Published in final edited form as:
Neuropsychopharmacology. 2006 December ; 31(12): 2739–2747. doi:10.1038/sj.npp.1301196.

Variations in the vesicular monoamine transporter 1 gene (VMAT1/SLC18A1) are associated with bipolar I disorder

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Abstract

The vesicular monoamine transporter 1 gene (VMAT1/SLC18A1) maps to the shared bipolar disorder (BPD) / schizophrenia (SZ) susceptibility locus on chromosome 8p21. Vesicular monoamine transporters are involved in transport of monoamine neurotransmitters which have been postulated to play a relevant role in the etiology of BPD and/or SZ. Variations in the VMAT1 gene might affect transporter function and/or expression and might be involved in the etiology of BPD and/or SZ. Genotypes of 585 patients with BPD type I and 563 control subjects were obtained for three missense SNPs (Thr4Pro, Thr98Ser, Thr136Ile) and 4 non-coding SNPs (rs988713, rs2279709, rs3735835, rs1497020). All cases and controls were of European descent. Allele frequencies differed significantly for the potential functional polymorphism Thr136Ser between BPD patients and controls ($p = 0.003$; $df = 1$; OR = 1.34; 95% CI: 1.11 – 1.62). Polymorphisms in the promoter region (rs988713: $p = 0.005$, $df = 1$; OR = 1.31; 95% CI: 1.09 – 1.59) and intron 8 (rs2279709: $p = 0.039$, $df = 1$; OR = 0.84; 95% CI: 0.71 – 0.99) were also associated with disease. Expression analysis confirmed that VMAT1 is expressed in human brain at the mRNA and protein level. Results suggest that variations in the VMAT1 gene may confer susceptibility to BPD in patients of European descent. Additional studies are necessary to confirm this effect and to elucidate the role of VMAT1 in CNS physiology.

Introduction

Bipolar disorder (BPD) is a common psychiatric illness that affects approximately 1% of the general population and is characterized by recurrent episodes of mania and depression. Family, adoption and twin studies show that BPD has a strong heritable component (Craddock and Jones 1999; Smoller and Finn 2003); however, genetic causes have been difficult to elucidate due to the complex mode of inheritance and genetic heterogeneity. Recent linkage studies have suggested that a susceptibility locus for BPD exists on chromosome 8p21–22 (Cichon *et al*, 2001; Ophoff *et al*, 2002; Park *et al*, 2004; Cheng *et al*, 2006). Interestingly, numerous linkage studies in schizophrenia (SZ) report a susceptibility locus on 8p21 (Pulver *et al*, 1995; Kendler *et al*, 1996; Straub *et al*, 1996; Blouin *et al*, 1998; Brzustowicz *et al*, 1999; Pulver *et al*, 2000; Gurling *et al*, 2001; Stefansson *et al*, 2002; Lewis *et al*, 2003; Suarez *et al*, 2006) and a candidate gene from this region,

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neuregulin 1 (NRG1), was associated with SZ (Stefansson *et al*, 2002; Stefansson *et al*, 2003; Williams *et al*, 2003; Yang *et al*, 2003; Corvin *et al*, 2004; Li *et al*, 2004; Tang *et al*, 2004; Petryshen *et al*, 2005); however, NRG1 is located about 10 – 15 cM away from the major linkage peaks and others could not confirm an association between the NRG1 gene and SZ (Bakker *et al*, 2004; Hong *et al*, 2004; Iwata *et al*, 2004; Thiselton *et al*, 2004; Duan *et al*, 2005; Liu *et al*, 2005). The report of multiple linkage studies in BPD and SZ supports the hypothesis of a shared susceptibility locus for BPD and SZ on 8p (Berrettini 2003; Berrettini 2004). This is especially remarkable since recent BPD linkage analyses found a peak on 8p in BPD patients with psychotic symptoms (Park *et al*, 2004; Cheng *et al*, 2006), suggesting that the phenotype of psychosis might influence susceptibility to BPD and SZ.

The vesicular monoamine transporter 1 gene (VMAT1), also known as SLC18A1, maps to this shared BPD/SZ susceptibility locus on chromosome 8p21 (Peter *et al*, 1993; Roghani *et al*, 1996). Vesicular monoamine transporters are involved in the packaging of dopamine, serotonin, adrenalin, and noradrenalin from the cytoplasm to their storage vesicles in presynaptic terminals. The vesicles ultimately discharge transmitters into the synaptic cleft by exocytosis following an action potential. Two different isoforms of the transporter are known, VMAT1 and VMAT2, both encoded by different genes (Peter *et al*, 1993). It was reported initially that VMAT1 is expressed exclusively in peripheral neurons and endocrine tissue and only the VMAT2 isoform was thought to be expressed in brain (Peter *et al*, 1995; Erickson *et al*, 1996; Eiden *et al*, 2004); however, other studies show that VMAT1 is expressed in rat brain (Hansson *et al*, 1998). Dysregulation of dopamine and serotonin neurotransmission has been long postulated to play a role in the etiology of BPD and SZ (Manji and Lenox 2000; Tamminga and Holcomb 2005), thus making VMAT1 a positional and functional candidate gene for these neuropsychiatric disorders.

Studies *in vitro* show that lithium and valproate, effective pharmacotherapies for BPD, increase the expression of VMAT1 (Cordeiro *et al*, 2000; Cordeiro *et al*, 2002; Cordeiro *et al*, 2004), suggesting that the VMAT1 gene might be a target for therapeutic drug action. Variations in the VMAT1 gene might alter transporter function and/or expression and therefore might play a direct role in the etiology of psychiatric disorders. Indirect evidence that the vesicular monoamine transporter (VMAT) is involved in psychiatric disorders stems from positron emission tomography (PET) imaging studies. Binding of radiolabled dihydrotetrabenazine, a catecholamine depleter with higher VMAT2 than VMAT1 affinity (DaSilva *et al*, 1993; DaSilva *et al*, 1994) was increased in thalamus and brainstem of BPD patients when compared to controls (Zubieta *et al*, 2000). Ventral brainstem binding was higher in BPD and SZ patients compared to controls (Zubieta *et al*, 2001). These experiments suggest that higher levels of VMAT expression may represent a trait-related abnormality in patients with BPD and SZ. In this study we test the hypothesis that VMAT1 is expressed in human brain and that variations in the VMAT1/SLC18A1 gene confer susceptibility to BPD.

Materials and methods

Subjects

Five hundred and eighty-five unrelated BPD type I patients participated in this study. Patients were collected at centers involved in the National Institute of Mental Health (NIMH) Genetics Initiative on BPD (http://zork.wustl.edu/nimh/bp.html) and carried a diagnosis of BPD type I as defined by DSM-IV criteria. The key criterion for admission of a family to the study was a diagnosis of BPD type I in two or more siblings. Background and detailed methodology for the NIMH Genetics Initiative are described elsewhere (Dick *et al*, 2003). All subjects were assessed with the Diagnostic Instrument for Genetic Studies (Nurnberger *et al*, 1994). Family history information was obtained through the Family

Interview for Genetic Studies (FIGS) and medical records were requested. Final best estimate diagnosis was made using all available information including medical records, information from relatives, and the DIGS interview, by two independent senior diagnosticians adhering to DSM-IV criteria. The patient group consisted of 38% males and 62% females. The average age at recruitment was 41.6 years. Psychotic symptoms were present in 66% of the probands at some point during their illness. Psychosis was defined as presence of auditory/visual hallucinations and/or paranoid or bizarre delusions.

The control subjects comprised 563 unrelated healthy individuals with no history of psychiatric or chronic neurological disease. The control group consisted of 51% males and 49% females with an average age of 38.5 years at recruitment. All cases and controls were of European descent. Informed consent was obtained from all individuals in accordance with Institutional Review Board (IRB) procedures.

DNA Analyses

The VMAT1/SLC18A1 gene encodes 525 amino acids and consists of 16 exons spanning 38346 bp. Review of the public database (http://www.ncbi.nlm.nih.gov/SNP), the Celera database (http://myscience.appliedbiosystems.com) and the literature revealed 13 non-synonymous and 10 synonymous single nucleotide polymorphisms (SNP) in the coding region of the SLC18A1 gene and at least 211 non-coding SNPs (Accession # NM 003053). SNPs for genotyping were chosen based on availability of Applied Biosystems Inc. (ABI) SNP assays, location in the gene and allele frequencies (Figure 1). Genotyping of three of the non-synonymous SNPs (Thr4Pro, Thr98Ser, Thr136Ile) and four intronic SNPs across the VMAT1/SLC18A1 gene, was performed using the ABI “Assays-on-demand” (ABI, Foster City, CA, USA) SNP genotyping assay as per manufacturers protocol. SNP1 = rs988713; SNP2 = rs2270641 (Thr4Pro); SNP3 = rs2270637 (Thr98Ser); SNP4 = rs1390938 (Thr136Ile); SNP5 = rs2279709; SNP6 = rs3735835; SNP7 = rs1497020.

Three additional missense mutations, rs17092144 (Gln11Arg), rs17840571 (Arg140Gly) and rs17092104 (Leu392Val) were genotyped in a subset of patients (n = 94) and controls (n = 190); however, because of low minor allele frequencies (<5%) these markers were not investigated in the entire sample. Genotyping failure rates for all markers were less then 1% for controls and probands. Accuracy of genotyping was ensured by independent genotyping of a subgroup of the sample at the University of Pennsylvania DNA Core Facility. Concordance rates were greater than 99.5 %.

Statistical Analyses

Genotypes and allele frequencies were compared between groups using Chi square contingency analysis. A two-tailed type I error rate of 5% was chosen for the analysis. Linkage disequilibrium (LD) and haplotype frequencies were estimated using the COCAPHASE program (Dudbridge 2003). The COCAPHASE program uses standard unconditional logistic regression analysis. Correction for multiple testing was performed using permutation correction by the COCAPHASE program. This approach corrects for multiple testing but takes into account the correlation between markers. It is thus less conservative than a Bonferroni correction, which is appropriate for independent tests such as unlinked markers. For the single-marker analyses, 10,000 permutations were carried out to estimate the significance of the best results, correcting for the seven loci tested. Haplotype analysis was performed using a 4 and 6 sliding marker window. Rare haplotypes were excluded from analysis since the EM algorithm does not accurately estimate haplotype frequencies <1% (Fallin and Schork 2000). The most significant p value was corrected by permutation analysis as described above.

Expression Analyses

Commercially available human brain cDNA was purchased from Clontech Laboratories, Inc. (amygdala, hippocampus, substantia nigra) and Invitrogen Corporation (adult frontal lobe, thalamus, fetal frontal lobe). Expression assays for VMAT1 and VMAT2 were chosen based on specificity after sequence alignment of both mRNAs (VMAT1: ABI assay ID: Hs_0091591, NM 003053, 2749 bp; VMAT2: ABI assay ID: Hs_00161858, NM 003054, 1898 bp). GAPDH was used as a normalizer (ABI assay ID: Hs_9999905). To avoid possible genomic DNA amplification, probes were designed so that they cross exon-exon junctions. Real-time quantitative PCR using 2ng of cDNA per reaction was performed using *Applied Biosystems* 7300 Sequence Detection System as per manufacturer's protocol and "no-template" control samples were included for each assay. Relative quantification was performed using the comparative C_T method ($\Delta\Delta C_T$).

Western analysis for VMAT1 was performed using post-mortem tissue samples of brain regions and peripheral tissues from an individual who carried a diagnosis of SZ and Alzheimer dementia. The patient died of acute bronchopneumonia and no information was available on the pharmacological regimen prior to death. Protein was extracted from the frozen samples, fractionated on a 4% to 12% gradient Bis-Tris Nu-PAGE gel and transferred to a nitrocellulose filter as outlined above. The membrane was blocked in TBS-T containing 5% normal donkey serum for one hour at room temperature. The membrane was incubated in a 1:100 dilution of an anti-VMAT1 polyclonal antibody (sc-15313, Santa Cruz Biotechnology, Santa Cruz, CA) for 2 hours at room temperature. The blot was washed for 30 min with TBS-T and then incubated with a 1:10,000 dilution of an anti-goat peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA). The blot was then washed for 1 hour at room temperature with TBS-T. Western blots were visualized using the Enhanced Chemiluminescence Plus kit (Amersham Biosciences).

An immunohistochemical survey of VMAT1 and VMAT2 in postmortem brain was conducted in sections of hippocampus, entorhinal cortex, hypothalamus, and midbrain-substantia nigra which were labeled with antibodies for VMAT1 (sc-7718, Santa Cruz Biotechnology) or VMAT2 (sc-7722, Santa Cruz Biotechnology). Immunohistochemistry was performed using standard procedures folllowing antigen retrieval by boiling in 1mM EDTA in 0.1M Tris buffer, pH 8.0 for 10 minutes, and using 0.25% NiSO46H2O to enhance the diaminobenzidine reaction product, as previously described (Talbot *et al*, 2004).

Results

All genotype counts were in Hardy-Weinberg equilibrium. Genotype and allele frequencies for the case-control study are shown in Table 1. The potential functional polymorphism Thr136Ile in VMAT1 was associated with BPD (p = 0.003; df = 1; OR = 1.34; 95% CI: 1.11 – 1.62; global significance p = 0.0145; standard error [SE]: 0.001195 after permutation correction). In addition our results show that two other SNPs in the VMAT1 gene are associated with disease: one SNP in the promoter region (SNP1, rs988713, −584A/G, p = 0.005, df = 1; OR = 1.31; 95% CI: 1.09 – 1.59) and one intronic SNP (SNP6, rs2279709, p = 0.038; df = 1; OR = 0.86; 95% CI: 0.71 – 0.99). We observed strong LD in the 5′ end of the gene and perfect LD between markers SNP1 and SNP4 (Table 2). Haplotype analysis shows association with a possible protective haplotype for BPD (Table 3); however, haplotypes do not reach a greater level of statistical significance than the single marker analysis (global significance: p = 0.0196; SE: 0.001386).

Real-time quantitative PCR expression analysis of human brain demonstrates VMAT1 and VMAT2 mRNA in various brain regions (Figure 2). Highest levels were observed for

VMAT1 in substantia nigra, followed by amygdala, hippocampus, thalamus, fetal frontal lobe and frontal lobe. VMAT2 expression was observed highest in substantia nigra.

Western analysis of VMAT1 in post mortem human brain confirms VMAT1 protein expression (Figure 3). VMAT1 immunoreactivity was detected at the expected molecular weight in all brain regions studied, including areas of particular interest in SZ and BPD, such as amygdala, hippocampus and nucleus accumbens.

Immunohistochemistry reveals that VMAT1 and VMAT2 are both expressed in a linear and punctuate pattern typical of axon fibers and terminals in hypothalamus and hippocampal formation (Figure 4). Similar to the qRT-PCR and Western data, we found robust expression of VMAT1 in hypothalamus and hippocampus. The substantia nigra shows heavy VMAT2 innervation, and very little VMAT1 immunoreactivity (data not shown).

Discussion

In the present study we show that the potential functional polymorphism Thr136Ile in the VMAT1 gene is associated with BPD. In addition our results suggest that two other SNPs in the VMAT1 gene may be associated with disease: one SNP in the promoter region (SNP1, rs988713) and one intronic SNP (SNP6, rs2279709). We observed strong LD in the 5′ end of the gene and weaker coverage in the 3′ end of the gene. All three associated SNPs appear to be in one haplotype block and no information is available on their functional effects. Due to the strong LD between markers, additional studies are necessary to elucidate the relevance of the tested variations. Haplotype analysis reveals association with a possible protective haplotype for BPD; however, haplotypes do not reach a greater level of statistical significance than the single marker analysis. The common variations, Thr98Ser and Thr136Ile, are located in the intravesicular loop 1. This region of the protein interacts with both the ligand (inhibitors and substrates) as well as the TMD10/11 region of the transporter (Sievert and Ruoho 1997). Although no information is available on the biological effect of variants in the VMAT1 gene, and phylogenetic analysis shows no conservation during evolution (data not shown), a direct functional effect of the Thr136Ile or a nearby variant must be considered. In particular, the variation in the promoter region, which is in perfect LD with the Thr136Ile polymorphism, could potentially modulate gene expression; however, functional relevance has not been tested. Increased VMAT1 expression might lead to excessive accumulation of neurotransmitter molecules in vesicles, with subsequent release in the synapse, contributing to mania and psychosis. Reduced VMAT1 expression might lead to insufficient accumulation of neurotransmitters in vesicles, and thus to decreased synaptic levels of neurotransmitters, resulting in depression. This hypothesis is consistent with evidence that reserpine, a VMAT inhibitor that depletes catecholamines, can precipitate episodes of depression and is used in animal models of depression (Slattery *et al*, 2004).

Expression analysis reveals that VMAT1 is widely expressed in human brain, in particular in substantia nigra, hippocampus, thalamus, amygdala and frontal lobe at the mRNA and protein level. This finding of VMAT1 expression in human brain is novel and contrary to previous reports (Peter *et al*, 1995; Erickson *et al*, 1996; Eiden *et al*, 2004) in which only VMAT2 was detected in brain. We used real-time PCR to detect VMAT1 mRNA in multiple brain regions. This method has been shown to detect minute amounts of mRNA and is more sensitive then other methods, with the advantage of being less observer dependent (Nakamura *et al*, 2003; Benoy *et al*, 2004). Differences in methodological approach might explain the discrepancy in findings since previous studies of rat and human brain utilized immunohistochemistry and in-situ-hybridization to analyze expression (Peter *et al*, 1995; Erickson *et al*, 1996; Eiden *et al*, 2004). To confirm VMAT1 expression in brain, we conducted western and immunohistochemistry analysis of postmortem brain tissue using

commercially available antibodies with no-cross reactivity. Our experiments provide evidence that VMAT1 is expressed in human brain on the protein level. Previous reports failed to detect VMAT1 protein in brain which might be due to probe/antibody specificity and/or tissue quality. VMAT1 expression in human brain is consistent with detection of VMAT1 in rat brain (Hansson *et al*, 1998). Furthermore, review of the public database, including expression profile information suggested by analysis of EST counts (UniGene Hs. 158322) and microarray expression data (Affymetrix GeneChip Human Genome U95 Set HG-U95A Accession # GDS181, Merck Rosetta Chip Accession # GDS833), indicates that VMAT1 is expressed in brain. Our results document co-expression of VMAT1 and VMAT2 in human brain. When comparing mRNA levels of multiple human brain regions between VMAT1 and VMAT2 (Figure 2), levels differed for substantia nigra (higher VMAT2) and fetal frontal lobe (higher VMAT1) but were similar between frontal lobe, hippocampus and thalamus. Interestingly, VMAT1 was found to be the predominant isoform in some brain regions during rat brain development (Hansson *et al*, 1998), coinciding with our observation of higher VMAT1 mRNA levels in the fetal frontal lobe when compared to VMAT2. VMAT1 predominance during early neurodevelopment might be important for migration of neurons, development of neurosecretory pathways and the survival of neurons (Leroux-Nicollet and Costentin 1998; Verney *et al*, 2002; Eells 2003). Variations in the VMAT1 gene might thus alter neurodevelopment and predispose individuals to neuropsychiatric diseases, consistent with the hypothesis that SZ and BPD are neurodevelopmental disorders (Lewis and Levitt 2002; Blumberg *et al*, 2004; Eastwood 2004). Taken together, our expression experiments provide evidence that VMAT1 is expressed in human brain at the mRNA and protein level. This finding is important since it stands in contrast to the current understanding of VMAT1/VMAT2 expression and could open a new avenue of research in neuropsychiatric disorders.

Our sample of BPD patients had a positive family history of affective disorder as a key criterion for admission, implicating higher genetic loading of the probands and thus a stronger genetic effect of the tested variation; however, no linkage to chromosome 8p has been reported in this sample (Segurado *et al*, 2003). Chromosome 8p has been suggested repeatedly as a linkage region for both SZ and BPD; however the underlying genes responsible for the linkage signal remain elusive. NRG1 was identified as one candidate gene from this region and was associated with SZ (Stefansson *et al*, 2002; Stefansson *et al*, 2003; Williams *et al*, 2003; Yang *et al*, 2003; Corvin *et al*, 2004; Li *et al*, 2004; Tang *et al*, 2004; Petryshen *et al*, 2005) and BPD (Green *et al*, 2005) in some studies but could not be confirmed by others (Bakker *et al*, 2004; Hong *et al*, 2004; Iwata *et al*, 2004; Thiselton *et al*, 2004; Duan *et al*, 2005; Liu *et al*, 2005). This inconsistency might be due to several factors including clinical heterogeneity, population stratification, different haplotype structure between populations and limited statistical power. Another possibility might be that several candidate genes contribute to the linkage finding in SZ and BPD and furthermore, that several candidate genes are contributing to a clinical phenotype like psychosis, rather then to a DSM-IV category. The phenotypic overlap between SZ, schizoaffective disorder and BPD has been the subject of extensive debate (Craddock and Owen 2005; Maier *et al*, 2005) and the possibility of shared underlying pathophysiologic mechanisms in SZ and BPD is further strengthened by convergent molecular genetic data (Berrettini 2003; Berrettini 2004). Previous studies have suggested that psychotic BPD may represent a genetically unique subtype (Potash *et al*, 2001; Potash *et al*, 2003). Dissection of the patient group into psychotic BPD shows that all three initially-associated markers remain statistically significant, despite reduction in sample size by subgrouping. This finding is consistent with the hypothesis that the phenotype of psychosis might contribute to the overall effect and might explain the presence of a shared susceptibility locus for BPD and SZ on chromosome 8p (Berrettini 2003; Berrettini 2004). However, there are no differences in allele frequencies at these SNPs between the psychotic and non-psychotic groups, suggesting that these SNPs

do not differentiate these two putative forms of BPD. These results require confirmation in an independent population of patients and controls and studies of VMAT1 in SZ are also warranted. In particular VMAT1 association studies in a SZ sample with reported linkage to 8p would be a logical extension of this work.

Although we report a positive association between the VMAT1 gene and BPD, it is possible that our finding might be a false positive result due to population stratification. Case-control association studies of subjects with self-reported ancestries are not immune to population stratification (Freedman *et al*, 2004), even though all cases and controls in this study were of European descent. A more accurate control would be the use of a family-based association design that matches the genotype of an affected offspring with those parental alleles not inherited by the offspring (Spielman and Ewens 1996). Ultimately, these results require confirmation in an independent population of patients and controls.

In summary, we show that VMAT1 mRNA and protein is present in many human brain regions and SNPs in the VMAT1 gene are associated with BPD. VMAT1 is thus another high ranking candidate gene for BPD and SZ on chromosome 8p. Our results require confirmation in other populations and additional studies are necessary to elucidate the role of VMAT1 in the pathophysiology of BPD.

Acknowledgments

This work was supported by the Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania. Financial support is gratefully acknowledged from National Institutes of Health grants MH59553, MH63876 (to W.H.B.) and R25 MH060490 (to F.W.L.), a grant from the National Alliance for Research on Schizophrenia and Depression (to W.H.B.), a grant from the Tzedakah Foundation (to W.H.B.) and a grant from the Deutsche Forschungsgemeinschaft (Sa434/3-1 to T.S.). We would like to thank Candice Schwebel, Jacqueline Scena and Andrew Weller for technical assistance and James Potash for help with clinical data on BPD patients.

Data and biomaterials utilized in this study were collected as part of ten projects that participated in the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. From 1999–03, the Principal Investigators and Co-Investigators were: Indiana University, Indianapolis, IN, R01 MH59545, John Nurnberger, M.D., Ph.D., Marvin Miller, M.D., Elizabeth Bowman, M.D., N. Leela Rau, M.D, P. Ryan Moe, M.D., Nalini Samavedy, M.D., Rif El-Mallakh, M.D, (at University of Louisville), Husseini Manji, M.D. (at Wayne State University), Debra A. Blitz, M.D (at Wayne State University), Eric T. Meyer, M.S., Carrie Smiley, R.N., Tatiana Foroud, Ph.D., Leah Flury, M.S., Danielle M. Dick, Ph.D., Howard Edenberg, Ph.D.; Washington University, St. Louis, MO, R01 MH059534, John Rice, Ph.D., Theodore Reich, M.D., Allison Goate, Ph.D., Laura Bierut, M.D.; Johns Hopkins University, Baltimore, MD, R01 MH59533, Melvin McInnis, M.D., J. Raymond DePaulo, Jr., M.D., Dean F. MacKinnon, M.D., Francis M. Mondimore, M.D., James B. Potash, M.D., Peter P. Zandi, Ph.D, Dimitrios Avramopoulos, Jennifer Payne; University of Pennsylvania, PA, R01 MH59553, Wade Berrettini, M.D., Ph.D.; University of California at Irvine, CA, R01 MH60068, William Byerley, M.D. and Mark Vawter, M.D.; University of Iowa, IA, R01 MH059548, William Coryell, M.D., Raymond Crowe, M.D.; University of Chicago, IL, R01 MH059535, Elliot Gershon, M.D., Judith Badner, Ph.D., Francis McMahon, M.D., Chunyu Liu, Ph.D., Alan Sanders, M.D., Maria Caserta, Steven Dinwiddie, M.D., Tu Nguyen, Donna Harakal; University of California at San Diego, CA, R01 MH59567, John Kelsoe, M.D., Rebecca McKinney, B.A.; Rush University, IL, R01 MH059556, William Scheftner, M.D., Howard M. Kravitz, D.O., M.P.H., Diana Marta, B.S., Annette Vaughn-Brown, M.S.N., R.N., Laurie Bederow, M.A.; NIMH Intramural Research Program, Bethesda, MD, 1Z01MH02810-01, Francis J. McMahon, M.D., Layla Kassem, PsyD., Sevilla Derta-Wadleigh, Ph.D., Lisa Austin, Ph.D., Dennis L. Murphy, M.D.

Most importantly, we thank the families who have participated in and contributed to these studies.

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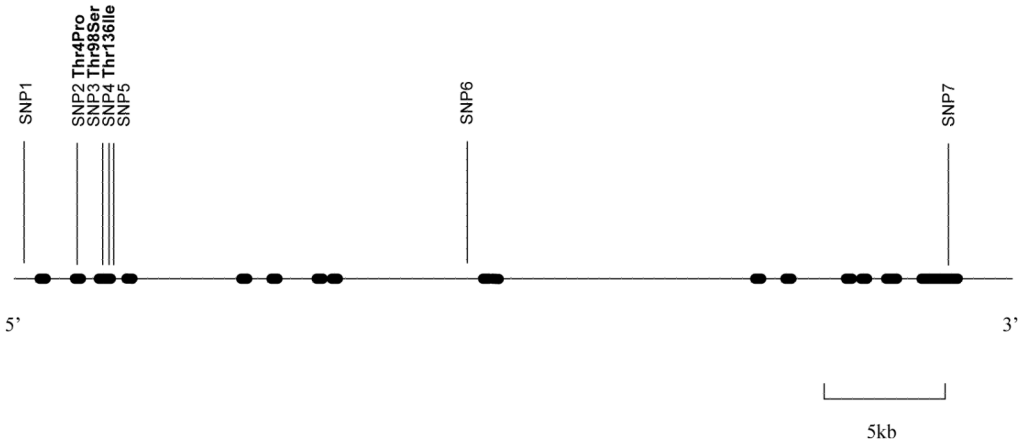

Figure 1.
Variations in the VMAT1 / SLC18A1 gene

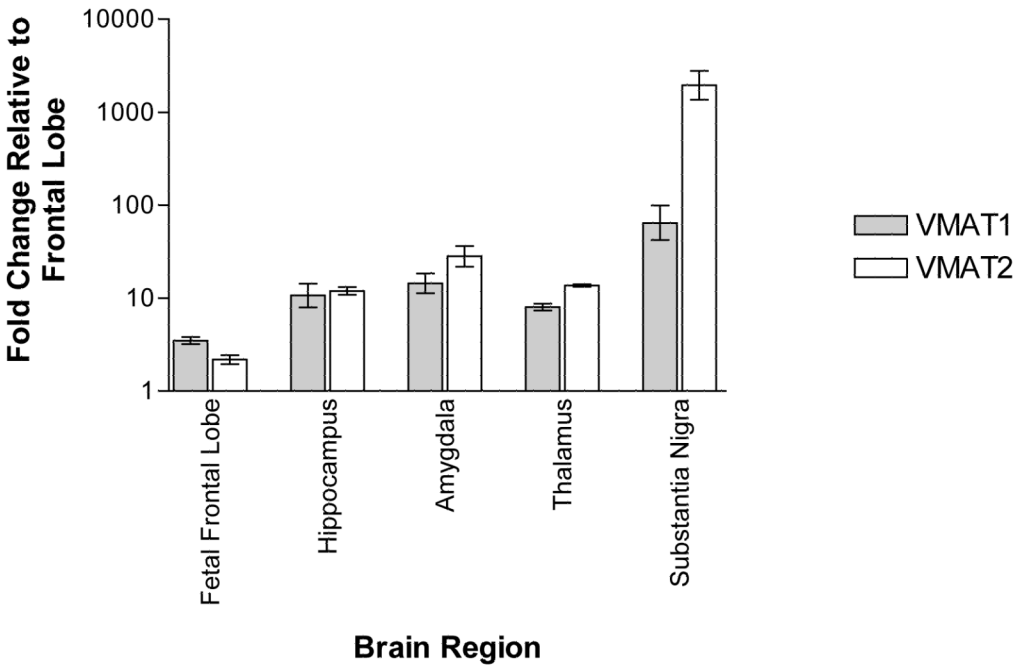

Figure 2.
real-time quantitative PCR determination of mRNA levels for VMAT1 and VMAT2 in human brain regions. Data shown as mean + s.e.m. of three independent experiments using the comparative Ct method.

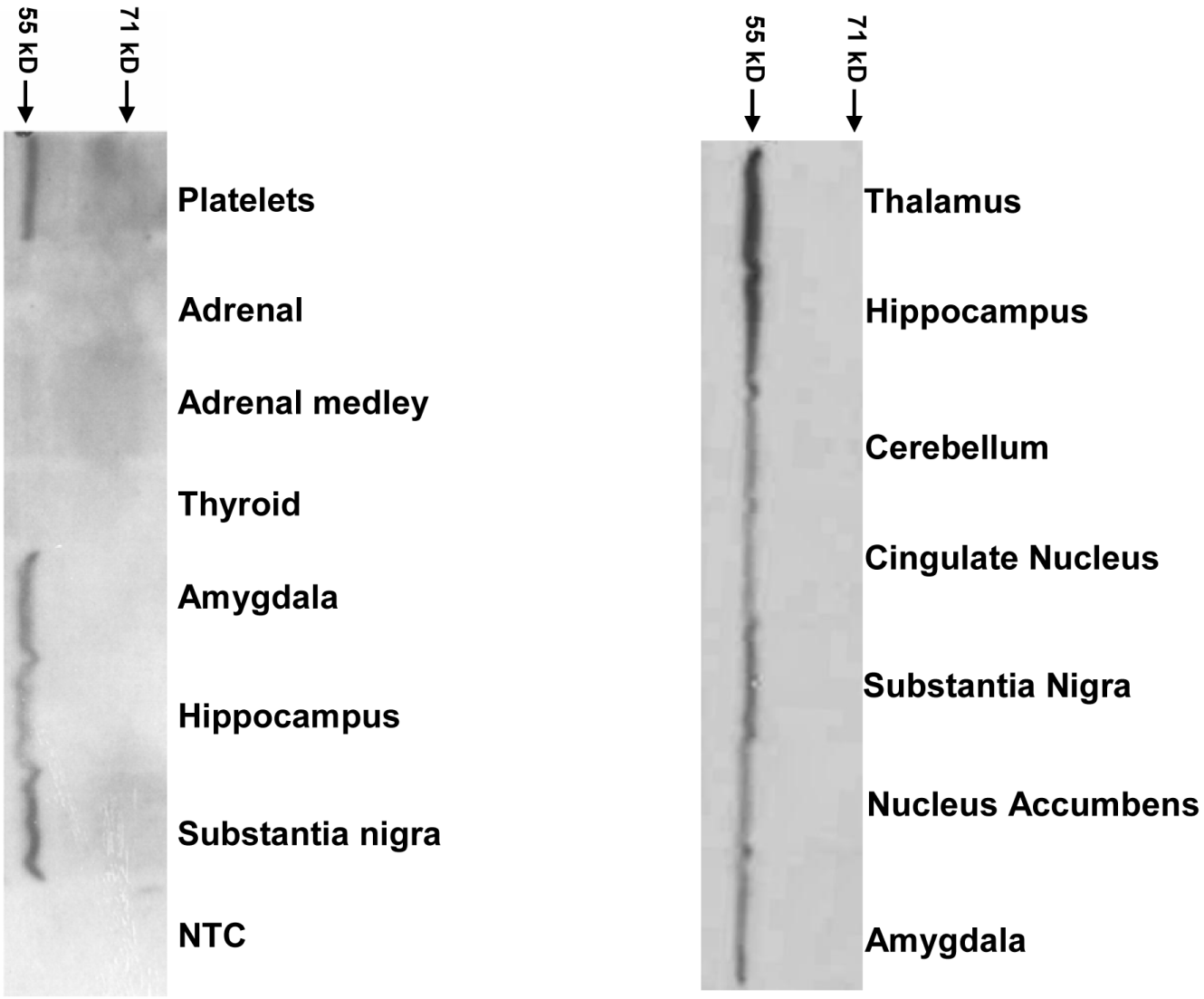

Figure 3.
Western blot of human VMAT1

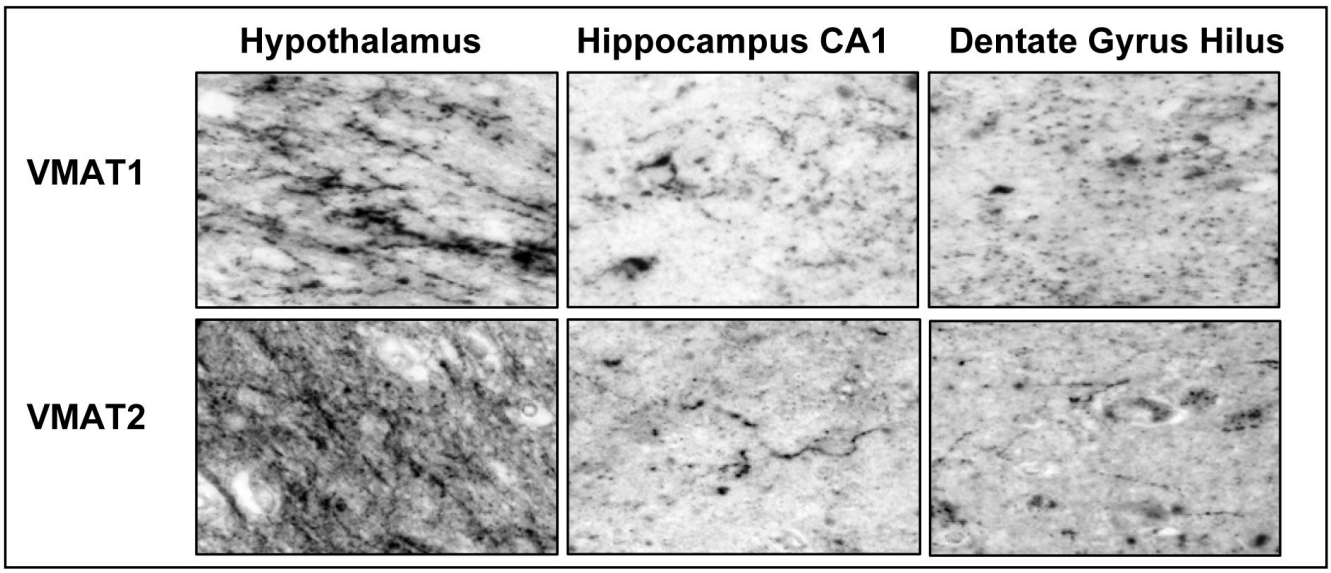

Figure 4.
VMAT1 and VMAT2 immunoreactivity in post-mortem human brain

Table 1

Genotype and Allele frequencies of variations in the VMAT1 / SLC18A1 gene

SNP		Sample	n	Genotype frequencies			*P**	Allele frequencies	*P**
SNP1	**rs988713**			**A/A**	**A/G**	**G/G**		**f(A)**	
		BP I	585	0.591	0.366	0.043	0.015	0.774	0.005
		BP I psychosis	392	0.584	0.367	0.048	0.085	0.768	0.029
		Controls	560	0.518	0.411	0.071		0.723	
SNP2	**rs2270641**			**Thr/Thr**	**Thr/Pro**	**Pro/Pro**		**f(Thr)**	
	Thr4Pro	BP I	581	0.418	0.449	0.133	0.848	0.643	0.623
		BP I psychosis	390	0.397	0.469	0.133	0.642	0.632	0.356
		Controls	560	0.427	0.452	0.121		0.653	
SNP3	**rs2270637**			**Thr/Thr**	**Thr/Ser**	**Ser/Ser**		**f(Thr)**	
	Thr98Ser	BP I	584	0.635	0.317	0.048	0.633	0.794	0.415
		BP I psychosis	391	0.657	0.299	0.043	0.847	0.807	0.984
		Controls	563	0.652	0.311	0.037		0.807	
SNP4	**rs1390938**			**Thr/Thr**	**Thr/Ile**	**Ile/Ile**		**f(Thr)**	
	Thr136Ile	BP I	580	0.597	0.360	0.043	0.009	0.777	0.003
		BP I psychosis	389	0.589	0.362	0.049	0.063	0.770	0.020
		Controls	560	0.518	0.409	0.073		0.722	
SNP5	**rs2279709**			**C/C**	**C/A**	**A/A**		**f(C)**	
		BP I	585	0.330	0.484	0.186	0.271	0.572	0.127
		BP I psychosis	392	0.332	0.482	0.186	0.322	0.573	0.159
		Controls	561	0.287	0.506	0.207		0.540	
SNP6	**rs3735835**			**G/G**	**G/C**	**C/C**		**f(G)**	
		BP I	585	0.287	0.480	0.232	0.069	0.527	0.038
		BP I psychosis	392	0.270	0.503	0.227	0.100	0.522	0.035
		Controls	561	0.319	0.503	0.178		0.570	
SNP7	**rs1497020**			**A/A**	**A/G**	**G/G**		**f(A)**	
		BP I	583	0.504	0.417	0.079	0.100	0.713	0.205
		BP I psychosis	391	0.496	0.417	0.087	0.323	0.705	0.450
		Controls	560	0.493	0.391	0.116		0.688	

* type-I error rates for comparison of genotype and allele frequencies between bipolar I patients and controls.

Table 2

Linkage Disequilibrium measures across the VMAT1 gene

		SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7
rs988713	SNP1	-	1.00	1.00	1.00	1.00	1.00	0.46
rs2270641	SNP2	0.18	-	1.00	1.00	1.00	1.00	0.49
rs2270637	SNP3	0.08	0.13	-	1.00	1.00	1.00	0.38
rs1390938	SNP4	1.00	0.18	0.08	-	1.00	1.00	0.45
rs2279709	SNP5	0.42	0.43	0.19	0.42	-	0.53	0.42
rs3735835	SNP6	0.27	0.65	0.20	0.27	0.19	-	0.56
rs1497020	SNP7	0.16	0.05	0.01	0.15	0.09	0.11	-

Note: D′ values are given above the diagonal, and r^2 values are given below the diagonal.

Table 3

Analysis of common haplotypes of SNP1–6 in the VMAT1 gene

Haplotype	Case	freq	Control	freq	OR	chisq	p
A-Thr-Thr-Thr-A-G	113	0.102	108.3	0.100	1	0.031	0.858
A-Thr-Thr-Thr-A-C	114	0.103	88.71	0.081	1.232	2.945	0.086
A-Thr-Ser-Thr-C-G	225	0.203	206	0.190	1.047	0.621	0.430
A-Pro-Thr-Thr-C-C	401	0.363	376	0.347	1.022	0.589	0.442
G-Thr-Thr-Ile-A-G	251	0.227	303	0.280	0.793	8.023	0.004[a]

[a] global significance: p = 0.0196; SE: 0.001386