Archival Report

Adenylate Cyclase 7 Is Implicated in the Biology of Depression and Modulation of Affective Neural Circuitry

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Background: Evolutionarily conserved genes and their associated molecular pathways can serve as a translational bridge between human and mouse research, extending our understanding of biological pathways mediating individual differences in behavior and risk for psychopathology.

Methods: Comparative gene array analysis in the amygdala and cingulate cortex between the serotonin transporter knockout mouse, a genetic animal model replicating features of human depression, and existing brain transcriptome data from postmortem tissue derived from clinically depressed humans was conducted to identify genes with similar changes across species (i.e., conserved) that may help explain risk of depressive-like phenotypes. Human neuroimaging analysis was then used to investigate the impact of a common single-nucleotide polymorphism (rs10644448) in a gene with identified conserved human-mouse changes, adenylate cyclase 7 (ADCY7), on threat-associated amygdala reactivity in two large independent samples.

Results: Comparative analysis identified genes with conserved transcript changes in amygdala (n = 29) and cingulate cortex (n = 19), both critically involved in the generation and regulation of emotion. Selected results were confirmed by real-time quantitative polymerase chain reaction, including upregulation in the amygdala of transcripts for ADCY7, a gene previously implicated in human depression and associated with altered emotional responsiveness in mouse models. Translating these results back to living healthy human subjects, we show that genetic variation (rs10644448) in ADCY7 biases threat-related amygdala reactivity.

Conclusions: This converging cross-species evidence implicates ADCY7 in the modulation of mood regulatory neural mechanisms and, possibly, risk for and pathophysiology of depression, together supporting a continuous dimensional approach to major depressive disorder and other affective disorders.

Key Words: Adenylate cyclase, amygdala, depression, human, mouse, serotonin transporter

Evolutionarily conserved genes and their emergent molecular pathways can serve as a translational bridge between human and mouse research, extending our understanding of biological pathways that mediate individual differences in behavior and risk for psychopathology (1). Our studies in major depressive disorder (MDD) and affect regulation have focused on the amygdala and anterior cingulate cortex (ACC) as critical components of a corticolimbic circuit of mood regulation (2) that is affected in MDD (3). Evidence supporting dysfunctions of these areas in MDD include decreased ACC volume and altered activity (4–7), decreased glial density and reduced (8) or no change in neuronal size (9) in ACC, decreased glial density (10) and fewer oligodendrocytes (11) in amygdala, and abnormal processing of emotional stimuli and sustained amygdala reactivity (12–15).

Animal research based on neuropsychiatric disorder candidate genes has the potential to inform disease mechanisms in human subjects. Prior investigations from our group have identified conserved gene changes between stress-based rodent models and human MDD, potentially identifying a subgroup of patients with cohesive underlying biological changes (16). A widely utilized genetic animal model replicating features of human depression is the serotonin transporter (SERT) knockout mouse (SertKO). Serotonin transporter, the protein responsible for the reuptake of serotonin from the synaptic cleft into presynaptic serotonergic neurons, is the therapeutic target of serotonin transporter reuptake inhibitors, which effectively treat MDD (17). Moreover, the vulnerability to develop MDD in response to stressful life events is modulated by a tandem-repeat polymorphism in SERT-linked promoter region (18).

Serotonin transporter knockout mice display a robust increased emotionality phenotype (19,20) (Supplement 1), which appears mediated by developmental events, as early-life SERT blockade results in a similar high emotionality phenotype in adults (21). These observations suggest that while altered serotonergic function (i.e., through altered Sert levels) may represent the initial biological trigger, the active mechanisms underlying altered mood states may be mediated by molecular and cellular adaptations that are remote from this original pathogenic event (22,23), hence, warranting the unbiased investigation of biological adaptations in these systems as putative mediators of the emotionality phenotype.

The overall strategy of this study was based on two main assumptions: one of cross-species translational conservation of changes (16), and one of a continuous dimensional approach to affect regulation between control subjects and subjects with MDD (1). First, due to the conservation of the structure and function of the corticolimbic circuit between humans and rodents, we posit that biological disturbances that are observed in this neural circuit...
both in human MDD and in a validated animal model of the human syndrome (SertKO mice) would represent core pathological features of the illness. Second, pathophysiological changes in affective disorders reside in the outer boundaries of a continuous distribution linking mechanisms of affect regulation in control and MDD subjects and are mediated by the same neural circuit. Accordingly, we used large-scale gene expression data to map molecular changes in the amygdala and cingulate cortex of SertKO mice. We then examined the extent to which these changes in SertKO mice were present in postmortem amygdala and cingulate cortex tissue from human subjects with MDD. Finally, we used imaging genetics to assess the potential functional neural correlates of a common human genetic polymorphism in one gene, ADCY7, exhibiting convergent expression findings across human and mouse transcriptomes.

Methods and Materials

Animals

Mice lacking the Sert gene (SertKO) and normal wild-type (WT) control C57BL/6 littermates (24) were obtained from Taconic (Hudson, New York) and bred via heterozygous breeding. All mice were maintained on a 12-hour light cycle with access to food ad libitum, and all procedures received Institutional Animal Care and Use Committee approval. Amygdala and cingulate cortex tissue samples were collected from five WT and five SertKO mice at 3 to 5 months of age for microarray analysis of gene expression. Additional amygdala and cingulate tissue samples were collected from 10 WT and 10 SertKO mice at 3 to 5 months of age for quantitative polymerase chain reaction (qPCR) validation of differential gene expression.

Microarray Analysis

Amygdala and cingulate cortex samples were collected from WT and SertKO mice by micropunch (n = 5 per area and per genotype group). A 1 mm diameter punch was used to dissect amygdala from 1 mm thick section starting at the level corresponding to figure 52 in the Paxinos and Franklin atlas (25) and including the basolateral and lateral nuclei. A 2 mm diameter punch was used for cingulate cortex from 1 mm thick section starting at the level corresponding to figure 52 in the same atlas. Total RNA was extracted from tissue homogenized in Trizol reagent (Invitrogen, Carlsbad, California) and processed according to manufacturer’s protocol. Labeled copy RNA from individual mouse and brain areas was hybridized to MOE430-Plus 2.0 microarray (Affymetrix, Santa Clara, California; n = 5 arrays per area per genotype; total, n = 20 samples) as described in Surget et al. (26). Signal intensities were extracted and normalized with the Robust Multi-Array algorithm (27). Analysis of gene expression changes was performed by two group student t tests separately in the two brain regions. For the purpose of exploratory analyses and due to the small sample size (n = 5/group) and large number of genes, statistical values were kept at moderate stringency (p < .05; changes greater than 20%) and not adjusted for multiple testing. Instead, comparative analyses were performed across experiments or species to identify genes with concordant and significant changes across species (i.e., conserved changes), and results were confirmed by qPCR performed on complementary DNA obtained from independent cohorts (i.e., biological replicates).

Real-Time Quantitative Polymerase Chain Reaction

Complementary DNA amplification using gene-specific primers was quantified in quadruplicates by SYBR green fluorescence signal (Invitrogen) using the Opticon Monitor DNA Engine (Bio-Rad, Berkeley, California). Validated primers for actin, glyceraldehyde 3-phosphate dehydrogenase, and cyclophilin messenger RNAs were used as internal control genes in mouse and human samples (16). For each gene of interest, the geometric mean of the three control genes was subtracted to provide ΔC(t) values (C[t], number of amplification cycles required for this gene’s signal to reach threshold), which can be converted to signal intensities in arbitrary units on a linear scale (signal intensities = 100 * 2-ΔC(t)). Because this is a nonlinear transformation that affects the detection of linear Pearson correlations, correlations of gene expression were calculated using signal intensities and not ΔC(t). Data are reported as fold changes in signal intensities across groups.

Imaging Genetics Participants

Two independent samples (sample 1: n = 82, sample 2: n = 98) were recruited from consecutive stages of the Adult Health and Behavior Study, which investigates a variety of behavioral and biological traits among nonpatient, middle-aged community volunteers (see Supplement 1 for demographics and screening details). Written informed consent according to the guidelines of the University of Pittsburgh’s Institutional Review Board was obtained from all participants upon their enrollment in the study.

Blood Oxygen Level-Dependent Functional Magnetic Resonance Imaging Paradigm

Both samples completed an archival challenge paradigm, which robustly and consistently elicits threat-related reactivity of the amygdala (28–30). The paradigm consists of four task blocks wherein participants match threat-related face stimuli expressing either anger or fear, interleaved with five sensorimotor control blocks of matching simple geometric shapes (see Supplement 1 for task details, functional magnetic resonance imaging [fMRI] acquisition parameters, and data analysis).

Genotyping

DNA was extracted from ethylenediaminetetraacetic acid-anticoagulated whole blood cells using a salting-out procedure. rs10644448 genotypes were derived from the Illumina 610-Quad BeadChip (Illumina Inc, San Diego, California) using the “list” command in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/). In sample 1, there were 24 participants homozygous for the G allele, 16 for the T allele, and 42 heterozygotes. In sample 2, there were 26 G allele and 28 T allele homozygotes and 44 heterozygotes. Genotype frequencies for both samples were in Hardy-Weinberg equilibrium (sample 1: χ² = .096, p = .76; sample 2: χ² = 1.013, p = .31). Allele frequencies did not deviate from those previously reported for Caucasians (50.8% G, 49.2% T; http://hapmap.ncbi.nlm.nih.gov/index.html.en) in either sample 1 (χ² = 1.09, p = .296) or sample 2 (χ² = .260, p = .610).

Results

Adenyate Cyclase 7 Transcripts in the Amygdala of Humans with MDD and SertKO Mice

We first generated large-scale gene expression data in the amygdala and cingulate cortex of SertKO and WT mice. Exploratory analyses revealed large numbers of changes affecting 2.2% and 8.0% of detected genes, respectively (Table S1 in Supplement 2). Fifty-five genes were affected in both regions with 38 genes in the same direction (Pearson correlation for the 55 genes, R = .77, p < 1 × 10-7), suggesting similar biological impacts of the SERT deletion across areas. We then compared gene expression changes in SertKO mice with changes observed in human postmortem expression data sets previously generated in a cohort of male subjects with familial MDD and matched control subjects (16). These unbiased comparisons revealed 31 genes in the amygdala and 20 genes in the cingulate (i.e., anterior subgenual cingulate
A widely utilized and well characterized blood oxygen level-dependent (BOLD) paradigm was employed in both samples to elicit robust bilateral threat-related amygdala reactivity (Figure 1A, B; sample 1: right amygdala \( y = 22, z = -4, r = -0.14 \); \( T = 13.59, p < .00001, k_e = 158 \); left amygdala \( x = -20, y = -6, z = -16 \); \( T = 13.32, p < .00001, k_e = 147 \); sample 2: right amygdala \( x = 20, y = -8, z = 16 \); \( T = 17.27, p < .00001, k_e = 103 \); left amygdala \( x = -20, y = -8, z = -16 \); \( T = 13.38, p < .00001, k_e = 106 \), familywise error corrected across amygdala region of interest). The single-subject mean blood oxygen level-dependent values from these amygdala clusters were entered as dependent variables in an independent-samples t test with ADCY7 rs1064448 genotype as the independent variable.

In both samples, we found a significant effect of rs1064448, wherein carriers of the T allele had greater left amygdala reactivity in comparison with G allele homozygotes (Figure 2B, D; sample 1: \( T = 80, p = .011 \); sample 2: \( T = 96, p = .017 \)). In addition, T allele carriers also exhibited significantly greater right amygdala reactivity in the larger second sample (sample 1: \( T = 2.40, p = .018 \); Figure 2D). These effects remained significant when controlling for gender (p values < .015), and there were no significant gender-genotype interactions (p values > .37). Importantly, the results did not change significantly when current alcohol use (number of drinks in past month), the biology of which has been linked to ADCY7 in prior research (24), was included as an additional covariate (sample 1: \( F(1.78) = 6.74, p = .011 \) for left amygdala; sample 2: \( F(1.94) = 6.09, p = .015 \) and \( F(1.94) = 5.40, p = .022 \) for right and left amygdala, respectively). Finally, the effect of genotype on amygdala reactivity was also independent of current depressive symptomatology, as assessed by the Beck Depression Inventory (sample 1: \( F(1.78) = 9.35, p = .003 \) for left amygdala; sample 2: \( F(1.94) = 6.07, p = .016 \) and \( F(1.94) = 6.39, p = .013 \) for right and left amygdala, respectively). It is worth noting, however, that while the depressive symptoms in the current sample were very low overall (sample 1: \( 2.66 \pm 2.93 \); sample 2: \( 3.91 \pm 4.09 \)), increased amygdala reactivity may be indicative of an underlying affective disorder vulnerability that is present before the development of any overt depressive symptomatology.

**Discussion**

Comparative gene expression analyses between human post-mortem samples of subjects with major depression and SERTKO mice, a rodent model replicating features of human depression, identified gene transcript changes in ADCY7 as a cross-species correlate of altered mood/emotionality. Further imaging genetics studies demonstrated a significant effect of a common human...
ADCY7 SNP (rs10644448) on threat-related amygdala reactivity in two independent samples. Together, the results suggest a role for ADCY7 in molecular and neuronal mechanisms regulating affect and mood regulation, as well as the pathophysiology of MDD.

Differential gene expression profiles identified an increased Adcy7 gene expression in Sert减肥 mice and humans with MDD. The Adcy7 gene encodes adenylate cyclase 7, a membrane-bound protein that catalyzes the synthesis of cyclic adenosine monophosphate (cAMP) and supports long-term amplified signal cascade within the cell. The cAMP pathway, including downstream targets protein kinase A and cAMP response element binding protein (CREB), has been implicated in depression previously (35). Various antidepressant treatments increase cAMP levels and CREB expression (36), leading to cAMP response element-mediated increases in brain-derived neurotrophic factor, among other molecules implicated in depression. Altered cAMP function in depression is also supported by reports of reduced CREB levels in postmortem brains of patients with MDD (37,38) and in suicide victims (32,39,40). Hence, since multiple redundant biological systems regulate cAMP levels in the brain, the information provided in this report participates in considerably narrowing down the region- and gene-specificity of effect for follow-up studies. Specifically, our cross-species studies point to the amygdala as a region of interest for adenylate cyclase 7 modulation of cAMP levels, potentially leading to emotion regulation and associated pathophysiology.

Here, we could not confirm changes in adenylate cyclase 7 expression at the protein level in human samples, despite repeated attempts using three different antibodies (Supplement 1). Thus, it is unclear how upregulation of ADCY7 could result in decreased cAMP signaling predicted in MDD. Typically, neuronal activity results in increased calcium influx, leading to altered activity in respective adenylate cyclase isoforms. Notably, an important contrast has to be made between adenylate cyclases that are activated by calcium (isoforms 1 and 8) and those that are inhibited by calcium. This latter group includes adenylate cyclase 5 (ADCY5) and 7 (ADCY7) isoforms (31), for which increased neuronal activity is expected to lead to decreased adenylate cyclase activity. Accordingly, combinatorial changes in adenylate cyclase isoforms may differentially modulate cAMP and alter depression-related behaviors in mutant mice. For instance, adenylate cyclase 1 (Adcy1) and Adcy8 double knockouts showed increased depressive-like behavior, while calcium-inhibited adenylate cyclase 5 (Adcy5) knockouts showed normal behavior in the sucrose preference test but decreased depressive-like behavior in the forced swim test and decreased anxiety-like behaviors (41). Interestingly, ADCY5 and ADCY7 are also targets of the mood stabilizer lithium (42). Using knockout and transgenic mutant lines, Hines et al. (31) showed higher Adcy7 expression associated with more depressive-like behavior and lower Adcy7 expression with less depressive-like behavior, suggesting that ADCY7 level may regulate the risk to develop MDD and that blocking or down-regulating ADCY7 may have an antidepressant effect. Together, the independent identification of ADCY7 by our cross-species unbiased microarray survey of gene expression provides supporting evidence for a role of ADCY7 in MDD and indirectly for altered cAMP signaling in the amygdala, a crucial hub region in emotion regulation that is affected in MDD.

Imaging genetics revealed robust effects of ADCY7 rs10644448 on threat-related amygdala reactivity in two independent samples of healthy middle-aged community volunteers. In comparison with G allele homozygotes, T allele carriers exhibited significantly increased left amygdala reactivity in both samples and right amygdala reactivity in the second larger sample. The G allele of rs10644448 is part of a previously reported ADCY7 haplotype, which includes the 7-repeat allele of a functional tetranucleotide polymorphism, and has been associated with increased risk for major depression in women (31). Our replicated finding of increased amygdala reactivity in T allele carriers may superficially seem at odds with this clinical association. However, it is important to highlight several conceptual and methodological issues regarding the mapping of common genetic polymorphisms onto neurobiological and clinical phenotypes.

First, genetic polymorphisms have more direct effects on proximate biological processes than on distal behavioral or clinical phenomena (41). This is clear in the high frequency of replicated associations between polymorphisms and neural phenotypes through imaging genetics, including the current replication of ADCY7 rs10644448 effects on amygdala reactivity, even in relatively small samples (32,42,43). In striking contrast, genetic associations with
clinical phenotypes, even in large samples, seldom replicate (44–46). Second, the nosological category of MDD is quite heterogeneous and comprises cases with diverse clinical profiles that likely reflect distinct underlying neural alterations. The use of the current clinical definition of MDD in genetic association studies might thus hinder our ability to effectively map genetically driven variability in neurobiology onto behavior (47). Of particular relevance to the current study and attesting to the putatively heterogeneous nature of MDD is a mixed literature showing heightened amygdala reactivity in some patient samples and in response to certain fMRI paradigms (14,48) but not others (49,50). Thus, it is possible that relative increased amygdala reactivity associated with the T allele of rs1064448 may predispose to a subtype of depression, the pathophysiology of which may only underlie a fraction of all cases meeting the DSM-IV diagnostic criteria (51). In addition, since threat-related amygdala reactivity correlates with trait anxiety (52), this bias may contribute to a broader risk endophenotype that cuts across nosological categories, leaving one vulnerable to not only depression but also a range of mood and anxiety disorders. Nonetheless, caution must be used when generalizing these results to vulnerability to MDD as it is defined in the DSM-IV (51).

Finally, the ADCY7 depression risk haplotype, as well as the ADCY7 overexpression effects, reported by Hines et al. (31) emerged only in females, whereas the rs1064448 T allele effect on amygdala reactivity in either of our samples was independent of gender. Thus, although the G allele may be part of a haplotype associated with increased risk in a gender-specific manner, rs1064448 might represent a regulatory locus with properties at least partially independent of those attributable to this risk haplotype. The lack of gender effects in the current study could be attributed to a number of additional differences between the current study design and that of Hines et al. (31). While the original study demonstrated that Adcy7 overexpression results in a host of depressive phenotypes in mice (31), here we demonstrate increased Adcy7 expression in the cingulate and amygdala of SERT knockout mice, which show a more generalized heightened emotionality phenotype. In addition, we found increased amygdala and anterior cingulate cortex ADCY7 transcripts in postmortem tissue of patients with depression and heightened in vivo amygdala reactivity as a function of ADCY7 genotype. Given the broader heightened emotionality phenotype of Sert knockout mice, along with the fact that anterior cingulate dysregulation and relatively increased reactivity of the human amygdala have been implicated in the pathophysiology of both anxiety and mood disorders (13,14,53–55), our findings identify adenylyl cyclase 7 as an important regulator of affective neural circuitry, which may contribute to individual variability in affective disorder risk more generally. Moreover, as has been established in epidemiologic samples, depression and anxiety are highly comorbid, likely reflecting a common biological basis (56). Thus, while prior studies showing gender-specific effects of adenylyl cyclase 7 have focused on depressive-like, as distinct from anxiety-like, phenotypes (31), here we associate adenylyl cyclase 7 with a broader heightened emotionality phenotype, for which its effects may be more equivalent between genders.

Regardless of this superficial incongruity, the interpretation of our current findings is limited because the functional effects of rs1064448 are unknown. Given the convergent findings showing increased ADCY7 expression levels in the amygdala of SERT knockout mice and human subjects with MDD, it is reasonable to hypothesize that the T allele of this 3’ untranslated region polymorphism may be associated with similarly increased ADCY7 levels, which may, in turn, result in the observed increase in threat-related amygdala reactivity. The current postmortem cohort (n = 28) is underpowered to detect changes in transcript/protein levels in association with genetic variants, so additional molecular genetics studies in large postmortem cohorts that are powered to detect genetic effects on transcript levels are needed to further test this conjecture and clarify the regulatory role of rs1064448 on ADCY7 expression and amygdala reactivity. Limitations notwithstanding, the current results provide cross-species evidence implicating adenylyl cyclase 7 in a promising biological mechanism of affective disorder vulnerability that merits further investigation.

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Supplementary material cited in this article is available online.


