated With Panic Disorder and Amygdala Structure and Function


Background: Individuals with panic disorder (PD) exhibit a hypersensitivity to inhaled carbon dioxide, possibly reflecting a lowered threshold for sensing signals of suffocation. Animal studies have shown that carbon dioxide–mediated fear behavior depends on chemosensing of acidosis in the amygdala via the acid-sensing ion channel ASIC1a. We examined whether the human ortholog of the ASIC1a gene, ACCN2, is associated with the presence of PD and with amygdala structure and function.

Methods: We conducted a case-control analysis (n = 414 PD cases and 846 healthy controls) of ACCN2 single nucleotide polymorphisms and PD. We then tested whether variants showing significant association with PD are also associated with amygdala volume (n = 1048) or task-evoked reactivity to emotional stimuli (n = 103) in healthy individuals.

Results: Two single nucleotide polymorphisms at the ACCN2 locus showed evidence of association with PD: rs685012 (odds ratio = 1.32, gene-wise corrected p = .011) and rs10875995 (odds ratio = 1.26, gene-wise corrected p = .046). The association appeared to be stronger when early-onset (age ≤ 20 years) PD cases and when PD cases with prominent respiratory symptoms were compared with controls. The PD risk allele at rs10875995 was associated with increased amygdala volume (p = .035) as well as task-evoked amygdala reactivity to fearful and angry faces (p = .0048).

Conclusions: Genetic variation at ACCN2 appears to be associated with PD and with amygdala phenotypes that have been linked to proneness to anxiety. These results support the possibility that modulation of acid-sensing ion channels may have therapeutic potential for PD.

Key Words: ACCN2, amygdala, ASIC1a, association, genetic, panic disorder

Panic disorder (PD) is a common psychiatric illness with highly stereotyped symptoms including a sense of shortness of breath or feelings of suffocation. Heightened sensitivity to carbon dioxide (CO2) is an established biological correlate of PD. Inhaled CO2 triggers panic attacks in most individuals with PD but only a minority of unaffected controls (1). The prevalence of panic attacks is elevated among patients with hypercapnia-associated respiratory diseases (2). A molecular basis for these phenomena has been proposed more recently. The amygdala, which is known to play a prominent role in fear circuitry, has been shown to be a chemosensor for the detection of hypercarbia, a function mediated by the acid sensing ion channel-1a subunit (ASIC1a) (3).

Although ASIC1a is expressed throughout the nervous system, particularly high levels are expressed in the amygdala (4,5). In rodents, CO2 inhalation reduces amygdala pH, inducing acidosis and fear behaviors (3–5). Conversely, disruptingasic1ain mice...
decreases acidosis-induced fear behavior, which can be restored through transgenic expression of ASIC1a in the amygdala (3). However, CO2 inhalation was found to induce panic attacks in three individuals with bilateral amygdala damage, suggesting that amygdala chemosensing is not required for the expression of CO2-triggered panic (6).

Nevertheless, the amiloride-sensitive cation channel 2 gene (ACCN2, the human ortholog of ASIC1a) remains a compelling candidate for involvement in the etiology of PD. We reported modest evidence of linkage between PD and a region of chromosome 12q encompassing ACCN2 in a large densely affected pedigree (7). The only published study examining association between ACCN2 variants and anxiety disorders (8) found a nominally significant association in a discovery sample that failed to replicate in a second cohort. However, the cases for this study included anxiety spectrum disorders, only a subset of which had PD.

We report here, in the largest analysis to date of ACCN2 variation and PD, an association between ACCN2 polymorphisms and PD risk. Given prior evidence that the amygdala is the key site for the effect of ASIC1a on fear behavior and CO2-induced anxiety (3) and that proneness to anxiety is associated with amygdala hypertrophy (9) and enhanced reactivity to emotional threat (10), we examined the relationship of ACCN2 variants with neuroimaging measures of amygdala structure and function. We hypothesized that individuals carrying ACCN2 risk alleles would exhibit increased susceptibility to threat-induced amygdala activation on the theory that amygdala reactivity to threatening stimuli may trigger fear circuitry in part through increased metabolic activity resulting in locally reduced pH and activation of ASIC1a (11).

Methods and Materials

Participants

PD Case-Control Analysis. To maximize power, we pooled samples from cohorts derived from genetic studies of anxiety, mood disorders, attention-deficit/hyperactivity disorder, treatment response, and healthy controls (Table 1). A description of each cohort is provided in Supplement 1. Inclusion and exclusion criteria for the genetic analyses are described subsequently and summarized in Figure 1.

Inclusion Criteria. The PD case-control analyses were restricted to individuals with self-reported European American (or in the case of the Brazilian sample, European-Brazilian) ancestry to minimize confounding by population stratification. For studies with family-based recruitment, genetically related individuals were excluded. All participants consented to participate in genetic studies of anxiety or a broader class of mental health conditions. The Partners Human Research Committee approved all aspects of the current study.

Cases met DSM-IV criteria for PD, with or without agoraphobia. Cases were recruited for studies of anxiety, with the exception of a subset of individuals recruited for the Massachusetts General Hospital Genetic Determinants of Behavioral Inhibition and Disinhibition Study, in which some parental probands were ascertained based on a history of PD, mood disorder, or attention-deficit/hyperactivity disorder. For families not ascertained based on PD in the parent-proband, we selected non-ascertained family members who met criteria for PD to minimize the risk of biased ascertainment.

Controls were drawn from individuals without a history of PD, including volunteers from a large-scale study of brain imaging and genetics (the Brain Genomics Superstruct Project; see Supplement 1 for details). When ascertainment was not based on anxiety disorder diagnosis (e.g., the Massachusetts General Hospital genetic studies of attention-deficit/hyperactivity disorder), controls were restricted to family members without a history of PD to minimize the risk of biased ascertainment. Controls were required to be at least age 26 (to increase the likelihood that they had passed through the window of risk for PD onset) and did not meet full DSM-IV criteria for any anxiety disorder, depression, or dysthymia.

Individuals with PD whose age of onset was ≤20 years (n = 179) were compared with controls as a secondary analysis. We selected age 20 as the cut point based on prior genetic epidemiologic evidence and because this was the median value observed in our data. Specifically, a prior large-interview family study documented a 17-fold familial risk of panic disorder using this cut point (12). The early-onset cases had a mean age of onset of 14.6 years (±4.3 years) and a mean age at assessment of 30.9 years (±11.5 years).

Given prior preclinical evidence associating ASIC1a with CO2-induced fear behavior and the hypothesis that CO2 hypersensitivity in PD may reflect an altered suffocation alarm system, we examined associations between ACCN2 variants and a putative respiratory subtype of PD. Although definitions of the respiratory subtype vary (13), most require a predominance of the following symptoms during panic attacks: 1) chest pain or discomfort, 2) shortness of breath, 3) feelings of choking, 4) paresthesias, and 5) fear of dying. We defined respiratory subtype cases as individuals identifying four or more of these panic symptoms on diagnostic interview. These data were available for only a subset of PD cases. Nonrespiratory subtype panic was defined as three or more of these symptoms and no endorsement of shortness of breath or choking feelings.

Exclusion Criteria. Individuals with a diagnosis of schizophrenia, psychosis, or bipolar disorder were excluded.

Genetic Methods

DNA from blood or saliva samples was extracted at the Massachusetts General Hospital Center for Human Genetic Research. Single nucleotide polymorphisms (SNPs) were selected to pairwise tag all common SNPs in the ACCN2 gene ±10 kb flanking regions using the Tagger module in Haploview (14) and based on the linkage disequilibrium (LD) structure of the HapMap Centre d’Etude du Polymorphisme Humain sample. An r2 threshold of .8 was used for tagging. Six SNPs captured 100% of the common variation (≥5% minor allele frequency) in the HapMap Centre d’Etude du Polymorphisme Humain sample. Two tag SNPs could not be designed into the primer group and were replaced with proxy SNPs (r2 ≥ .9).

We used iPLEX Gold chemistry and the MassARRAY System (Sequenom Bioscience, San Diego, California) to perform SNP genotyping. Genotyping of the six ACCN2 SNPs was conducted as part of a larger study of multiple anxiety disorders and related phenotypes that comprised 139 SNPs (including 40 ancestry informative markers that differentiate individuals into continental populations) in 2976 samples (including 38 duplicate sample pairs). We made use of this larger dataset to examine genotyping performance and remove poor-quality SNPs and samples. Samples were excluded based on missingness >1. SNPs were excluded based on at least one of the following: missingness >1, minor allele frequency <.01, or Hardy Weinberg p value < 10^-4 as calculated in PLINK (15). Using these thresholds, 66 samples and 12 markers were excluded. The concordance rate for
the 37 duplicate pairs was .99. After quality control, 2873 individuals and 127 markers remained, including five of the six ACCN2 SNPs (rs706793 failed because of missingness and deviations from Hardy Weinberg equilibrium) (Figure 2).

To control for ancestry, we restricted analyses to European American non-Hispanic individuals. To define this subset of our sample, we used multidimensional scaling analysis of data from the 36 ancestry informative markers (AIMs) SNPs that passed

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cases (n)</th>
<th>Controls (n)</th>
<th>Age (Mean, SD)</th>
<th>Sex (% Female)</th>
<th>Diagnostic Instrument</th>
<th>Ascertainment</th>
<th>Recruitment Sites</th>
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<tr>
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<td>130</td>
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<td>72</td>
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<td>Anxiety disorders (cases only)</td>
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<td>57</td>
<td>SCID or K-SADS</td>
<td>Parental PD, MDD, ADHD or none (cases and controls)</td>
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<td>76</td>
<td>91</td>
<td>39.0, 9.6</td>
<td>72</td>
<td>MINI</td>
<td>PD (cases and controls)</td>
<td>Porto Alegre, Brazil</td>
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<tr>
<td>MGH Predictors of Response and/or Relapse in Anxiety Disorders</td>
<td>107</td>
<td>0</td>
<td>36.5, 12.5</td>
<td>56</td>
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<td>PD treatment study (cases only)</td>
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<td>Improving Outcomes in Pharmacotherapy of Social Phobia</td>
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<td>SCID or MINI</td>
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<td>0</td>
<td>37.7, 14.5</td>
<td>37</td>
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<td>Social phobia (cases)</td>
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<td>315</td>
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<td></td>
<td>Healthy controls</td>
<td>Cambridge, MA</td>
</tr>
</tbody>
</table>

ADHD, attention-deficit/hyperactivity disorder; CBT, cognitive-behavioral therapy; HCPA, Hospital de Clinicas de Porto Alegre; K-SADS, Schedule for Affective Disorders and Schizophrenia for School-Age Children; MDD, major depressive disorder; MGH, Massachusetts General Hospital; MINI, Mini International Neuropsychiatric Interview; PD, panic disorder; SCID, Structured Clinical Interview for DSM-IV.

Figure 1. Case-control Consolidated Standards of Reporting Trials diagram. Ascertainment of cases and controls for the association analysis of ACCN2 variants and panic disorder. ADHD, attention-deficit/hyperactivity disorder; QC, quality control.

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quality control, along with self-reported race/ethnicity. Using the first two dimensions of variation and self-reported race/ethnicity for all 2873 individuals, we created a scatterplot that allowed us to visualize the population structure of our sample. A clear clustering of European American and non-Hispanic individuals was revealed (Figure 3). We defined the European American non-Hispanic sample set as individuals who 1) fell within or near the main European American non-Hispanic cluster and 2) self-reported race/ethnicity as European American (with ethnicity unreported), European American non-Hispanic, missing, unknown, or non-Hispanic. Of 2693 individuals who met these criteria, a subset of 1260 met the panic case or control criteria as outlined previously.

**PD Case-Control Genetic Analysis**

We tested an additive genetic model on case or control status using the logistic regression function in PLINK, adjusting for sex. Correction for multiple testing was performed using the PLINK \text{max}(T)\ procedure with 10,000 permutations. This procedure reassigns case or control labels for subjects, maintaining patterns of LD between SNPs. Each observed SNP test statistic is compared with the maximum of all permuted statistics over all SNPs to calculate the corrected \( p \) value.

**Neuroimaging Methods**

**Participants.** Native English-speaking young adults from the Brain Genomics Superstruct Project (18–30 years old) with normal or corrected-to-normal vision were included for structural magnetic resonance imaging (MRI) analyses (\( n = 1048 \); age, 21.09 ± 2.59 years old; female, 54.80%; right-handed, 91.70%; years of education, 14.59 ± 1.90). A subset of participants (\( n = 103 \)) performed task-based functional MRI allowing examination of the relationship between \( ACNN2 \) genotype and task-evoked amygdala reactivity (age, 20.37 ± 2.45 years old; female, 52.40%; right-handed, 100%; years of education, 14.07 ± 1.75). Participants were genotyped concurrently with the case-control sample and underwent identical quality control procedures. Estimated slice-based signal-to-noise ratio and maximum absolute motion did not differ by \( ACNN2 \) genotype (\( ts < .84; ps > .41 \)).

**MRI Data Acquisition.** Imaging data were acquired on matched 3-tesla Tim Trio scanners (Siemens Medical Solutions, Erlangen, Germany) at Harvard University and Massachusetts General Hospital using the vendor-supplied 12-channel phased-array head coil. Structural data included a high-resolution multi-echo T1-weighted magnetization-prepared gradient echo image using the following parameters: repetition time = 2200 msec, inversion time = 1100 msec, echo time = 1.54 msec for image 1–7.01 msec for image 4, flip angle = \( 7^\circ \), 1.2 × 1.2 × 1.2 mm, and field of view = 230. Task-evoked functional MRI data using a gradient echo sequence were collected using the following parameters: time points = 68, repetition time = 3000 msec, echo time = 30 msec, flip angle = \( 85^\circ \), 3 × 3 × 3 mm voxels, and field of view = 216. There were 47 anterior commissure–posterior commissure aligned slices that employed interleaved acquisition and no gap between slices. Variance associated with site and software upgrades that occurred during data acquisition was partialled out of analyses.

**Functional Task.** A matching task previously shown robustly to activate the amygdala was used (16). Four blocks of emotional face matching were interleaved with five sensorimotor control blocks. During emotion blocks, participants were presented with trios of faces (angry, afraid) derived from a standard set of pictures of facial affect (17) and were instructed to select one of two faces (bottom) that matched the target face (top). During sensorimotor control blocks, participants viewed trios of geometric shapes (circles, ellipses) and were instructed to select one of two shapes (bottom) identical to the target shape (top). Each block consisted of six images, presented sequentially for 3000 msec.

**MRI Data Analyses.** Structural data were analyzed using FreeSurfer version 4.5.0 software (http://surfer.nmr.mgh.harvard.edu). FreeSurfer provides automated algorithms for the volumetric segmentation of subcortical structures and estimation of cortical thickness (18,19). Cortical thickness was calculated as the closest distance from the gray matter/white matter boundary to the gray matter/cerebrospinal fluid boundary at each vertex on the tessellated surface (19). After surface based registration, but before the analysis of cortical thickness, a 22-mm full width at half maximum smoothing kernel was applied to each participant’s data. Follow-up surface-based analyses assessed the specificity of the observed effects. Surface effects were thresholded at \( p < .001 \) uncorrected for multiple comparisons. Estimated intracranial volume was calculated using the approach of Buckner et al. (20) as implemented in FreeSurfer.

Task data preprocessing included 1) removing the first four volumes to allow for T1 equilibration; 2) compensation of systematic, slice-dependent time shifts; and 3) motion correction. Data were spatially normalized to the Montreal Neurological Institute standard anatomic atlas space (2-mm isotropic voxels) using a T2-weighted echo planar image blood oxygen level--
dependent (BOLD) contrast atlas in SPM2 (21). A 6-mm full width at half maximum smoothing kernel was applied to each participant’s data. After preprocessing, a general linear model was conducted in SPM8, modeling each condition as a boxcar regressor convolved with a hemodynamic response function and a high-pass (1/128 Hz) filter for linear drift. Group-level analyses were conducted, selecting contrasts of interest for each participant (faces > shapes) and entering them in second-level random-effects t tests.

Analysis of the task-evoked data revealed significant bilateral amygdala response during the emotion task (p < .0001 family-wise error-corrected) (Figure 4B). Hypothesis-driven region of interest analyses were performed by defining a 6-mm-radius region of interest centered on the maximally increased BOLD response in the left and right amygdalae (x = −22, y = −4, z = −22 and x = 22, y = −4, z = −22). Average response was extracted from each region of interest, and follow-up analysis of variance (ANOVA) was conducted in SPSS (IBM Corp., Armonk, New York).

Imaging Genetic Analysis. Analyses of subcortical anatomic variability and task-evoked reactivity targeted the amygdala. We examined associations between these anatomic and functional phenotypes and the two SNPs associated with PD from the case-control analysis (rs10875995 and rs685012). For morphometric analyses, a repeated-measures ANOVA was conducted partialing out variance associated with site, console software version, age, sex, handedness, and intracranial volume and examining relations between amygdala volumes and ACCN2 genotype. To examine specificity of the observed effects, follow-up analyses were conducted on the remaining subcortical structures and the surface-based estimates of cortical thickness. For analyses of task-evoked reactivity, repeated-measures ANOVA was conducted examining relations between genotype and amygdala reactivity, partialing out variance associated with console software version, handedness, age, and sex. For the sake of brevity, only effects involving genotype are presented (other findings are available on request).

Results

Association with PD

Three of the five SNPs tested demonstrated nominally significant association with PD. Two survived gene-wise correction for multiple testing: rs685012 (odds ratio [OR] for C allele = 1.32, 95% confidence interval [CI] = 1.1–1.57, p_corrected = .011) and rs10875995 (OR for C allele = 1.26, 95% CI = 1.06–1.51, p_corrected = .046). These models were adjusted for sex, but results were virtually unchanged when models were not adjusted for sex (Table 2).

Effect sizes and statistical significance increased in the secondary analysis of early age-of-onset cases versus controls. Three SNPs were significant in sex-adjusted models after correcting for the number of SNPs tested: rs10875995 (OR for C allele = 1.58, 95% CI = 1.24–2.00, p_uncorrected = .0002, p_corrected = .0006),
rs685012 (OR for C allele = 1.49, 95% CI = 1.17–1.90, \( p_{uncorrected} = 0.009 \), \( p_{corrected} = 0.0042 \)), and rs17124367 (OR for T allele = 1.80, 95% CI = 1.22–2.66, \( p_{uncorrected} = 0.0034 \), \( p_{corrected} = 0.013 \)) (Table S1 in Supplement 1 for genotype frequencies).

In a second exploratory analysis, we identified 43 respiratory-subtype PD cases and compared them with controls. The SNPs most strongly associated with PD overall were also associated with respiratory-subtype PD: rs685012 (OR for C allele = 1.84, 95% CI = 1.17–2.89, \( p_{uncorrected} = 0.008 \), \( p_{corrected} = 0.027 \)) and rs10875995 (OR for C allele = 1.90, 95% CI = 1.22–2.96, \( p_{uncorrected} = 0.0046 \), \( p_{corrected} = 0.017 \)). Neither rs685012 nor rs10875995 was associated with PD when non-respiratory-subtype cases (n = 87) were compared with controls (both \( p > 0.70 \)).

**Association of ACCN2 Variants with Brain Structure and Function**

The ANOVA examining the relation between rs10875995 genotype and amygdala volume revealed a main effect of genotype \( F_{1,1046} = 4.46, p = .04, \eta_p^2 = .01 \), driven by bilaterally greater amygdala volumes in the C allele (PD associated) carriers (1808.81 ± 7.11 mm\(^3\)) relative to T/T homozygotes (1787.10 ± 7.41 mm\(^3\)) (Figure 4A). As suggested by the absence of an rs10875995 genotype-by-hemisphere interaction \( F_{1,1046} = .81, p = .37, \eta_p^2 < .01 \), this effect was present when separately considering the right amygdala \( F_{1,1040} = 5.12, p = .02, \eta_p^2 = .01 \) and in the same direction, although not significant, when considering the left amygdala \( F_{1,1040} = 2.56, p = .11, \eta_p^2 < .01 \). Follow-up tests examined the specificity of the relations between rs10875995 and amygdala volume across each subcortical structure. The volumes of the remaining subcortical structures (caudate, globus pallidus, nucleus accumbens, putamen, and thalamus) did not display relations with genotype \( \text{all } F < 3.39, \text{all } p > .07 \). No significant relations emerged between rs685012 genotype and amygdala volume \( \text{all } F < 1.04, \text{all } p > .30 \). Cortical thickness estimates did not significantly differ by ACCN2 genotype.

The ANOVA examining the relation between rs10875995 genotype and amygdala reactivity revealed a main effect of genotype \( F_{1,95} = 6.93, p = .01, \eta_p^2 = .07 \). Follow-up analyses restricted to the voxels displaying maximally increased BOLD response confirmed the observed bilateral increase in the amygdala response of C allele carriers, relative to T/T homozygotes \( x = -22, y = -4, z = -22 \) and \( x = 22, y = -4, z = -22 \) \( F_{1,98} = 7.96, p = .0058, \eta_p^2 = .08 \).

The ANOVA examining the relation between rs685012 genotype and amygdala reactivity revealed a trend toward a main effect of genotype \( F_{1,97} = 2.90, p = .09, \eta_p^2 = .03 \), driven by heightened bilateral amygdala response in C allele carriers \( .65 ± .04 \) relative to T/T homozygotes \( .54 ± .05 \). After partialing out left and right amygdala volume (corrected for intracranial volume), there was no significant association between rs685012 genotype and amygdala reactivity \( F_{1,94} = 2.13, p = .14, \eta_p^2 = .02 \).

**Discussion**

Although PD is heritable, success in identifying susceptibility genes has been limited relative to progress in other domains of psychiatric genetics (22). Nevertheless, genetic studies of anxiety disorder phenotypes have the advantage that animal models and neuroimaging research have established molecular and neuroanatomic underpinnings of panic and related anxiety phenotypes. Among psychiatric disorders, PD is one of the few disorders in which symptoms can be reliably provoked by a biological challenge (CO\(_2\) inhalation) (23). In the present study, we capitalize on this evidence, focusing on variation in ACCN2, a gene previously associated with amygdala-mediated anxiety and implicated in anxiety-related hypersensitivity to CO\(_2\) (3). Our results suggest that variants in ACCN2 are associated both with PD and with alterations in amygdala structure and function.

In case-control analyses, the minor alleles of 2 SNPs—rs685012, located in the S’ putative promoter region of ACCN2, and rs10875995, located in intron 3—were associated with PD after correcting for the number of markers tested. We are unable to determine whether either of these association signals directly reflects a causal variant or LD with such a variant. We observed a stronger effect of ACCN2 alleles on early-onset PD, a subtype that has been shown to be associated with increased familial risk of PD (12), and among cases of PD exhibiting a respiratory subtype, which has been associated with increased sensitivity to inhaled CO\(_2\) (13).

Wemmie et al. (4,5) demonstrated that ASIC1a is required for normal conditioned and unconditioned fear behavior and that
this effect is mediated by ASIC1a sensing of reduced pH (3). Mirroring human studies of PD, inhalation of CO2 evokes fear behavior in mice, an effect that is reduced by deletion or blockade of ASIC1a within the amygdala (3). Overexpression of ASIC1a in the amygdala was sufficient to trigger CO2-induced fear behavior. In light of this evidence and several prior studies showing that increased proneness to anxiety is associated with increased amygdala volume in healthy volunteers (9, 24, 25), we examined the association between amygdala volume and variation in ACCN2. Consistent with our observation that the C allele of rs10875995 SNP contributes to PD risk, we found evidence of association between this variant and both amygdala volume and reactivity to emotional faces. The association with brain structure appeared to be specific to amygdala volume in that no significant association was seen with other subcortical structures or with cortical thickness. We reported more recently an association between increased amygdala volume and measures of trait anxiety in the same sample used in the present report (N = 1050) (9). The present results indicate that the increased amygdala volume phenotype is related in part to ACCN2 alleles that are also associated with risk for PD. Although MRI studies of PD have more often found reduced amygdala volume (26), our results suggest that ACCN2 may contribute to the relative enlargement of the amygdala. Although this finding might appear to be discrepant with prior MRI studies that find reduced amygdala volume among individuals with PD, our findings do not imply that risk allele carriers with PD should have large amygdalae. In addition, it is possible that the reduction in amygdala volume previously reported with PD itself is a consequence of the illness—we would not have seen this because our imaging sample was free of Axis I pathology. In healthy volunteers, subsyndromal levels of anxiety may be associated with increased amygdala volume, as we found in our prior work (9). The mechanism by which ACCN2 alleles could increase amygdala volume is unclear and warrants further study. However, disruption of ASIC1a attenuates hippocampal brain-derived neurotrophic factor (BDNF) suppression in response to stress (27). Stress has been shown to increase BDNF in the amygdala (28), an effect opposite to that seen in the hippocampus (29), and BDNF increases amygdala dendritic and axonal growth (30). One hypothesis that warrants further investigation is that enhanced ASIC1a activity increases amygdala volume by promoting stress-induced amygdala BDNF secretion.

We also found that the same ACCN2 allele associated with both PD and amygdala volume is associated with increased amygdala reactivity to emotional faces, a phenotype linked to PD in previous studies (26). Wemmie et al. (31) suggested that amygdala activation by fear-inducing stimuli may trigger fear responses by lowering local pH (e.g., by synaptic release of H+ and metabolic effects of neural activity). If this is so, the observed association between allelic variants and amygdala reactivity may reflect enhanced sensitivity to reduced pH secondary to neuronal activity that mediates the processing of emotional stimuli. Such activation would clearly be less direct (and less intense) than that induced by direct CO2 inhalation.

We previously reported modest evidence of linkage (NPL = 4.96) between the region on chromosome 12q encompassing the ACCN2 locus and PD in a large pedigree densely affected with the disorder (7). In a prior association study between ACCN2 SNPs and anxiety disorders, Hettema et al. (8) reported a two-stage analysis comprising twin pairs. They used structural equation modeling to identify a latent genetic factor underlying susceptibility to neuroticism, major depression, generalized anxiety disorder, PD, agoraphobia, and social phobia. Twin pairs scoring at the extremes on this factor were selected for case-control analyses. In the first stage (n = 188 cases, 188 controls), the investigators found modest evidence of association for the C allele of rs685012 (p = .077) and the T allele of rs1108923 (p = .023) but no association in a second-stage sample (n = 401 cases, 351 controls) or in the subset of cases with PD (n = 122). The relatively small sample size limits the interpretability of this null finding with respect to PD. Additional evidence for a role of acid-sensing ion channels in PD comes from a study in the Faroe Islands population isolate that observed nominal association with variants in ACCN1 (the gene encoding ASIC2a) (32). Prior research suggests that ASIC2a may interact with ASIC1a and may facilitate the synaptic function of ASIC1a (31).

In conjunction with preclinical studies of ASIC1a, our results support the possibility that variants in ACCN2 contribute to PD risk by lowering the threshold for amygdala sensing of acidosis. The association between ACCN2 and PD would be consistent with a leading biological model of PD pathogenesis. Klein (33) and others proposed that panic attacks and the heightened sensitivity to CO2 seen in PD represent the abnormal triggering of an evolved suffocation alarm system. Alternatively, CO2 hypersensitivity might trigger panic attacks by inducing somatic symptoms that individuals with PD are more likely to fear. Individuals with

Table 2. Association Results for the Panic Disorder Case-Control Analysis: Genotype Data and Association Statistics

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<th>Genotypesa</th>
<th>Case-Control Analysis</th>
<th>Sex-Adjusted Case-Control Analysis</th>
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</tbody>
</table>

CI, confidence interval; OR, odds ratio; SNPs, single nucleotide polymorphisms.

aA1/A1 = homozygote minor allele; A1/A2 = heterozygote; A2/A2 = homozygote major allele.
heightened amygdala acid sensing might be more prone to experience interoceptive cues in response to internal (e.g., metabolic) or external (e.g., CO₂) stimuli, which might further trigger panic responses among individuals with increased anxiety sensitivity. This mechanism provides a potential link between our findings and cognitive-behavioral models of the pathogenesis of panic. The more recent observation of rare individuals with amygdala damage who nevertheless experienced panic in response to CO₂ inhalation suggests that amygdala ASIC1a chemosensors are not required for panic (6).

Our results should be interpreted in light of the limitations of this study. First, although our sample is large relative to most prior genetic studies of PD, we had >80% power to detect moderate allelic effects (e.g., genotypic risk ratio >1.32 for allele frequency ≥.20 at α < .01) but not smaller effects. Second, to obtain adequate sample sizes, the case-control samples were drawn from multiple studies that had varying ascertainment procedures. However, phenotypic heterogeneity would be expected to obscure true effects rather than increase the risk of spurious association. Third, the markers we tested are not known to have functional effects, and the association signals might reflect LD with the causal variation. Replication of our findings is needed to establish an association between ACCN2 and PD.

Taken together, our results suggest that variants of ACCN2 contribute to PD risk as well as influence amygdala structure and function. These findings build on prior preclinical research that has strongly implicated this gene in amygdala-mediated fear behavior. They also support the possibility that inhibitors of ASIC1a might have therapeutic potential in the treatment of PD and related disorders involving dysregulation of amygdala-mediated fear responses. Preclinical studies suggest that acid-sensing ion channel inhibitors produce anxiolytic effects comparable to a benzodiazepine (34). Given this, proof-of-concept studies of ASIC1a inhibitors may be warranted.

This work was supported in part by National Institutes of Health (NIH) Grant No. K24MH094614 (JWS), NIH Grant No. T32-MH16259 (LMM), Jonathan Edwards Brooking Mental Health Fellowship and NIH Grant No. T32MH017119 (LED), NIH Grant No. R01 47077 (JFR), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) Grant 306053/2006-7, Fundo de Incentivo a Pesquisa e Eventos-Hospital de Clínicas de Porto Alegre (FIPe-HCPA) (GGM), NIH Grant No. R01 63683 (DRH-B), NIH Grant No. MH64122 (MBS), NIH Grant No. R01 MH081116 (MWO), the Highland Foundation (MHP, NMS), and the Frazier Research Institute at McLean (BMC). We thank Ahmad Hariri for assistance with constructing the functional magnetic resonance imaging task.

JWS is on the Scientific Advisory Board of PsyBrain, Inc. DÖ is a Principal Investigator (PI) on research contract with Myriad Genetics, Inc, and served on the advisory board for Lilly Pharmaceuticals. DFT has received research support from Merck, Endo Pharmaceuticals, and Eli Lilly. MVA has received grant/research support from Forest Laboratories, Hamilton Academic Health Sciences Organization Innovation Grant (Academic Funding Plan [AFP] Innovation Grant), Janssen-Ortho, Inc, National Institutes of Health, Pfizer, Inc, and Servier; is part of the Speaker’s Bureau for Janssen-Ortho, Inc, Lundbeck, Pfizer, Inc, and Shire; and is on advisory boards for Forest Laboratories, Janssen-Ortho, Inc, Labo Pharm, Lundbeck, Pfizer, Inc, Servier, Shire, Sunovion, and Valeant. JFR has equity in Medavante. JR has received research support from Pamlab. JB currently is receiving research support from American Professional Society of ADHD and Related Disorders, U.S. Department of Defense, ElMindA, Janssen, McNeil, Shire, and VayaPharma/Enzymotec; in 2012, he received an honorarium from Massachusetts General Hospital Psychiatry Academy and The Children’s Hospital of Southwest Florida/Lee Memorial Health System for tuition-funded continuing medical education courses; in 2011, he gave a single unpaid talk for Juste Pharmaceutical Spain, received honoraria from Massachusetts General Hospital Psychiatry Academy for a tuition-funded continuing medical education course, and received honoraria for presenting at an international scientific conference on attention-deficit/hyperactivity disorder; he received an honorarium from Cambridge University Press for a chapter publication; and he received departmental royalties from a copyrighted rating scale used for attention-deficit/hyperactivity disorder diagnoses, paid by Eli Lilly, Shire, and AstraZeneca to the Department of Psychiatry, Massachusetts General Hospital. RLB is a paid consultant for Pfizer and Johnson & Johnson. MHP is on advisory boards and serves as a consultant for Corcept Therapeutics, Eli Lilly, Ironwood Pharmaceuticals, Medavante, Otsuka, Targia Pharmaceuticals, and Transcept Pharmaceuticals; has received research grants from Bristol-Myers Squibb, Euthymics, Forest Laboratories, GlaxoSmithKline, Eli Lilly, National Center for Complementary and Alternative Medicine, National Institute on Drug Abuse, and National Institute of Mental Health; participated in continuing medical education–supported activities from Pfizer; has equity in Doyen Medical, Mensante Corporation, Mindsite, and Targia Pharmaceuticals; and has received royalty/patent from Structured Interview Guide for the Hamilton Anxiety Scale and SAFER interviews. NMS has received research support in the past 3 years from American Foundation for Suicide Prevention, Forest Laboratories, National Institutes of Health, Department of Defense, and Highland Foundation, and honoraria for speaking/continuing medical education from Massachusetts General Hospital Psychiatry Academy; her spouse has equity in Eli Lilly, Ironwood, and Shire, and is a Gatekeeper. MWO has served in the past 3 years as a paid consultant for MicroTransponder, Inc, Concert Pharmaceuticals, and ProPhase and received royalty support for use of the Structured Interview Guide for the Hamilton Anxiety Scale from ProPhase. The remaining authors report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2013.12.018.

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