Genetic Variation in Cholinergic-Muscarinic-2 Receptor Gene Modulates Muscarinic 2-Receptor Binding In Vivo and Accounts for Reduced Binding in Bipolar Disorder

DM Cannon1,2,*, JK Klaver2, SK Gandhi2, G Solorio2, SA Peck2, K Erickson2, J Savitz, N Akula2, WC Eckelman3, ML Furey2, BJ Sahakian4, FJ McMahon2, and WC Drevets2

1 Clinical Neuroimaging Lab, Department of Psychiatry, National University of Ireland, Galway, Co. Galway, Ireland 2 Mood & Anxiety Disorders Program, National Institutes of Mental Health, NIH, DHHS, Bethesda, MD 20892, U.S.A 3 Molecular Tracer LLC, Bethesda MD 20814, U.S.A 4 Department of Psychiatry, University of Cambridge School of Clinical Medicine, Addenbrooke’s Hospital, Cambridge CB2 2QQ, U.K

Abstract

Genetic variation in the cholinergic-muscarinic2 (M2) receptor gene (CHRM2) has been associated with the risk for developing depression. We previously reported that M2-receptor distribution volume (VT) was reduced in depressed subjects with bipolar disorder (BD) relative to depressed subjects with major depressive disorder (MDD) and healthy controls (1). In the current study we investigated the effects of six single nucleotide polymorphisms (SNP) for CHRM2 on M2-receptor binding to test the hypotheses that genetic variation in CHRM2 influences M2-receptor binding and that a CHRM2 polymorphism underlies the deficits in M2-receptor VT observed in BD. The M2-receptor VT was measured using PET and [18F]FP-TZTP in unmedicated, depressed subjects with BD (n=16) or MDD (n=24) and healthy controls (n=25), and the effect of genotype on VT was assessed. In the controls one SNP (with identifier rs324650, in which the ancestral allele adenine (A) is replaced with one or two copies of thymine (T), showed a significant allelic effect on VT in the pregenual and subgenual anterior cingulate cortices in the direction AA<AT<TT. In contrast, in BD subjects with the TT-genotype VT was significantly lower than in BD subjects with the AT-genotype in these regions. The BD subjects homozygous for the T-allele also showed markedly lower VT (by 27 to 37% across regions) than healthy controls of the same genotype. Post hoc analyses suggested that T homozygosity was associated with a more severe illness course, as manifested by lower socioeconomic function, poorer spatial recognition memory and a greater likelihood of having attempted suicide. These data represent novel preliminary evidence that reduced M2-receptor VT in BD is associated with genetic variation within CHRM2. The differential impact of the M2-receptor polymorphism at rs324650 in the BD and HC samples suggests interactive effects with an unidentified vulnerability-factor for BD.

Disclosure Statement: No author has potential conflicts of interest related to this work.
Keywords
Depression; Anxiety; Muscarinic M2 binding; CHRM2; G-protein coupled receptor; [18F]FP-TZTP; Positron Emission Tomography

Introduction

A variety of indirect evidence has implicated the central muscarinic-cholinergic system, and more specifically the type-2 muscarinic (M2) receptor, in the pathophysiology of depressive symptoms arising in major depressive disorder (MDD) and bipolar disorder (BD) [reviewed in (1)]. We previously used positron emission tomography (PET) and [18F]FP-TZTP (3-(3-((3-fluoropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyridine) to investigate muscarinic cholinergic receptor binding in MDD and BD, and found that this radioligand’s distribution volume (VT) was reduced in the cingulate cortex in BD subjects relative to both healthy controls and MDD subjects (1). In the cingulate cortex (and in most brain regions) [18F]FP-TZTP binding is relatively selective for M2-receptors (2). Moreover, [18F]FP-TZTP binds to M2-receptors as an agonist, putatively accounting for this radioligand’s sensitivity to intrasynaptic ACh concentrations (3). The abnormal reduction in [18F]FP-TZTP binding in the cingulate cortex of BD subjects thus suggested that either the intrasynaptic acetylcholine concentration was increased or the density or affinity of M2-receptors was decreased in bipolar depression.

Conceivably, these observations in BD may reflect genetic variation in the gene coding for the M2-receptor (CHRM2). CHRM2 contains several single nucleotide polymorphisms (SNPs) that have been associated with the risk for developing major depressive episodes (4–6). Genetic variation in the 3′ region of the CHRM2 gene (A/T 1890, rs8191992) has been associated with MDD in females (5). In families with both an alcohol-dependent proband and relatives with MDD, Wang et al. (4) showed an association between two SNPs in intron 4 of the CHRM2 gene and depression. Wang et al. (4) also identified a T-T-T haplotype (rs1824024-rs2061174-rs324650) that was under-transmitted to individuals who manifested both alcohol dependence and co-morbid MDD, and this group subsequently identified the risk-influencing locus for affective disorders as rs324650 in European-Americans (6). In a study of MDD cases not selected on the basis of having co-morbid alcoholism, however, no association was identified between this haplotype and MDD (7). Although no direct association between CHRM2 and BD has been reported, linkage and sib-pair studies found associations between the risk for BD and genetic variation in the vicinity of CHRM2. The CHRM2 gene is located in the q31–35 region of chromosome 7 (8) and evidence for linkage was reported for 7q31 (LOD=2.08)(9, 10) and 7q34 (LOD=2.78) in affected sib-pair analyses of families of BD probands (11). Moreover, a study of 27 SNPs across the CHRM2 gene demonstrated an association between CHRM2 and “externalizing psychopathology” as a broader conceptualization of psychiatric disorders that encompassed symptoms or syndromes that occur comorbidly with mood disorders, such as substance dependence (12).

In addition, CHRM2 function has been associated with cognitive domains that are impaired in individuals with BD. Neuropsychological studies have shown that BD subjects manifest
impairments in attention, memory and social cognition, which in some cases appear trait-like, insofar as they are evident in unaffected relatives of bipolar probands (13). For example, deficits in verbal memory have been identified in currently depressed subjects with MDD or BD, as well as in unaffected twin and non-twin siblings of BD subjects (14–16). The CHRM2 gene conceivably may influence function across a range of cognitive domains through its role in generating or modulating evoked electrophysiological oscillations (17–19), as the development of theta and delta event-related oscillations which play critical roles in decision making (20, 21), selective attention (22), recognition memory and episodic retrieval (23–27) are dependent upon muscarinic cholinergic receptor stimulation. Consistent with such a far-reaching influence, genetic variation in CHRM2 has been shown to influence performance intelligence quotients (PIQ)(28–32).

Acetylcholine neurotransmission has been linked to the regulation of mood (33–35), sleep (36, 37) and neuroendocrine function (38–41) by preclinical and clinical evidence. In studies of MDD and BD, increasing cholinergic transmission via administration of muscarinic receptor agonists or acetylcholinesterase inhibitors exacerbates depressive symptoms in both illnesses and reduces manic symptoms in BD (42–44). Moreover, neurophysiological responses to muscarinic receptor–agonist challenge are exaggerated both in subjects with current depression and in subjects with remitted MDD or BD relative to controls (45, 46). Since the muscarinic cholinergic system has been shown to play roles in evaluating and learning the salience of sensory stimuli (47), the increased muscarinic sensitivity evidenced in individuals with mood disorders conceivably may contribute to the altered perceptions of emotionally-valenced events reported in these conditions (48).

In healthy humans, administration of the M2 antagonist procaine, which putatively increases intrasynaptic ACh concentrations, elicits a spectrum of robust emotional responses, ranging from sadness, anxiety and fear to euphoria (49), resembling the spectrum of emotional symptoms manifested in BD. These responses were associated with physiological activation of limbic structures, primarily the anterior cingulate cortex (ACC)(50), a region densely innervated by cholinergic projections from the basal forebrain that also has been implicated in the pathophysiology of MDD and BD by neuroimaging and neuropathological evidence. In this and other structures the M2-receptor is expressed both presynaptically and postsynaptically, and the presynaptic M2-receptor constitutes one of the predominant muscarinic inhibitory autoreceptor subtypes (i.e., receptor stimulation decreases ACh release)(51), conferring it with a major influence over cholinergic transmission.

The current study characterized relationships between the V_T values obtained in our previous study and six SNPs within CHRM2 to test the hypothesis that genetic variation in this gene influences M2-receptor receptor binding to [18F]FP-TZTP in healthy humans. For SNPs where such an effect was demonstrated we additionally examined interactions between genetic variation in CHRM2, diagnosis and regional [18F]FP-TZTP V_T to test the hypothesis that a genotype-by-diagnosis interaction accounts for the abnormal reduction in regional V_T observed in BD (52). Finally, post hoc analyses examined the effects of variation in CHRM2 on performance on tests of intelligence (28–31), memory, attention or executive function (53–55) and explored interactions between V_T, genetic variation, cognitive function and mood disorders.
Materials and Methods

Participants

Subjects ages 18 to 50 who either were psychiatrically healthy (n=25) or met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for a current major depressive episode (n=40) were recruited through advertisements in local media, the NIMH Outpatient Clinic or the Howard University School of Medicine. The depressed subjects additionally met criteria for either BD (n=16) or recurrent-MDD (n=24) using the DSM-IV criteria. Exclusion criteria included exposure to psychotropic drugs including nicotine or medications with anticholinergic activity within the three weeks prior to scanning, major medical or neurological illnesses, lifetime history of substance dependence including nicotine, substance abuse within 1 year, and current pregnancy or breast feeding. Additional exclusion criteria applied to the healthy control sample included having a personal or family history of a major psychiatric disorder. Subjects provided written informed consent as approved by the NIMH IRB.

Clinical Assessments

Mood and anxiety symptoms were assessed using the Montgomery-Asberg Depression (MADRS)(56), the Hamilton-Anxiety (HAM-A)(57) and the Young Mania Rating scales (YMRS)(58). Socioeconomic status (SES) scores were determined based on the level of education and employment attained (59). The family history of psychiatric disorders was assessed using the Family Interview for Genetics Studies (60). All assessments were performed at the time of scanning.

Genotyping

Blood was sampled in all subjects for genotyping. Genotype data spanning the coding region and ~2 kb of flanking sequence were downloaded from the International HapMap Project (version 1.0, accessed 11/2004). Based on these data, a relatively uncorrelated set ($r^2 < 0.8$) of common markers (minor allele frequency $>7.5\%$ in persons of European descent) was selected for genotyping. Six SNPs were selected to assess variation in the CHRM2 gene: rs7810473; rs1824024; rs2061174; rs2350786; rs324650 and rs8191992. These SNPs were included on a chip that included a larger set of 768 SNPs in 68 candidate genes, as detailed in McMahon et al. (61). Samples were shipped to Illumina, Inc., San Diego, California, where they were genotyped on an assay(62) with >99% success and >99% of possible genotypes returned, including blind duplicate genotypes, all of which matched exactly.

A set of 344 unlinked SNPs was used to control for ethnic differences. Using STRUCTURE (63), we estimated probability of membership in each of three ancestral populations (20,000 burn-in steps followed by 20,000 replications). These values then were used as covariates in subsequent analysis.

PET image acquisition and processing

A detailed account of the image acquisition and processing and of the modeling of distribution volume ($V_T$) was reported in Cannon et al[1]. Briefly a 120 minute dynamic PET scan was acquired using a GE Advance scanner in 3D mode (3D spatial resolution=6
mm full-width at half-maximum) following injection of 352–389 MBq of high specific activity $[^{18}\text{F}]$FP-TZTP(3, 64). Arterial blood was sampled during scanning. MRI scans were obtained using a GE Signa Scanner (3.0 Tesla) and co-registered to the PET images to provide an anatomical framework for image analysis. PET data were corrected for partial-volume effects frame-by-frame before kinetic modeling(3). The primary outcome parameter was the $[^{18}\text{F}]$FP-TZTP $V_T$, which is proportional to the product of receptor density and affinity. The arterial input function for $[^{18}\text{F}]$FP-TZTP was generated by quantifying the plasma concentration of parent $[^{18}\text{F}]$FP-TZTP using a hexane-extraction procedure(65) in 28 serial blood samples drawn at increasing intervals from a radial artery cannula (number_frame duration [in minutes]: 6_0.25, 5_0.5, 2_1, 3_2, 1_3, 5_5, 2_10, and 4_15). Using quantitative tracer kinetic modeling and a one-tissue compartment model the regional $V_T$ (modeled as $K_1/k_2$ where $K_1$ is the rate of delivery of $[^{18}\text{F}]$FP-TZTP and $k_2$ is the rate of clearance) and $K_1$ values were obtained from the arterial input function and the regional tissue time radioactivity concentration curves (3). $V_T$ was corrected for protein binding of the parent radioligand by dividing by the plasma free fraction ($f_p$). The $V_T$ was modeled from the averaged radioactivity concentrations within 10 regions-of-interest (ROI): whole brain, subgenual anterior cingulate (sgACC), pregenual anterior cingulate (pgACC), posterior and dorsal cingulate cortices, amygdala, hippocampus, ventral striatum, lateral orbital cortex and primary visual cortex.

**Cognitive Assessments**

The intelligence quotient (IQ) was assessed by the Weschler Abbreviated Intelligence Scale(WAIS; performance and verbal T-scores). Attention and executive function were evaluated using the computerized Rapid Visual Information Processing (RVIP) and Intradimensional/Extradimensional Shift (ID/ED) tasks, respectively. We assessed memory using the Delayed Match to Sample (DMS) and spatial recognition memory (SRM) tasks. These computerized tasks were from the Cambridge Neuropsychological Test Automated Battery (CANTAB; Cambridge Cognition Ltd, Cambridge, UK) and were presented on an Advantech computer (Model PP-120T-RT) with a 10.5 inch touch-screen monitor.

**Statistics**

The hypothesis that genetic variation in $CHRM2$ influenced $M_2$-receptor binding was tested in healthy controls by examining effects on $V_T$ at each SNP allele-wise using two-sample t-tests in the 10 ROI listed above. The hypothesis that an interaction between diagnosis and genetic variation in $CHRM2$ accounted for the lower $V_T$ in depressed BD subjects (1) was tested using a linear mixed-model with an unstructured model for covariance in the regions where the greatest $M_2$-receptor binding deficits were observed in the BD subjects in our original study: the pgACC and sgACC. Corrections for multiple testing in these two regions were performed by calculating the false discovery rate (FDR) adjusted p-value (66). Results with $p_{FDR}<0.05$ were considered significant. All uncorrected p-values given are denoted $p_{UNC}$. The specificity of these findings to the ACC was assessed post hoc by examining the relationships between genotype and $V_T$ in the other eight ROI examined in the HC sample. The normality of the $V_T$ data was assessed using the Shapiro-Wilk test, which showed the data were normally distributed ($0.98<W<0.99, 0.23<p<0.92$) without influential outliers (based on an individual $V_T$ value $>\text{mean}\pm3\times\text{SD}$). Gender distribution across genotype was
examined using Pearson’s Chi-squared test and mean age across diagnostic groups was examined using ANOVA.

For any SNP where an interaction between diagnosis and $V_T$ was significant, post-hoc exploratory analyses were conducted using Pearson’s Chi-squared tests to assess the relationships between genotype and: 1) the likelihood of having a past suicide attempt, 2) the presence of a first-degree relative with BD, and 3) current psychosocial function, as reflected by the socioeconomic status scores. In addition, secondary analyses explored relationships between $V_T$, genotype and performance on tasks of intelligence (28–31), memory, attention or executive function (53–55) using Pearson’s or Spearman’s correlations depending on the normality of distribution of the performance variables. Eight variables were assessed: performance IQ (PIQ T-score), verbal IQ (VIQ T-score), attention performance (RVIP: correct detections of the target sequences and omission errors), memory performance (DMS: % total correct; SRM: % correct), and executive function performance (ID/ED Shift-completed stage trials [i.e. number of trials taken to complete a stage, adjusted for stages completed] and errors [adjusted for trials completed]). IQ (PIQ $p_{UNC}=0.26$) and performance on the task of attention (RVIP correct detections $p_{UNC}=0.76$, RVIP omission errors $p_{UNC}=0.63$) were normally distributed. Therefore, their relationship to $V_T$ was examined using Pearson’s bivariate correlations. Performance on tasks of memory (DMS percent correct $p_{UNC}=0.051$, SRM percent correct $p_{UNC}=0.003$), executive function (ID/ED shift stage trials $p_{UNC}=0.0001$, and errors $p_{UNC}=0.0001$) and verbal IQ ($p_{UNC}=0.061$) were considered non-normally distributed and Spearman’s correlations performed thereafter. Due to the large number of comparisons, results were not reported unless p-values would remain significant after applying FDR corrections for eight tests.

Finally, based on the literature reporting relationships between the CHRM2 gene and intelligence, an exploratory analysis was conducted to assess associations between average IQ (PIQ+VIQ T-scores) and genotype for all six CHRM2 SNPs.

**Results**

Mean age (HC 33±6.5, MDD 34±8.6, BD 32±7.7, $F=0.28$, $p_{UNC}=0.77$) and gender distribution (group{n female/total n}, HC{14/25}, MDD{18/24}, BD{12/16}) did not differ significantly across diagnostic groups ($\chi^2=2.54$, $p_{UNC}=0.28$). Genotype frequencies did not differ significantly across subject samples for any of the six SNPs (table 1). Three of the BD subject had BD Type I. Only one BD subject had a psychotic episode in the past. On ratings of depression severity and anxiety symptoms the MDD and BD groups did not differ significantly from each other (p>0.2) but rated higher than the HC group (p<0.001) (MADRS MDD: 22±6.9, BD: 25±8.1, HC: 0.3±0.7; HAMA MDD:13±5, BD: 16±6, HC: 0.3±0.7). The BD group had a higher YMRS score (4.8±3.5) than the MDD (3.2±2.0) and HC groups (0.2±0.6, $p_{UNC}=1.15\times10^{-8}$). The HC group had a higher SES score (53±8.6) than the BD (42±10) or MDD (44±12, $p_{UNC}=0.002$) groups and the MDD and BD group did not differ significantly from each other.

Genotype in five of the six CHRM2 SNPs assessed did not relate significantly to $[^{18}\text{F}]$FP-TZTP binding among the HC subjects. In healthy controls the allele-wise testing revealed
higher $V_T$ associated with the T-allele for SNP rs324650 in the pgACC and sgACC (Fig. 1). Similar associations were observed in the whole brain, amygdala, ventral striatum and lateral orbital cortex ($p_{\text{uncorrected}}<0.05$) but these did not remain significant after applying corrections for multiple comparisons. In contrast, in the MDD or BD samples no significant allele-based difference existed in any region.

The interaction between diagnosis, rs324650 genotype and $V_T$ was significant in the pgACC ($F=3.62$, $p_{\text{FDR}}=0.04$) and reached a trend level in the sgACC ($F=3.89$, $p_{\text{FDR}}=0.06$). Post-hoc exploratory analyses showed similar relationships in the amygdala ($F=4.30$, $p_{\text{UNC}}=0.02$), hippocampus ($F=5.42$, $p_{\text{UNC}}=0.01$; Fig. 2) and lateral orbital cortex ($F=3.68$, $p_{\text{UNC}}=0.04$). The interaction was accounted for by reduced $[^{18}\text{F}]$FP-TZTP $V_T$ in BD subjects who were T-homozygotes relative to HC-subjects of the same genotype ($0.013<p_{\text{UNC}}<0.022$ in the regions listed above). The T-homozygous BD subjects also showed lower $V_T$ relative to both BD-heterozygotes ($0.031<p_{\text{UNC}}<0.046$) and to HC-heterozygotes ($0.006<p_{\text{UNC}}<0.019$, Fig. 2). Post-hoc assessments revealed no other genotype-by-diagnosis interactions involving the five other $CHRM2$ SNPs examined ($p>0.1$).

Of the clinical variables considered the proportion of cases with past suicide attempts differed by genotype at rs324650 (Table 2). Five of the six BD subjects homozygous for the T-allele previously had attempted suicide, compared to only three of the ten BD subjects who were A-carriers ($p=0.039$). The proportion of BD subjects who had a first-degree relative with BD showed a non-significant trend toward being higher in T-homozygotes than in A-carriers ($p=0.053$). Finally, the BD subjects homozygous for the T-allele showed lower socioeconomic status scores (mean±SD: 33±9.7, $t=3.8$, $p=0.002$) than A-carrier BD subjects (mean±SD: 48±6.0). The MADRS scores did not differ significantly between the T-homozygotes and A-carriers from the BD sample ($t=-0.65$, $p_{\text{UNC}}=0.52$).

Performance on tests of attention and memory was impaired in the BD and MDD groups versus the control group (Fig. 3A). The number of omission errors on the RVIP task and the percentage of correct responses on the DMS task differed across groups ($F=5.12$, $p=0.009$ and $F=3.58$, $p=0.035$, respectively). These differences were attributable to poorer performance in the MDD and BD groups relative to the HC group (Fig. 3A) and not to differences between the MDD and BD groups. Performance on the ID/ED Shift task, intelligence, memory or attention tests did not correlate significantly with $V_T$ in any region in the HC, BD or MDD groups.

Assessments of the relationship between $CHRM2$ rs324650 genotype and cognitive performance revealed that BD subjects homozygous for the T-allele showed poorer spatial recognition memory on the SRM ($t=3.36$, $p=0.005$; Fig. 3B) relative to A-carrier BD subjects. These subgroups did not differ on the other neuropsychological test measures considered. In MDD, spatial working memory performance (DMS percent correct) was poorer in those possessing the rs324650 AA-genotype relative to T-carriers ($F=7.81$, $p=0.003$). In healthy controls none of the cognitive performance measures differed significantly across $CHRM2$ rs324650 genotypic variants.

*Mol Psychiatry.* Author manuscript; available in PMC 2011 October 01.
Exploratory analyses of the association between genetic variation at other CHRM2 markers and cognitive performance showed that in the entire study sample, individuals homozygous for the T-allele of SNP rs2061174 had a higher average IQ (122±12, F=3.9, p=0.026) than C-carriers (CT: 113±13; CC: 112±11).

Discussion

Genetic variation within the CHRM2 gene influenced the binding of [18F]TZTP to M2-receptors in healthy controls and accounted for the abnormal reduction in M2-receptor binding previously reported in subjects with bipolar depression. This genetic variance was associated with a SNP involving an adenine-to-thymine substitution at marker rs324650 within the CHRM2 gene. Our data thus implicate either this SNP or a distinct possibly nearby variant in high linkage-disequilibrium with rs324650. The rs324650 SNP was associated with an allelic effect on M2-receptor binding (VT) in healthy humans such that the T-allele was associated with higher VT than the A-allele (figure 1). This effect accounted for 20% (partial eta^2=0.20) of the total variance in VT. This is similar to the 28% contribution that variance in the HTR2A gene coding for the 5-HT2A receptor accounted for 5-HTT binding ([11C]DASB PET) in the thalamus (67) in a recent study of a similar design. The rs324650 SNP also was associated with an interaction between VT and diagnosis such that while BD subjects with either AA or AT genotypes did not differ from their respective control subgroups, BD subjects with the TT-genotype showed 27 to 37% reductions in VT relative to TT controls across brain regions (figure 2). This difference among the T-homozygote’s appeared to account for the abnorma reduction in [18F]FP-TZTP VT found previously in the entire BD sample relative to the healthy control sample [1]. This effect accounted for 27% (partial eta^2=0.27) of the total variance in VT. Post hoc analyses suggested that within the BD sample the TT-genotype was associated with a more severe illness course, as manifested by lower socioeconomic function, poorer spatial recognition memory and a greater likelihood of having attempted suicide.

The effects of genetic variation within CHRM2 on VT were evident in brain regions where [18F]FP-TZTP binding is relatively selective for M2-receptors (2). The in vitro affinity of [18F]FP-TZTP is highest for M2-receptors (K_i=2.2 nmol/l), lower for M1-receptors (K_i=7.4 nmol/l) and negligible for other muscarinic receptors (K_i ≥80 nmol/l). Studies in muscarinic-receptor knock-out mice have shown that [18F]FP-TZTP is relatively selective for M2-receptors in most brain tissues, excepting the amygdala and hippocampus, where 20% to 23% of binding is attributable to M1-receptors. Thus to our knowledge this report that [18F]FP-TZTP binding in the ACC is influenced by genetic variation in CHRM2 constitutes the first direct link between a single altered nucleotide among a sequence coding for a receptor and radioligand binding to that receptor in vivo in the human brain.

Nevertheless, since [18F]FP-TZTP is sensitive to intrasynaptic concentrations of acetylcholine, the effect of genetic variance in rs324650 on M2-receptor binding conceivably may be attributable to differences either in M2-receptor density or affinity, or in intrasynaptic ACh-concentrations. In some brain regions the M2-receptor functions as an autoreceptor that exerts inhibitory regulation over acetylcholine release (68, 69), so genetic variation that affects autoreceptor function may influence neurotransmitter release.
Nevertheless, a several hundred percent change in endogenous acetylcholine concentrations would be required to produce the magnitude of difference in \( V_T \) found between T-homozygous BD subjects and T-homozygous controls (3), making it unlikely that the effect of genetic variation on \( V_T \) is accounted for by differences in neurotransmitter concentration alone. The effect of variation in or near rs324650 on M2-receptor binding more likely reflects an influence of this polymorphism on the regulation of gene expression or splicing to an extent that alters M2-receptor density, or on the G-protein coupling or intracellular trafficking of the receptor to the cell membrane to an extent that alters M2-receptor affinity.

A recent post-mortem study using the antagonist radioligand \[^{3}H\]AFDX detected reduced M2M-receptor binding in the dorsolateral frontal cortex (BA46) of subjects with MDD and BD while no change was detected in rostral prefrontal (BA10) and parietal (BA46) cortices and ACC was not tested (70). Another post-mortem study focused on the ACC observed no significant difference in BD relative to controls again using the antagonist radioligand \[^{3}H\]AFDX (71). The latter studies are measuring the total (high- and low-affinity state) \( M_2 \) and \( M_4 \)-receptor density (\( B_{\text{max}} \)) whereas the present study using a M2-receptor agonist, \[^{18}F\]FP-TZTP is preferentially measuring the pool of high-affinity M2-receptors present rather than the total pool (72)(discussed in Cannon et al., 2006) and therefore is more sensitive to the functional state of the M2-receptor system. Moreover, the \( V_T \) parameter is proportional to the product of density and affinity. Thus consideration of our data within the context of these post mortem data would lead to the hypothesis that the effect of the BD diagnosis on \( V_T \) reflects affinity rather than density at least in the ACC.

Despite \( CHRM2 \) having been cloned (73, 74) the regulatory regions involved in neuronal expression and the nature of their influence over promoter activity and splice variant generation are only partly understood. The SNP rs324650 resides in intron 5 of the \( CHRM2 \) gene on chromosome 7, located within a transposable element, a short interspersed repeat (SINE). SINE repeats can participate in the process of reverse transcription, whereby they drive transcription of their own transposase and cause aberrant expression of linked genes (29, 75–79). The \( CHRM2 \) gene expression also is regulated by elements within a large 5′ untranslated region (UTR) encoded by multiple exons and by intronic regions upstream of the neuronal-specific promoter 5′UTR (80). Regulators of \( CHRM2 \) expression may potentially act through transcriptional regulation, altered translation, epigenetic factors, heterodimerization, indirect interaction with other genes and endogenous regulators of receptor density, any of which may influence \( V_T \).

Notably one post-mortem study assessed the influence of \( CHRM2 \) SNPs rs324650, rs2061174 or rs324640 on gene expression in the superior and inferior parietal lobe and found no significant effect in a sample of 50 individuals (29). The in vivo measure of \( V_T \) is sensitive to a several factors (M2 receptor density, affinity, endogenous neurotransmitter concentrations) that may have not been reflected by mRNA concentrations. Nevertheless, our data further suggest that the sensitivity of future post mortem studies of the effects of the rs324650 polymorphism on \( CHRM2 \) expression may be enhanced by specific assessment of limbic structures such as the anterior cingulate cortex, amygdala and hippocampus. Genetic variation in \( CHRM2 \) does not appear to uniformly affect all brain regions. Possible explanations include currently unidentified region specific factors possibly including.
epigenetic or other spatially localized modulators of transcription, translation and/or 
expression. Several studies support this by documenting tissue specific control of gene 
expression by cis-acting SNPs (81).

Since the M₂-receptor plays a major role in the regulation of acetylcholine release, genetic 
variation within CHRM2 that alters the function of this autoreceptor could in turn alter 
cholinergic neurotransmission, and thus exert far-reaching effects on a variety of emotional 
and cognitive domains. Consistent with this expectation, associations have been reported 
previously between the M₂R gene and depression, IQ, alcoholism and Alzheimer’s Disease. 
Wang et al. (2004) and Jones et al. (2004) reported associations between two SNPs within 
intron 4 (upstream of the coding sequence) and major depressive episodes arising within the 
context of alcohol dependence (4, 18). Downstream SNPs reportedly influenced the risk for 
alcohol dependence (independently of depression) and electrophysiological event-related 
oscillations (18). Moreover, a polymorphism in the 3’ UTR of CHRM2 has been associated 
with the vulnerability for developing MDD in women (5) and with general intelligence (31). 
However, negative studies for an association between CHRM2 and MDD have also been 
reported (7). The latter may be consistent with the lack of relationship detected between 
genotype, M₂-receptor binding and the MDD group.

With respect to the CHRM2 SNP rs324650, in healthy humans this polymorphism reportedly 
influences personality traits including agreeableness, conscientiousness and openness (82). 
This latter correlation was noteworthy, since lower openness scores have been associated 
with a greater risk for developing depression (82) and, within the context of BD, suicidal 
ideation (83). Luo et al (2007) associated greater openness scores with the T-allele of 
rs324650 relative to the A-allele (p=0.029 unpublished data). Both the CHRM2 gene (28– 
32) and BD (84–87) are associated with altered cognitive function, and reduced protection 
against suicide and disability or reduced resilience (88). Therefore, we examined the 
relationship between genotype of rs324650 and suicide attempts, cognition and 
socioeconomic status (SES). BD subjects with the TT variant at rs324650 were more likely 
to have attempted suicide (table 2) and had lower SES scores and poorer spatial recognition 
memory. Nevertheless, the T-allele was not associated significantly with the likelihood of 
receiving the BD diagnosis, and in alcoholism with secondary depression a haplotype that 
included the T-allele of rs324650 appeared under-transmitted to affected individuals (4). 
Thus our data suggest the preliminary hypothesis that the TT-genotype of rs324650 or a 
polymorphism in high LD with rs324650 is associated with a more severe or disabling 
phenotype of BD, as characterized by a higher risk of attempting suicide, poorer social-
occupational function and greater cognitive impairment, without clearly altering the 
vulnerability for developing depression. Taken together these deficits in the BD group who 
are homozygous for the T-allele may reflect poorer cognitive reserve, hypothesized to confer 
protection against more severe symptoms in neuropsychiatric disorders (88, 89). However, 
due to the small sample size studied these associations should be considered preliminary and 
warrant investigation in a larger sample.

Although we did not detect a significant association or interaction with V₇ or diagnosis 
involving the CHRM2 SNPs rs7810473, rs1824024, rs2061174, rs2350786 or rs8191992, 
these negative results may have reflected power limitations due to our small samples. It is
also the case that we will not have captured some proportion of common variation and a large range of rare alleles in the present approach. We capture 49% of the common (5%) alleles at rsq>0.3 across the coding region +/- 34 kb, or 57% of common alleles across the coding region +/- 10 kb. Of the SNPs we did investigate rs8191992 previously was associated with depression among females (31). This SNP is in LD with rs324650 ($r^2=0.607$) (18), a relationship which we confirmed independently in the CEPH sample from the International HapMap Project (version 21, accessed 2/2009; $r^2=0.561$, $D^{' }=0.78$).

The relationship detected between CHRM2 and BD but not MDD add to the body of knowledge regarding genetic overlap between the two depressive disorders. A number of markers appear to confer risk for both BD and MDD such as the gene coding for the alpha-1C subunit of the L-type voltage-gated calcium channel (CACNA1A) (90) and some that appear to be selective for BD but not MDD such as the neuregulin-1 gene (NRG1) (91). The present data suggest that muscarinic cholinergic neurotransmission may be more affected by CHRM2 in BD than in MDD. Indeed it is not clear based on the current BD sample whether these findings extend to BD with psychosis or other subtypes not represented. Our data for the rs324650 T-homozygotes show an interaction with diagnosis such that the BD-TT subjects showed significantly lower V_T than the HC-TT subjects. In contrast, the TT subjects from the MDD group showed V_T values that were intermediate between, and not significantly different from, those of the HC-TT subjects and the BD-TT subjects. Our findings thus would appear to support a hypothesis that some, but not all MDD subjects may have a common genetic background with BD cases.

The secondary analyses detected an association between intelligence and the CHRM2 SNP rs2061174. This SNP previously was associated with performance intelligence, along with the CHRM2 SNPs rs2350786, rs8191992, rs2061174, rs324640 and rs324650 (28, 32). In addition, multiple SNPs spanning several LD blocks within the CHRM2 gene (intron 4–5 and intron 5–6) have been associated with performance IQ. Given the evidence that variation in CHRM2 plays a role in general cognitive performance, we cannot exclude the possibility that the apparent effect of the rs324650 TT variant on the severity of bipolar illness may be mediated by a more general effect on intelligence.

Compatible with this hypothesis, spatial recognition memory in BD individuals possessing the TT-genotype was impaired relative to A-carriers. Muscarinic cholinergic function previously has been implicated in other types of memory formation as well, including inhibitory avoidance formation (92) and consolidation of memory for salient events (93). Thus the interaction between BD and variation in the CHRM2 gene may be associated more specifically with the cognitive deficits observed with BD (84, 86, 87, 94, 95). In contrast, in MDD poorer performance on the delayed match to sample task was evident in subjects with the AA-genotype relative to T-carriers (rs324650).

In BD, increasing cholinergic transmission via administration of muscarinic-receptor agonists or acetylcholinesterase-inhibitors exacerbates depressive symptoms in both illnesses and reduces manic symptoms in BD. In addition, neurophysiological responses to muscarinic-receptor agonist challenge are exaggerated both in currently-depressed and currently-remitted MDD or BD-subjects relative to controls (46). The muscarinic-
cholinergic system generally has been shown to play roles in evaluating and learning the
salience of sensory stimuli\(^{(47)}\), suggesting that disturbances of muscarinic-function may
alter the perception of emotionally-valenced events \(^{(48)}\). It might be hypothesized that such
disturbances \((1, 4, 5, 50)\) underlie the mood dysregulation associated with BD as a result of
aberrant regulation brought about by SINE repeats within the CHRM2 gene.

In summary, we detected an allelic effect of the CHRM2 SNP rs324650 on M\(_2\)-receptor
binding in vivo in healthy humans. In addition, we found an interaction between this SNP,
M\(_2\)-binding and bipolar depression, in which the TT-genotype of rs324650 was associated
with abnormally decreased M\(_2\)-receptor binding in T-homozygotes with BD \([1]\). The
mechanism underlying the contrasting effects of the rs324650 SNP in bipolar depressives
versus healthy controls remains unclear, but conceivably may reflect an interaction between
this SNP and another genetic or environmental factor associated with bipolar disorder. If
confirmed in a larger sample, these preliminary data hold the potential to identify a subgroup
of bipolar disordered cases in which aberrant M\(_2\)-receptor expression or function plays a
major role in pathogenesis.

Acknowledgments

We acknowledge Peter Herscovitch, M.D. and the Clinical Center PET Department for providing the radioligand
and for technical assistance, Michele Drevets, R.N. and Joan Williams, R.N., for evaluation and recruitment of the
research subjects, the staff of the NIH Clinical Center, Allison Nugent, Stephen Fromm, Kelly Anastasi and
Laurentina Cizza for assistance in data management, and Dave Luckenbaugh, Ph.D. for advice regarding the
statistical analysis of the PET data.

Funding: This study was supported by the intramural research program of the NIH/NIMH and a National Alliance
for Research on Schizophrenia and Depression Young Investigator Award.

References

1. Cannon DM, Carson RE, Nugent AC, Eckelman WC, Kiesewetter DO, Williams J, et al. Reduced
muscarinic type 2 receptor binding in subjects with bipolar disorder. Arch Gen Psychiatry. 2006 Jul;
63(7):741–747. [PubMed: 16818863]
2. Jagoda EM, Kiesewetter DO, Shimoji K, Ravasi L, Yamada M, Gomez J, et al. Regional brain
uptake of the muscarinic ligand, \([18F]FP-TZTP\), is greatly decreased in M2 receptor knockout mice
but not in M1, M3 and M4 receptor knockout mice. Neuropharmacology. 2003 Apr; 44(5):653–661.
[PubMed: 12668051]
3. Carson RE, Kiesewetter DO, Jagoda E, Der MG, Herscovitch P, Eckelman WC. Muscarinic
cholinergic receptor measurements with \([18F]FP-TZTP\): control and competition studies. J Cereb
Blood Flow Metab. 1998 Oct; 18(10):1130–1142. [PubMed: 9778190]
4. Wang JC, Hinrichs AL, Stock H, Budde J, Allen R, Bertelsen S, et al. Evidence of common and
specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene
with alcohol dependence and major depressive syndrome. Hum Mol Genet. 2004 Sep 1; 13(17):
1903–1911. [PubMed: 15229186]
5. Comings DE, Wu S, Rostamkhani M, McGue M, Iacono WG, MacMurray JP. Association of the
muscarinic cholinergic 2 receptor (CHRM2) gene with major depression in women. Am J Med
Genet. 2002 Jul 8; 114(5):527–529. [PubMed: 12116189]
6. Luo X, Kranzler HR, Zuo L, Wang S, Blumberg HP, Gelernter J. CHRM2 gene predisposes to
alcohol dependence, drug dependence and affective disorders: results from an extended case-control
structured association study. Hum Mol Genet. 2005 Aug 15; 14(16):2421–2434. [PubMed:
16003161]
7. Cohen-Woods S, Gaysina D, Craddock N, Farmer A, Gray J, Gunasinghe C, et al. Depression Case
Control (DeCC) Study fails to support involvement of the muscarinic acetylcholine receptor M2

\textit{Mol Psychiatry. Author manuscript; available in PMC 2011 October 01.}
8. Saffen D, Mieda M, Okamura M, Haga T. Control elements of muscarinic receptor gene expression. Life Sci. 1999; 64(6–7):479–486. [PubMed: 10069513]

9. Detera-Wadleigh SD, Badner JA, Yoshikawa T, Sanders AR, Goldin LR, Turner G, et al. Initial genome scan of the NIMH genetics initiative bipolar pedigrees: chromosomes 4, 7, 9, 18, 19, 20, and 21q. Am J Med Genet. 1997 May 31; 74(3):254–262. [PubMed: 9184307]

10. Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G, et al. A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. Proc Natl Acad Sci U S A. 1999 May 11; 96(10): 5604–5609. [PubMed: 10318931]

11. Liu J, Juo SH, Dewan A, Grunn A, Brito M, et al. Evidence for a putative bipolar disorder locus on 2p13–16 and other potential loci on 4q31, 7q34, 8q13, 9q31, 10q21–24, 13q32, 14q21 and 17q11–12. Mol Psychiatry. 2003 Mar; 8(3):333–342. [PubMed: 12660806]

12. Dick DM, Aliev F, Wang JC, Gruca RA, Schuckit M, Kuperman S, et al. Using dimensional models of externalizing psychopathology to aid in gene identification. Arch Gen Psychiatry. 2008 Mar; 65(3):310–318. [PubMed: 18316677]

13. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain structure & function. 2008 Sep; 213(1–2):93–118. [PubMed: 18704495]

14. Gourovitch ML, Torrey EF, Gold JM, Randolph C, Weinberger DR, Goldberg TE. Neuropsychological performance of monozygotic twins discordant for bipolar disorder. Biol Psychiatry. 1999 Mar 1; 45(5):639–646. [PubMed: 10088052]

15. Keri S, Kelemen O, Benedek G, Janka Z. Different trait markers for schizophrenia and bipolar disorder: a neurocognitive approach. Psychol Med. 2001 Jul; 31(5):915–922. [PubMed: 11459389]

16. MacQueen GM, Galway TM, Hay J, Young LT, Joffe RT. Recollection memory deficits in patients with major depressive disorder predicted by past depressions but not current mood state or treatment status. Psychol Med. 2002 Feb; 32(2):251–258. [PubMed: 11866320]

17. Jones KA, Porjesz B, Almasy L, Bierut L, Dick D, Goate A, et al. A cholinergic receptor gene (CHRM2) affects event-related oscillations. Behav Genet. 2006 Sep; 36(5):627–639. [PubMed: 16823639]

18. Jones KA, Porjesz B, Almasy L, Bierut L, Goate A, Wang JC, et al. Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. Int J Psychophysiol. 2004 Jul; 53(2):75–90. [PubMed: 15210286]

19. Rangaswamy M, Porjesz B. Uncovering genes for cognitive (dys)function and predisposition for alcoholism spectrum disorders: A review of human brain oscillations as effective endophenotypes. Brain Res. 2008 Jun 24.

20. Basar E, Basar-Eroglu C, Karakas S, Schurrmann M. Are cognitive processes manifested in event-related gamma, alpha, theta and delta oscillations in the EEG? Neurosci Lett. 1999 Jan 15; 259(3): 165–168. [PubMed: 10025584]

21. Schurrmann M, Basar-Eroglu C, Kolev V, Basar E. Delta responses and cognitive processing: single-trial evaluations of human visual P300. Int J Psychophysiol. 2001 Jan; 39(2–3):229–239. [PubMed: 11163900]

22. Mitrofanis J, Guillery RW. New views of the thalamic reticular nucleus in the adult and the developing brain. Trends Neurosci. 1993 Jun; 16(6):240–245. [PubMed: 7688166]

23. Basar E, Basar-Eroglu C, Karakas S, Schurrmann M. Gamma, alpha, delta, and theta oscillations govern cognitive processes. Int J Psychophysiol. 2001 Jan; 39(2–3):241–248. [PubMed: 11163901]

24. Doppelmayr M, Klimesch W, Schweiger J, Auinger P, Winkler T. Theta synchronization in the human EEG and episodic retrieval. Neurosci Lett. 1998 Nov 20; 257(1):41–44. [PubMed: 9857961]
25. Gevins A, Smith ME, Leong H, McEvoy L, Whitfield S, Du R, et al. Monitoring working memory load during computer-based tasks with EEG pattern recognition methods. Human factors. 1998 Mar; 40(1):79–91. [PubMed: 9579105]

26. Klimesch W, Doppelmayr M, Yonelinas A, Kroll NE, Lazzara M, Rohm D, et al. Theta synchronization during episodic retrieval: neural correlates of conscious awareness. Brain research. 2001 Aug; 12(1):33–38. [PubMed: 11489606]

27. Klimesch W, Schimke H, Schwaiger J. Episodic and semantic memory: an analysis in the EEG theta and alpha band. Electroencephalogr Clin Neurophysiol. 1994 Dec; 91(6):428–441. [PubMed: 7529682]

28. Dick DM, Aliev F, Kramer J, Wang JC, Hinrichs A, Bertelsen S, et al. Association of CHRM2 with IQ: converging evidence for a gene influencing intelligence. Behav Genet. 2007 Mar; 37(2):265–272. [PubMed: 17160701]

29. Gosso FM, de Geus EJ, Polderman TJ, Boomsma DI, Posthuma D, Heutink P. Exploring the functional role of the CHRM2 gene in human cognition: results from a dense genotyping and brain expression study. BMC medical genetics. 2007; 8:66. [PubMed: 17996044]

30. Gosso MF, van Belzen M, de Geus EJ, Heutink P, Boomsma DI, et al. Association between the CHRM2 gene and intelligence in a sample of 304 Dutch families. Genes, brain, and behavior. 2006 Nov; 5(8):577–584.

31. Comings DE, Wu S, Rostamkhani M, McGue M, Lacono WG, Cheng LS, et al. Role of the cholinergic muscarinic 2 receptor (CHRM2) gene in cognition. Mol Psychiatry. 2003 Jan; 8(1):10–11. [PubMed: 12556901]

32. Posthuma D, Luciano M, Geus EJ, Wright MJ, Slagboom PE, Montgomery GW, et al. A genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. Am J Hum Genet. 2005 Aug; 77(2):318–326. [PubMed: 16001363]

33. Janowsky DS, el-Yousef K, Davis JM, Sekerke HJ. Parasympathetic suppression of manic symptoms by physostigmine. Arch Gen Psychiatry. 1973 Apr; 28(4):542–547. [PubMed: 4692153]

34. Janowsky DS, el-Yousef MK, Davis JM. Acetylcholine and depression. Psychosom Med. 1974 May-Jun; 36(3):248–257. [PubMed: 4829619]

35. Janowsky DS, el-Yousef MK, Davis JM, Hubbard B, Sekerke HJ. Cholinergic reversal of manic symptoms. Lancet. 1972a Jun 3; 1(7762):1236–1237. [PubMed: 4113219]

36. Gillin JC, Sitaram N, Duncan WC. Muscarinic supersensitivity: a possible model for the sleep disturbance of primary depression? Psychiatry Res. 1979 Jul; 1(1):17–22. [PubMed: 233154]

37. Gillin JC, Sitaram N, Mendelson WB. Acetylcholine, sleep, and depression. Hum Neurobiol. 1982; 1(3):211–219. [PubMed: 6764466]

38. Risch SC, Janowsky DS, Gillin JC. Muscarinic supersensitivity of anterior pituitary ACTH and B-endorphin release in major depressive illness. Peptides. 1983b Sep-Oct; 4(5):789–792. [PubMed: 6318208]

39. Risch SC, Janowsky DS, Gillin JC, Rausch IL, Loevinger BL, Huey LY. Muscarinic supersensitivity of anterior pituitary ACTH release in major depressive illness, adrenal cortical dissociation. Psychopharmacol Bull. 1983c; 19(3):343–346. [PubMed: 6314421]

40. Risch SC, Janowsky DS, Mott MA, Gillin JC, Kalir HH, Huey LY, et al. Central and peripheral cholinesterase inhibition: effects on anterior pituitary and sympathomimetic function. Psychoneuroendocrinology. 1986; 11(2):221–230. [PubMed: 3018822]

41. Risch SC, Kalin NH, Janowsky DS. Cholinergic challenges in affective illness: behavioral and neuroendocrine correlates. J Clin Psychopharmacol. 1981 Jul; 1(4):186–192. [PubMed: 7028800]

42. Janowsky, D.; Overstreet, D. The role of acetylcholine mechanisms in Affective Disorders. In: Bloom, Floyd E.; Kupfer, David J., editors. Psychopharmacology, The Fourth Generation of Progress. Lippincott Williams and Wilkins, Raven Press; 2000.

43. Janowsky DS, el-Yousef MK, Davis JM, Sekerke HJ. A cholinergic-adrenergic hypothesis of mania and depression. Lancet. 1972b Sep 23; 2(7778):632–635. [PubMed: 4116781]

44. Risch SC, Cohen RM, Janowsky DS, Kalin NH, Sitaram N, Gillin JC, et al. Physostigmine induction of depressive symptomatology in normal human subjects. Psychiatry Res. 1981a Feb; 4(1):89–94. [PubMed: 7012883]
45. Riemann D, Hohagen F, Bahro M, Lis S, Stadmuller G, Gann H, et al. Cholinergic neurotransmission, REM sleep and depression. J Psychosom Res. 1994; 38(Suppl 1):15–25. [PubMed: 7799246]
46. Dilsaver SC. Pathophysiology of “cholinoceptor supersensitivity” in affective disorders. Biol Psychiatry. 1986 Jul; 21(8–9):813–829. [PubMed: 3015271]
47. McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci. 2004; 27:1–28. [PubMed: 15217324]
48. Phillips ML, Drevets WC, Rauch SL, Lane R. Neurobiology of emotion perception II: Implications for major psychiatric disorders. Biol Psychiatry. 2003 Sep 1; 54(5):515–528. [PubMed: 12946880]
49. Ketter TA, Andreasen PJ, George MS, Lee C, Gill DS, Parekh PI, et al. Anterior paralimbic mediation of procaine-induced emotional and psychosexual experiences. Arch Gen Psychiatry. 1996 Jan; 53(1):59–69. [PubMed: 8540778]
50. Benson BE, Carson RE, Kiesewetter DO, Herscovitch P, Eckelman WC, Post RM, et al. A potential cholinergic mechanism of procaine’s limbic activation. Neuropsychopharmacology. 2004 Jul; 29(7):1239–1250. [PubMed: 14997171]
51. Langer SZ. 25 years since the discovery of presynaptic receptors: present knowledge and future perspectives. Trends Pharmacol Sci. 1997 Mar; 18(3):95–99. [PubMed: 9137779]
52. Cannon DM, Ichise M, Rollis D, Klaver JM, Gandhi SK, Charney DS, et al. Elevated serotonin transporter binding in major depressive disorder assessed using positron emission tomography and [11C]DASB; comparison with bipolar disorder. Biol Psychiatry. 2007 Oct 15; 62(8):870–877. [PubMed: 17678634]
53. Burk JA, Sarter M. Dissociation between the attentional functions mediated via basal forebrain cholinergic and GABAergic neurons. Neuroscience. 2001; 105(4):899–909. [PubMed: 11530228]
54. McGaughy J, Everitt BJ, Robbins TW, Sarter M. The role of cortical cholinergic afferent projections in cognition: impact of new selective immunotoxins. Behav Brain Res. 2000 Nov; 115(2):251–263. [PubMed: 11000424]
55. Sarter M, Bruno JP. Abnormal regulation of corticopetal cholinergic neurons and impaired information processing in neuropsychiatric disorders. Trends Neurosci. 1999 Feb; 22(2):67–74. [PubMed: 10092046]
56. Montgomery S, Asberg M. A new depression scale designed to be sensitive to change. Brit J Psychiat. 1979; 134:382–389.
57. Hamilton M. The assessment of anxiety states by rating. Brit J Med Psychol. 1959:50–55. [PubMed: 13635808]
58. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. British Journal of Psychiatry. 1978; 133:429–435. [PubMed: 728692]
59. Hollingshead, A. Four factor index of social status. New Haven (Connecticut): Department of Sociology, Yale University; 1975.
60. NIMH Genetics Initiative. Family Interview for Genetic Studies.
61. McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, et al. Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. Am J Hum Genet. 2006 May; 78(5):804–814. [PubMed: 16642436]
62. Gunderson KL, Kruglyak S, Graige MS, Garcia F, Kermani BG, Zhao C, et al. Decoding randomly ordered DNA arrays. Genome Res. 2004 May; 14(5):870–877. [PubMed: 15078854]
63. Pritchard JK, Stephens M, Rosenberg NA, Donnelly P. Association mapping in structured populations. Am J Hum Genet. 2000 Jul; 67(1):170–181. [PubMed: 10827107]
64. Kiesewetter DO, Vuong BK, Channing MA. The automated radiosynthesis of [18F]FP-TZTP. Nucl Med Biol. 2003 Jan; 30(1):73–77. [PubMed: 12493545]
65. Ma Y, Kiesewetter DO, Jagoda EM, Huang BX, Eckelman WC. Identification of metabolites of fluorine-18-labeled M2 muscarinic receptor agonist, 3-(3-(3-fluoropropyl)thio)-1,2,5-thiadiazol-4-yl)-1,2,5,6-tetrahydro-1-methylpyrididine, produced by human and rat hepatocytes. J Chromatogr B Biomed Sci Appl. 2002 Jan 25; 766(2):319–329.
66. Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage. 2002 Apr; 15(4):870–878. [PubMed: 11906227]
67. Laje G, Cannon D, Drevets W, McMahon FIP. Genetic Variation in HTR2A Influences Serotonin Transporter Binding Potential as Measured using PET and [11C]DASB. International Journal of Neuropsychopharmacology. 2009

68. Yan Z, Surmeier DJ. Muscarinic (m2/m4) receptors reduce N- and P-type Ca2+ currents in rat neostriatal cholinergic interneurons through a fast, membrane-delimited, G-protein pathway. J Neurosci. 1996 Apr 15; 16(8):2592–2604. [PubMed: 8786435]

69. Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G. Blockade of M2-like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. Eur J Neurosci. 1998 Sep; 10(9):3020–3023. [PubMed: 9758172]

70. Gibbons AS, Scarr E, McLean C, Sundram S, Dean B. Decreased muscarinic receptor binding in the frontal cortex of bipolar disorder and major depressive disorder subjects. J Affect Disord. 2009 Aug; 116(3):184–191. [PubMed: 19103464]

71. Zavitsanou K, Katsifis A, Yu Y, Huang XF. M2/M4 muscarinic receptor binding in the anterior cingulate cortex in schizophrenia and mood disorders. Brain Res Bull. 2005 May 15; 65(5):397–403. [PubMed: 15833594]

72. Ross, E. Pharmacodynamics. In: Hardman, JG.; Limbird, LE.; Gilman, AG., editors. Goodman & Gilman’s The Pharmacological Basis of Therapeutics. 9. New York, NY: McGraw-Hill Professional; 1996.

73. Bonner TI, Buckley NJ, Young AC, Brann MR. Identification of a family of muscarinic acetylcholine receptor genes. Science. 1987 Jul 31; 237(4814):527–532. [PubMed: 3037705]

74. Peralta EG, Winslow JW, Peterson GL, Smith DH, Ashkenazi A, Ramachandran J, et al. Primary structure and biochemical properties of an M2 muscarinic receptor. Science. 1987 May 1; 236(4801):600–605. [PubMed: 3107123]

75. Bejerano G, Lowe CB, Ahituv N, King B, Siepel A, Salama SR, et al. A distal enhancer and an ultraconserved exon are derived from a novel retroposon. Nature. 2006 May 4; 441(7089):87–90. [PubMed: 16625209]

76. Han JS, Boeke JD. LINE-1 retrotransposons: modulators of quantity and quality of mammalian gene expression? Bioessays. 2005 Aug; 27(8):775–784. [PubMed: 16015595]

77. Hellmann-Blumberg U, Hintz MF, Gatewood JM, Schmid CW. Developmental differences in methylation of human Alu repeats. Molecular and cellular biology. 1993 Aug; 13(8):4523–4530. [PubMed: 8336699]

78. Muratani K, Hada T, Yamamoto Y, Kaneko T, Shigeto Y, Ohue T, et al. Inactivation of the cholinesterase gene by Alu insertion: possible mechanism for human gene transposition. Proc Natl Acad Sci U S A. 1991 Dec 15; 88(24):11315–11319. [PubMed: 1662391]

79. McClintock B. The origin and behavior of mutable loci in maize. Proc Natl Acad Sci U S A. 1950 Jun; 36(6):344–355. [PubMed: 15430309]

80. Krejci A, Bruce AW, Dolezal V, Tucek S, Buckley NJ. Multiple promoters drive tissue-specific expression of the human M muscarinic acetylcholine receptor gene. J Neurochem. 2004 Oct; 91(1):88–98. [PubMed: 15379890]

81. Heinzen EL, Ge D, Cronin KD, Maia JM, Shianna KV, Gabriel WN, et al. Tissue-specific genetic control of splicing: implications for the study of complex traits. PLoS biology. 2008 Dec 23;6(12):e1. [PubMed: 19222302]

82. Luo X, Kranzler HR, Zuo L, Zhang H, Wang S, Gelernter J. CHRM2 variation predisposes to personality traits of agreeableness and conscientiousness. Hum Mol Genet. 2007 Jul 1; 16(13): 1557–1568. [PubMed: 17468496]

83. Allen MH, Chessick CA, Miklowitz DJ, Goldberg JF, Wisniewski SR, Miyahara S, et al. Contributors to suicidal ideation among bipolar patients with and without a history of suicide attempts. Suicide Life Threat Behav. 2005 Dec; 35(6):671–680. [PubMed: 16552982]

84. Taylor Tavares JV, Clark L, Cannon DM, Erickson K, Drevets WC, Sahakian BJ. Distinct profiles of neurocognitive function in unmedicated unipolar depression and bipolar II depression. Biol Psychiatry. 2007 Oct 15; 62(8):917–924. [PubMed: 17825802]

85. Tavares JV, Drevets WC, Sahakian BJ. Cognition in mania and depression. Psychol Med. 2003 Aug; 33(6):959–967. [PubMed: 12946080]
86. Roiser JP, Cannon DM, Gandhi SK, Taylor Tavares J, Erickson K, Wood S, et al. Hot and cold cognition in unmedicated depressed subjects with bipolar disorder. Bipolar Disord. 2009 Mar; 11(2):178–189. [PubMed: 19267700]

87. Holmes M, Erickson K, Luckenbaugh D, Drevets W, Bain E, Cannon D, et al. A comparison of cognitive functioning in medicated and unmedicated subjects with bipolar depression. Bipolar Disorder. 2008; 10:806–815.

88. Elliott, R.; Sahakian, B.; Charney, D. The Neural Basis of Resilience. Foresight Mental Capital and Wellbeing Project; 2008. State of Science Review: E7.

89. Barnett JH, Salmond CH, Jones PB, Sahakian BJ. Cognitive reserve in neuropsychiatry. Psychol Med. 2006 Aug; 36(8):1053–1064. [PubMed: 16854246]

90. Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, et al. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. Mol Psychiatry. 2009 Jul 21.

91. Schosser A, Cohen-Woods S, Gaysina D, Chow PC, Martucci L, Farmer A, et al. NRG1 gene in recurrent major depression: No association in a large-scale case-control association study. Am J Med Genet B Neuropsychiatr Genet. 2009 Apr 14.

92. Giovannini MG. The role of the extracellular signal-regulated kinase pathway in memory encoding. Rev Neurosci. 2006; 17(6):619–634. [PubMed: 17283607]

93. Power AE, Roozendaal B, McGaugh JL. Glucocorticoid enhancement of memory consolidation in the rat is blocked by muscarinic receptor antagonism in the basolateral amygdala. Eur J Neurosci. 2000 Oct; 12(10):3481–3487. [PubMed: 11029617]

94. Clark L, Chamberlain SR, Sahakian BJ. Neurocognitive mechanisms in depression: implications for treatment. Annu Rev Neurosci. 2009; 32:57–74. [PubMed: 19400725]

95. Erickson K, Drevets WC, Clark L, Cannon DM, Bain EE, Zarate CA Jr, et al. Mood-congruent bias in affective go/no-go performance of unmedicated patients with major depressive disorder. Am J Psychiatry. 2005 Nov; 162(11):2171–2173. [PubMed: 16263859]
Figure 1. Association between regional distribution volume values ($V_T$) and genetic variation in the single nucleotide polymorphism at marker rs324650 on the muscarinic 2 receptor gene, \textit{CHRM2} for healthy controls.

After applying the false discovery rate correction for multiple testing the results in the pregenual anterior cingulate cortex (pgACC) is significant ($p_{\text{corrected}} < 0.05$).

Abbreviations: pgACC pregenual anterior cingulate cortex, sgACC subgenual prefrontal cortex.
Figure 2. Reduced hippocampal $^{[18}F$FP-TZTP V$_T$ in subjects with BD and the TT-genotype of rs324650 in the CHRM2 gene

A significant interaction was detected between rs324650 genotype and group in the hippocampus (F=5.42, p=0.01) that is accounted for by reduced $^{[18}F$FP-TZTP V$_T$ in BD subjects homozygous for the non-ancestral T-allele of rs324650 relative to heterozygotic BD-subjects (T=2.50, p=0.03) and relative to controls of the same genotype (T=2.74, p=0.019).
Figure 3. Cognitive performance A) impairment in BD and MDD groups relative to healthy controls, B) impairment in BD subjects homozygous for the T-allele versus A-carriers for rs324650

A. BD and MDD groups showed impairment in attention and memory evidenced by a greater number of errors of omission (*, RVIP, F=2.48, p=0.009), and reduced percentage correct responses (**, DMS, F=3.447, p=0.039) relative to controls, respectively. The HC group has no error bars because they are performing at 100%. B. In BD, spatial recognition memory performance is impaired in subjects homozygous for the T-allele versus A-carriers ($, T=3.36, p=0.005)
Table 1

Genotype frequencies for the CHRM2 single nucleotide polymorphisms investigated

Genotype frequency for each of the six SNPs examined did not differ significantly across groups.

| CHRM2 SNP | HC    | MDD   | BD    | Spearman’s Chi-Square(p) |
|-----------|-------|-------|-------|--------------------------|
| rs7810473 |       |       |       |                          |
| Genotype  |       |       |       |                          |
|           | AA    | 8 (35%) | 4 (27%) | 9 (56%)                  |
|           | AT    | 11 (48%) | 9 (60%) | 3 (19%)                  |
|           | TT    | 4 (17%) | 2 (13%) | 4 (25%)                  |
|           | Total | 23     | 15     | 16                       | 5.86 (0.21)  |
|           |       |       |       |                          |
| rs1824024 |       |       |       |                          |
| Genotype  |       |       |       |                          |
|           | GG    | 10 (40%) | 16 (67%) | 7 (44%)                  |
|           | GT    | 14 (56%) | 6 (25%) | 6 (38%)                  |
|           | TT    | 1 (4%) | 2 (8%) | 3 (19%)                  |
|           | Total | 25     | 24     | 16                       | 7.23 (0.12)  |
| rs2061174 |       |       |       |                          |
| Genotype  |       |       |       |                          |
|           | CC    | 5 (20%) | 9 (38%) | 2 (13%)                  |
|           | CT    | 15 (60%) | 5 (21%) | 8 (50%)                  |
|           | TT    | 5 (20%) | 10 (42%) | 6 (38%)                  |
|           | Total | 25     | 24     | 16                       | 9.34 (0.05)  |
| rs2350786 |       |       |       |                          |
| Genotype  |       |       |       |                          |
|           | AA    | 6 (24%) | 9 (38%) | 8 (50%)                  |
|           | AG    | 15 (60%) | 7 (29%) | 6 (38%)                  |
|           | GG    | 4 (16%) | 8 (33%) | 2 (13%)                  |
|           | Total | 25     | 24     | 16                       | 9.83 (0.13)  |
| rs324650  |       |       |       |                          |
| Genotype  |       |       |       |                          |
|           | AA    | 5 (20%) | 7 (29%) | 4 (25%)                  |
|           | AT    | 12 (48%) | 10 (42%) | 6 (38%)                  |
|           | TT    | 8 (32%) | 7 (29%) | 6 (38%)                  |
|           | Total | 25     | 24     | 16                       | 0.89 (0.93)  |
| rs8191992 |       |       |       |                          |
| Genotype  |       |       |       |                          |
|           | AA    | 6 (24%) | 4 (17%) | 6 (38%)                  |
|           | AT    | 11 (44%) | 8 (33%) | 4 (25%)                  |
|           | TT    | 8 (32%) | 12 (50%) | 6 (38%)                  |
|           | Total | 25     | 24     | 16                       | 3.77 (0.44)  |
Table 2
Chi-square for clinical variables significantly more frequent in the SNP rs324650 TT genotype versus A-carriers among BD subjects. Post-hoc exploratory – p< 0.05

One participant with BD was adopted and no family history was available.

| Clinical Variable          | Absence | Presence | Chi-Square | Z   | P   |
|---------------------------|---------|----------|------------|-----|-----|
| Suicide Attempt           |         |          |            |     |     |
| A-carriers                | 7       | 3        |            |     |     |
| TT group                  | 1       | 5        | 4.27       | 0.039|
| First Degree Relative with BD |       |          |            |     |     |
| A-carriers                | 5       | 5        |            |     |     |
| TT group                  | 0       | 5        | 3.75       | 0.053|