Multiple Regulatory Variants Modulate Expression of 5-Hydroxytryptamine 2A Receptors in Human Cortex

Supplemental Information

Supplemental Methods

Immunohistochemistry

HEK293 cells were grown in complete media (DMEM/F12 50:50 supplemented with 10% fetal bovine serum and 1% penecillin/streptomycin) at 37°C in 4-well Lab-Tek II chamber slides (Thermo Fisher Scientific, Inc.) coated in BD Matrigel (BD Biosciences) and transfected in antiboiotic-free complete media with 500 ng of construct using Lipofectamine 2000 (Life Technologies, Inc.), following manufacturer protocol. After 6 hours, the antibiotic-free media was replaced with complete media to prevent infection. Twenty-four hours after transfection, complete cell media was replaced with serum-free media and cells were incubated for 3.5 hours, after which additional media was added containing no 5-HT (serum-starved) or 1 uM 5-HT (final concentration) and incubated for 30 minutes. Immediately following, cells were lightly washed in warm Dulbecco's phosphate-buffered saline (DPBS) and fixed in 4% paraformaldehyde for 20 minutes. Following fixation, cells were again washed in DPBS and permeabilized by incubation with 0.1% Triton-X diluted in PBS for 10 minutes on ice. Cells were then blocked in 10% goat serum for one hour at room temperate and subsequently incubated with primary antibodies overnight at 4°C (SR-2A (H-75) rabbit anti-human polyclonal (Santa Cruz Biotechnology, Inc.) diluted 1:150 for 5-HT2A and c-Myc (9E10) mouse anti-human monoclonal (Santa Cruz)1:400 for c-Myc in 2% goat serum/DPBS). The next day, cells were washed in DPBS three times for 5 minutes and incubated in secondary antibody (Alexa Fluor 488 goat anti-mouse or Alexa Fluor 568 goat anti-rabbit (Life Technologies, Inc.) each diluted 1:1000 in 2% goat serum/PBS) and DRAQ5 (eBioscience, Inc.) nuclear counterstain (diluted 1:1000). Finally, cells were washed in PBS three times for 5 minutes and coverslipped with 90% glycerol.

CpG Methylation in Human Prefrontal Cortex

Genomic DNA from 223 dorsolateral prefrontal cortex samples was isolated with phenol-chloroform, bisulfite converted with the EZ DNA methylation kit (Zymo Research Corp., Irvine, CA) and methylation status measured with the Infinium HumanMethylation27 BeadChips (Illumina, Inc., San Diego, CA). Single nucleotide polymorphism genotypes extending 100 kb

upstream and downstream of the *HTR2A* gene locus, measured using HumanHap650Y_V3 or Human 1M-Duo_V3 BeadChips (Illumina, Inc.), were regressed against methylation status of a CpG site in exon 2 of *HTR2A* (chr13:47,469,654 of build GRCh37/hg19), while accounting for age, sex, and race.

HTR2A Untranslated Region (UTR) Cloning

Short (sUTR), medium (mUTR), and long 5′UTR (lUTR) amplicons were polymerase chain reaction amplified (primers in Table S2) from a single complementary DNA sample homozygous for the WT sequence of the 5′UTR and inserted immediately adjacent to the luciferase start codon in the pGL4.23 luc2/minP luciferase vector (Promega Corp., Madison, WI) using Clontech In-Fusion HD cloning (Takara Bio, Inc., Mountain View, CA). Primers for the UTR amplicons, corresponding to the UTR transcription start sites (TSS), were -2368 (lUTR), -1128 (mUTR), and -328 (sUTR) base pairs upstream of the translation start codon for 5-HT2A, each paired a common reverse primer beginning at the -1 position. The UTR lengths correspond to TSS observed in our transcriptome sequencing study and those mapped in the UCSC Genome Browser (1), supported by previous studies (2,3) and *in silico* analysis of TSS by Eponine (4). rs6311/A was introduced into the lUTR construct using QuikChange II XL Site-Directed Mutagenesis (Agilent).

Clinical Associations with STAR*D

Statistical analyses considered these potential covariates: sex, race, age at study entry, current marital status, years of schooling, highest degree obtained, current employment status, weight change immediately prior to study entry, total weight change after study entry, menopausal/post-hysterectomy, final study citalopram dose, initial Quick Inventory of Depressive Symptomatology (QIDS) score, treatment-emergent suicidal ideation phenotype (ref), pre-existing conditions and severity from the Cumulative Illness Rating Scale (see Table S4 for specific conditions), and cumulative side effects experienced. We tested the following outcome variables for STAR*D Level 1 (statistical test performed): depression severity (univariate analysis of variance for initial QIDS), change in depression score (repeated-measures analysis of variance for initial versus final QIDS score), and all self-reported side effects on the Patient Rated Inventory of Side Effects (see Table S4 for specific side effects) (case/control logistic regression). For side effects, patients were scored as cases if they reported experiencing the side effect at any point during treatment.

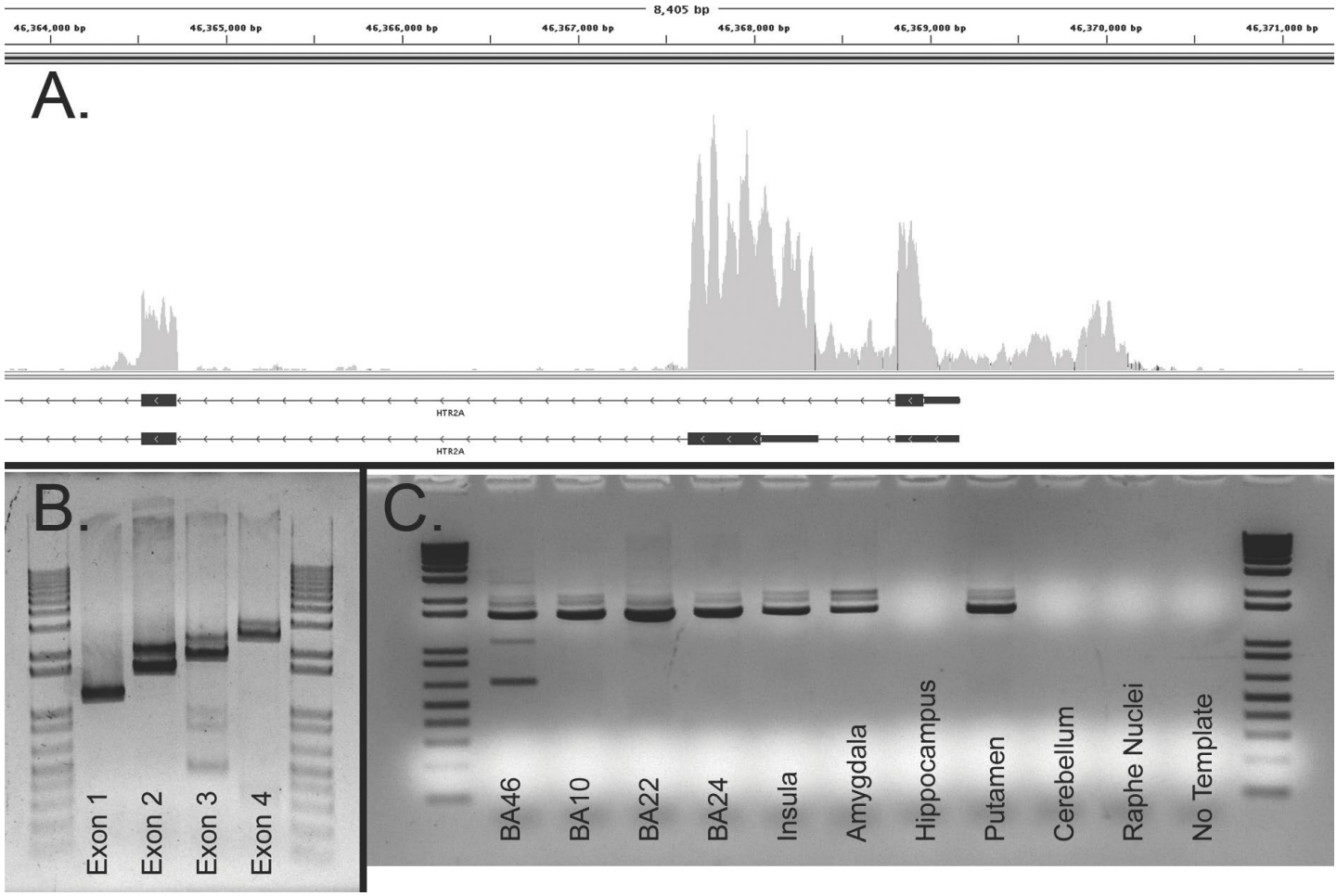

Figure S1. *HTR2A* 5′UTR usage in human brain. (**A**) Massively parallel sequencing reads from a single representative BA46 sample. Read depth is represented as a histogram above the annotated *HTR2A* gene, encoded right to left. (**B**) PCR amplification of PFC cDNA from the extended 5′UTR into exons 1, 2, 3, or 4 confirms mRNAs constituting the extended 5′UTR and all coding exons. The less abundant higher molecular weight band in exon 2-4 reactions represent retention of intron 1, as confirmed by Sanger sequencing and observed in RNA-Seq. Sizes for each amplicon match predicted sizes for mature mRNA plus the unspliced extended 5′UTR (Exon 1 = 1191bp, Exon 2 = 1615bp, Exon 3 = 1907bp, Exon 4 = 2459bp). (**C**) PCR amplification of the extended 5′UTR through exon 2 in multiple brain regions. Most brain regions express the extended 5′UTR, although we find no evidence of expression in the hippocampus, cerebellum, or Raphe nuclei. Again, in all regions where expression is observed, we also see evidence of intron 1 retention. BA, Brodmann area; cDNA, complementary DNA; mRNA, messenger RNA; PCR, polymerase chain reaction; PFC, prefrontal cortex; UTR, untranslated region.

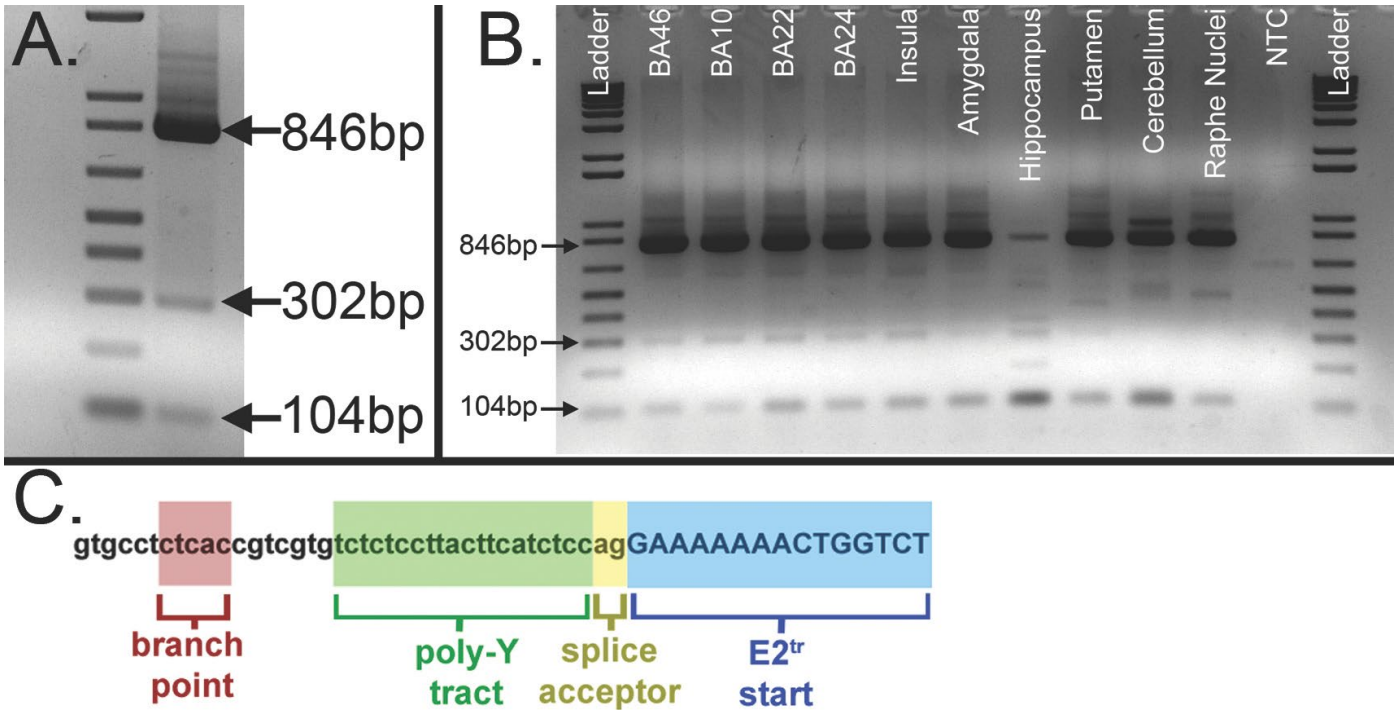

Figure S2. Alternative exon 2 splicing of *HTR2A* in human brain. (**A**) Exon 2 splice variants observed by PCR amplification of BA46 samples. The lanes correspond to a no template control, 1kb Plus DNA Ladder (Invitrogen), and cDNA from a single representative sample amplified using primers in exon 1 and 3, respectively. The full-length $E2^{+}$ variant (846bp) is most abundantly expressed, but $E2^{tr}$ (302bp) and $E2^{-}$ (104bp) are also apparent. (**B**) Exon 2 splice variant expression in ten different brain regions. Most brain regions express all three splice variants identified in BA46. However, the relative expression varies greatly in the hippocampus, and other regions appear to express uncharacterized novel splice variants (putamen, cerebellum, Raphe nuclei). (**C**) *Cis*-regulatory elements adjacent to the $E2^{tr}$ alternative exon 2 in genomic DNA. BA, Brodmann area; cDNA, complementary DNA; NTC, no template control; PCR, polymerase chain reaction.

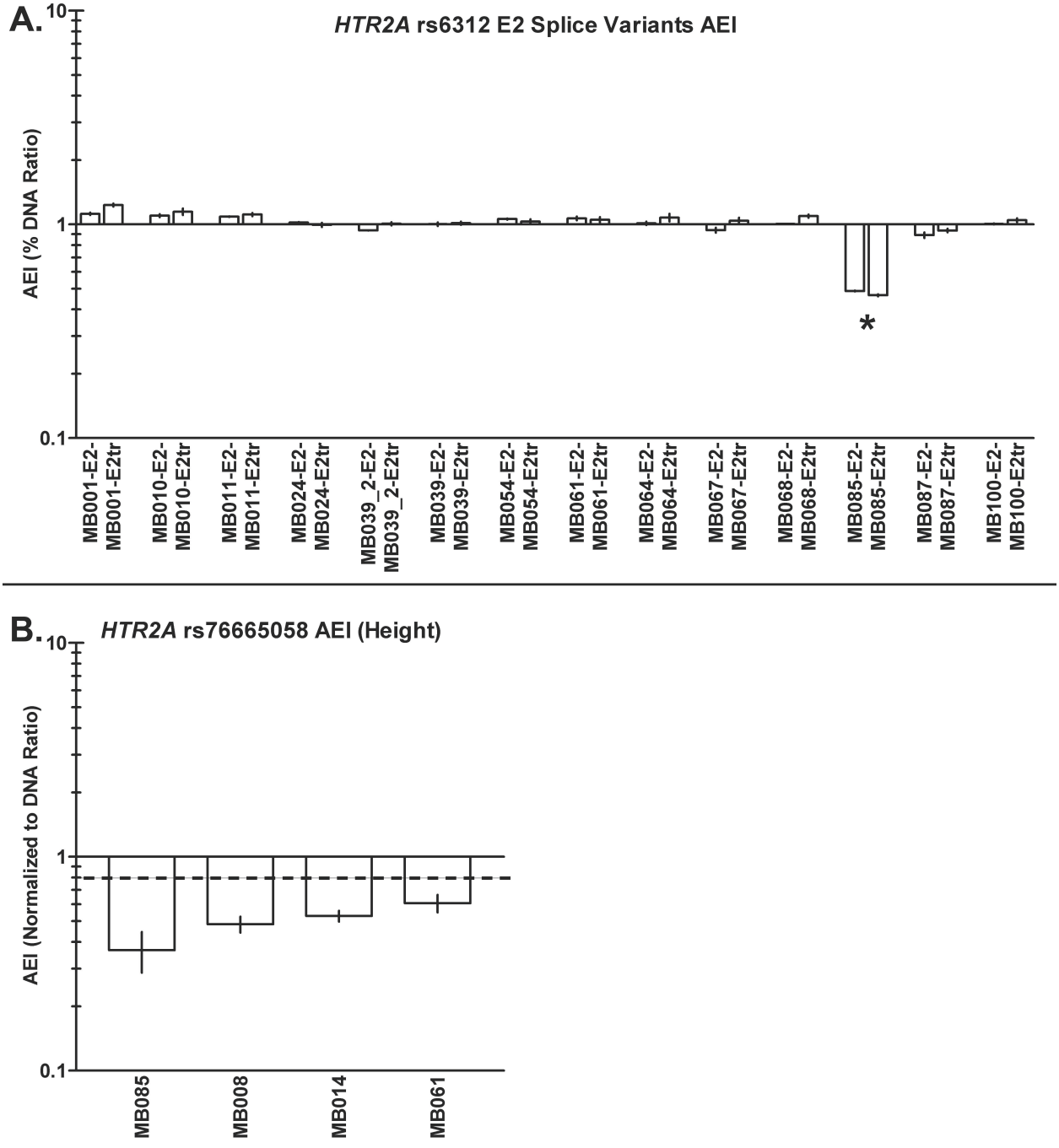

Figure S3. (**A**) AEI for exon 2 splice variants. Sample MB085 (*) displayed significant AEI for both $E2^{tr}$ and $E2^{-}$ splice variants, whereby the major allele of rs6312 express 2-fold less mRNA compared to the minor allele. (**B**) AEI for the 3′UTR SNP rs76665058. All samples heterozygous for rs76665058 display significant AEI ranging from 1.6 to 2.7-fold differences across alleles. The dotted line represents 2 standard deviations of within-sample variability. AEI, allelic expression imbalance; SNP, single nucleotide polymorphism; UTR, untranslated region.

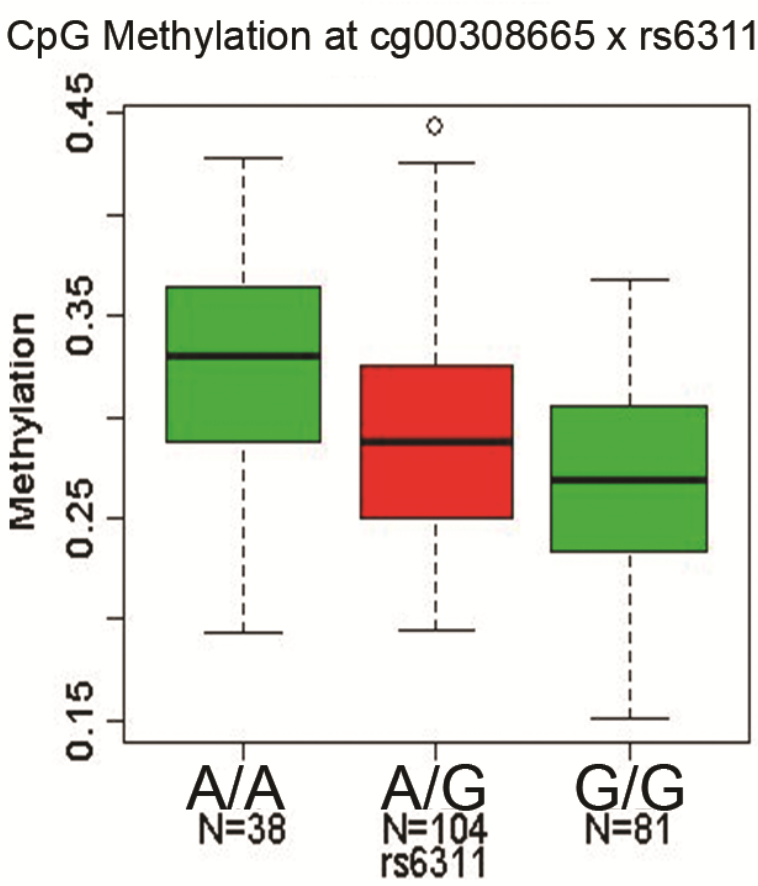

Figure S4. CpG methylation status in *HTR2A* across rs6311 genotype. Homozygous A/A minor allele carriers of rs6311 had a significantly higher percentage of CpG methylation at cg00308665 (chr13:47,469,654 of build GRCh37/hg19) compared to homozygous G/G major allele carriers ($p = 6.34 \times 10^{-7}$).

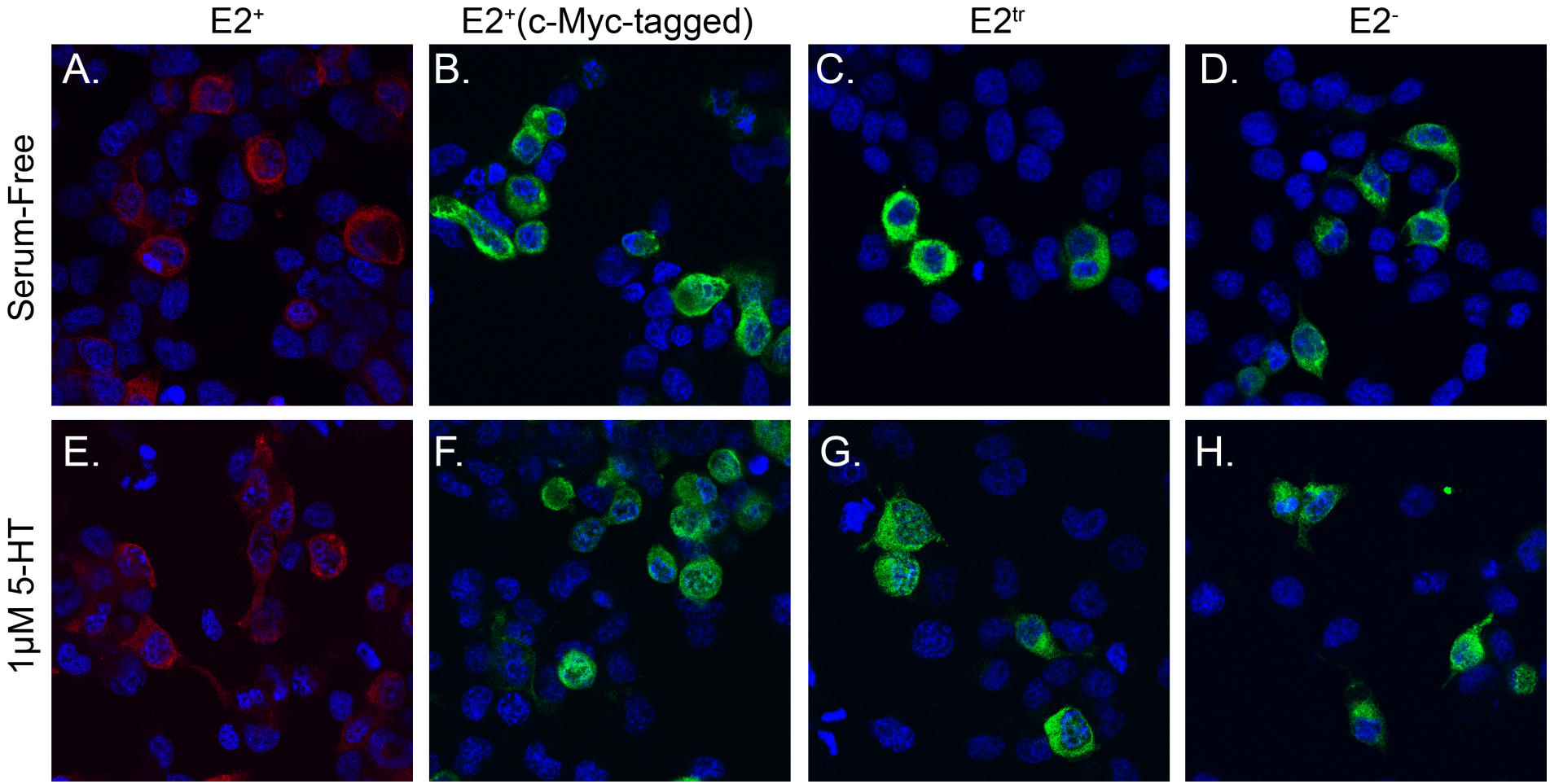

Figure S5. Immunohistochemical staining of exon 2 splice variants overexpressed in HEK293 cells following serum starvation and reintroduction of 5-HT. Following serum starvation, which removes 5-HT from the cell media, staining for the $E2^{+}$ isoform appears more pronounced on the cell surface (**A**,**B**), although it is also evident in the cytoplasm. Reintroduction of 5-HT into the media appears to decrease membrane expression of $E2^{+}$ (**E**,**F**). Truncated splice variants appear to be more evenly distributed throughout the cytoplasm under serum-free conditions (**C**,**D**) and the distribution does not change in response to 5-HT (**G**,**H**).

Table S1. Brain Tissue Demographics

Total Cohort					
Race *(n)*	**Sex (M:F)**	**Age Yrs. (Avg. ± SD)**	**PMI Hrs. (Avg. ± SD)**	**RIN (Avg. ± SD)**	**Cocaine:Control**
Caucasian (46)	31:15	41.4 ± 12.7	14.4 ± 5.2	7.7 ± 0.8	21:25
African-American (12)	12:0	28.4 ± 7.0	16.5 ± 5.0	7.6 ± 0.9	5:7
Mixed/Other[a] (16)	8:8	43.9 ± 15.5	17.9 ± 4.2	7.4 ± 1.0	4:12
Total (74)	51:23	39.8 ± 13.5	15.5 ± 5.1	7.6 ± 0.9	30:44
Prefrontal Cortex (BA46) Transcriptome Samples					
Caucasian (8)	6:2	35.0 ± 5.1	12.6 ± 4.1	7.8 ± 0.8	4:4
African-American (1)	1:0	25.0	20.5	8.2	0:1
Mixed/Other[a] (1)	0:1	32.0	16.0	9.4	1:0
Brain Region Transcriptome Survey[b]					
African-American	Male	20	12	5.0-7.1	Control

Avg., average; BA, Brodmann area; F, female; M, male; PMI, post-mortem interval; RIN, RNA integrity number.
[a] Includes both mixed race and Hispanic tissues.
[b] Tissues originated from different regions in the same individual, including frontopolar cortex (BA10), Wernicke's Area (BA22), ventral anterior cingulate cortex (BA24), insular cortex, amygdala, hippocampus, putamen, cerebellum, and pontine raphe nuclei.

Table S2. Primers Used in the Current Study

Primer	Region	Assay	Sequence (5′ --> 3′)
rs4941575 Seq F	Upstream	sequencing	TCTGGCTTGTTCCAAACCTAAGT
rs4941575 Seq R	Upstream	sequencing	CAGAGCTTCTAGGTTAATAGCATGAGG
rs73175539 Seq F	Upstream	sequencing	TCTCAGATCAACACCGGCAG
rs73175539 Seq R	Upstream	sequencing	TGGTTACTCTCCCAGTCAGCC
rs731244 Seq F	Upstream	sequencing	GCCTGCTCGCCAGCGT
rs731244 Seq R	Intron 1	sequencing	AAGATTAGCAGACAACTTTCCTCCC
rs1328685 F	5′UTR	SNaPshot, genotyping	CTTGGCCACAAACATATTGAAGG
rs1328685 R	5′UTR	SNaPshot, genotyping, cDNA synthesis	TGGGTTTTGCTACAGTTCTATCACC
rs1328685 PER	5′UTR	SNaPshot[1], genotyping	TTCTGTTCTTCACATTCTCCCT
rs6311 F	5′UTR	SNaPshot	GTAATTCCACTCTGGACACAAACACT
rs6311 R	5′UTR	SNaPshot, cDNA synthesis	AATTTTTTAGGCTGAAGGGTGAAG
rs6311 PEF	5′UTR	SNaPshot[1]	GCTTTGGATGGAAGTGCC
rs6311 RFLP F	5′UTR	genotyping	TTCCACTCCGGACACAAACACTGT
rs6311 RFLP R	5′UTR	genotyping	[6FAM]CCCATTAAGGTAGGTAAGTGGCACTGT
rs6312 Seq F	Exon 1	sequencing	AGCTGGCTCAGCTCTTGCA
rs6312 Seq R	Intron 2	sequencing	TCCTGAAGGCTGGAATATTGG
rs6312 F	Exon 1	SNaPshot, genotyping	CTGTGAGAGATGCAGCGAGTC
rs6312 R	Intron 1	SNaPshot, genotyping	AAGCATGATTTCAAACCGGAA
rs6312 PEF	Exon 1	SNaPshot[1], genotyping	GAATAACAAATGTATCTCATGTGTG
rs6313 F	Exon 2	qPCR, SNaPshot, genotyping	CTCAACTACGAACTCCCTAATGCAA
rs6313 R	Exon 2	qPCR, SNaPshot, genotyping, cDNA synthesis	TTGGTTCGATTTTCAGAGTCGA
rs6313 PER	Exon 2	SNaPshot[1], genotyping	CATCAGAAGTGTTAGCTTCTCC
E3 Splice R	Exon 3	exon 2 splicing, cDNA synthesis	CCAGACTGCACAAAGCTTGC
E1 Splice F	Exon 1	exon 2 splicing, qPCR	CTGTGAGAGATGCAGCGAGTC
$E2^{+}$ R	Exon 2 – Exon 1	qPCR	TCGGGAAGATAAATGTCATTTGTC
$E2^{-}$ R	Exon 3 – Exon 1	qPCR	GCCACCGGTACCATTTGTC
$E2^{tr}$ R	Exon 2tr – Exon1	qPCR	CAGACCAGTTTTTTTCATTTGTCTTC

rs2070040 Seq F	Intron 2	sequencing	CATGATTTAATTGGGCTGGGT
rs2070040 Seq R	Intron 3	sequencing	TCCCTCCAAGCTACAGCACAT
rs6304 RFLP F	Exon 3	genotyping	CGCCATCCAGAATCCCATC
rs6304 RFLP R	Exon 3	genotyping	[HEX]GCACGAACTGTCATTTCAAATGA
rs1328684 RFLP F	Intron 3	genotyping	CTACTGTTTTGGGTGGTGCAAG
rs1328684 RFLP R	Intron 3	genotyping	[6FAM]CAAAGTGGTCTGCATCCTTACGT
rs2760351 F	Intron 3	SNaPshot, genotyping	CCCTGCTTCATCCCTGGT
rs2760351 R	Intron 3	SNaPshot, genotyping	GGTATTTGCATTTATTTCAAATCTTTTCT
rs2760351 PEF	Intron 3	SNaPshot[1], genotyping	GCTCTCCTATCTTTTGTAAGAGTAC
rs655888 F	Intron 3	SNaPshot, genotyping	TCCACTCTACTTCCAATCCTGAAA
rs655888 R	Intron 3	SNaPshot, genotyping	AAATACCCATGCTACCGATGACT
rs655888 PER	Intron 3	SNaPshot[1], genotyping	GACCCCCCAGCTCAGTC
Antisense PreAmp F	Intron 3	qPCR, sequencing	ACCTTGGTTTTGGCCTGGTG
Antisense PreAmp R	Intron 3	qPCR, sequencing	AAATACCCATGCTACCGATGACT
Antisense Nested F	Intron 3	qPCR, sequencing	GACTTTTCACTTCCAAAACTGTTTAAA
Antisense Nested R	Intron 3	qPCR, sequencing	CAGGATTGGAAGTAGAGTGGAGTTG
rs7330461 F AS Short	Intron 3	genotyping	ACCCTCAGAGACCCaGCa
rs7330461 F AS Long	Intron 3	genotyping	aaaaACCCTCAGAGACCCgGCt
rs7330461 R Common	Intron 3	genotyping	GAGAGGTCAGCAGAGCCACAT
rs7997012 Seq F	Intron 3	sequencing	CTTTCTAATCAATGAGCAACTGTGC
rs7997012 Seq R	Intron 3	sequencing	AAAAAAGAGAGGAAACATGAATCAAGTA
rs6314 F[2]	Exon 4	qPCR, SNaPshot, genotyping	GCAAGATGCCAAGACAACAGATAA
rs6314 R[2]	Exon 4	qPCR, SNaPshot, genotyping, cDNA synthesis	TCACACACAGCTCACCTTTTCAT
rs6314 PEF	Exon 4	SNaPshot[1], genotyping	TGGTTGCTCTAGGAAAGCAG
rs3803189 F	3′UTR	SNaPshot	GCAATACAGATTTTATAACACTGACCTTAGT
rs3803189 R	3′UTR	SNaPshot, cDNA synthesis	GATGACATGGGATTGAGTTGGTTAC
rs3803189 PEF	3′UTR	SNaPshot[1]	CCATTATATTCAATAAAATTTTCACTATT
rs3803189 F Common	3′UTR	genotyping	[HEX]TGGAAACCTTGCTGCTATGCT
rs3803189 R AS Short	3′UTR	genotyping	GCCATTATATTCAATAAAATTTTCACTtTTg
rs3803189 R AS Long	3′UTR	genotyping	ttaaGCCATTATATTCAATAAAATTTTCACTAcTt

rs7324017 F	3′UTR	SNaPshot, genotyping	GGAAGTGTCATTGTGTAATTTGGAA
rs7324017 R	3′UTR	SNaPshot, genotyping, cDNA synthesis	GGAGTAGTTCAGTTCAAATGCAGC
rs7324017 PEF	3′UTR	SNaPshot[1], genotyping	GATATGTTGAAAGATGGTTCACT
rs73473857 F	3′UTR	SNaPshot	ATATGGACGAAAAGCAAGTCAATG
rs73473857 R	3′UTR	SNaPshot, cDNA synthesis	TTGCAGCAATGGAAGGTCATAG
rs73473857 PER	3′UTR	SNaPshot[1]	TAAATAAACATGATACAAACATGCAC
rs73473857 RFLP F	3′UTR	genotyping	AAAAGCAAGTCAATGAAAACACTCAGTA
rs73473857 RFLP R	3′UTR	genotyping	[HEX]TCTTCCACAAAATTAGATTAATTTCCAG
rs61948307 F	3′UTR	SNaPshot	CCAAATTGAACTAAGTCACTGTACTGCT
rs61948307 R	3′UTR	SNaPshot	TTTTGAGTCTACTTTATTTACAGTTATTTATCCTTT
rs61948307 PEF	3′UTR	SNaPshot[1]	AACTTATTTTAATCAAGGCGATG
rs61948307 RFLP F	3′UTR	genotyping	TTTTATGAACTTATTTTAATCAAGGCGAT
rs61948307 RFLP R	3′UTR	genotyping	[6FAM]GTTCTGAGTCTGATGACCTGGAAGA
rs76665058 F	3′UTR	SNaPshot, genotyping	AATGAATGAATTTTGTGTGAGTCCA
rs76665058 R	3′UTR	SNaPshot, genotyping, cDNA synthesis	TGCTCTCGAATATCAGGATGATACC
rs76665058 PER	3′UTR	SNaPshot[1], genotyping	ATTCCGTTTAGTAGACACAGCT
pGL4.23 Luc F	Luciferase gene	qPCR	ACGGTAAAACCATGACCGAGA
pGL4.23 Luc R	Luciferase gene	qPCR	TTGCCGGTCAGTCCTTTAGG
pGL4.23 sUTR F	short UTR	qPCR	CTTTTTTGTCCTCGGTTTGGTG
pGL4.23 sUTR R	short UTR	qPCR	AAAGAACTGAACTGTGGTGGCTG
pGL4.23 mUTR F	medium UTR	qPCR	AGCTGGCTCAGCTCTTGCAT
pGL4.23 mUTR R	medium UTR	qPCR	TGTGACTCGCTGCATCTCTCA
pGL4.23 lUTR F	long UTR	qPCR	GAAGAGTCGCGGATAACAGCA
pGL4.23 lUTR R	long UTR	qPCR	CCTTGACCTCAGCATCTTCCC
pGL4.23 sUTR Clone F	short UTR	cloning	GGTAAAGCCACCATGGGACATTTATCTTCCCGAGCGCT
pGL4.23 mUTR Clone F	medium UTR	cloning	GGTAAAGCCACCATGGGTGGAAACCAGGAGTCCCTTG
pGL4.23 lUTR Clone F	long UTR	cloning	GGTAAAGCCACCATGGTGATAGAGCTAGGATCCAAAACCAAG
pGL4.23 Common R	pGL4.23 clone	cloning	TTGGCATCTTCCATGGGTCTAAGCCAGAACTTGTAGCAGATGA

cDNA, complementary DNA; qPCR, quantitative polymerase chain reaction; UTR, untranslated region.
[1]Primer used only in primer extension SNaPshot reaction.
[2]Primer used for estimation of total *HTR2A* via qPCR.

Table S3. Luciferase Activity and mRNA Expression for Estimating Translation Efficiency

Cell Line	Construct – Replicate	Luc RLU Avg.	Luc RLU SD	Luc CT Avg.	Luc CT SD	RLU/Transformed CT Avg.[a]	RLU/Transformed CT SD[a]	% of NoUTR	Construct	Avg. % NoUTR
HEK293T	NoVector – 1	49.25	6.65	-	-	-	-	-	No Vector	-
HEK293T	NoVector – 2	47.75	4.19	-	-	-	-	-		
HEK293T	sUTR – 1	3358.00	284.75	21.06	0.32	72.57	7.23	0.07	sUTR	**0.05**
HEK293T	sUTR – 2	2892.00	265.83	20.31	0.33	37.84	4.14	0.03		
HEK293T	mUTR – 1	1910.75	70.13	22.09	0.12	85.03	3.78	0.08	mUTR	**0.05**
HEK293T	mUTR – 2	2243.00	311.06	20.52	0.25	33.90	5.74	0.03		
HEK293T	lUTRG – 1	3331.00	610.65	21.53	0.21	99.66	22.45	0.09	lUTRG	**0.13**
HEK293T	lUTRG – 2	6813.25	553.47	21.44	0.08	190.31	17.24	0.17		
HEK293T	lUTRA – 1	8297.00	315.00	21.75	0.43	288.60	10.81	0.26	lUTRA	**0.16**
HEK293T	lUTRA – 2	7446.50	608.17	19.76	0.19	64.80	5.56	0.06		
HEK293T	NoUTR – 1	2719.75	128.40	25.12	0.27	999.38	51.80	0.91	No UTR	**1.00**
HEK293T	NoUTR – 2	2931.50	234.69	25.32	0.23	1204.26	108.75	1.09		
SH-SY5Y	NoVector – 1	432.00	62.54	-	-	-	-	-	No Vector	**-**
SH-SY5Y	NoVector – 2	379.25	23.33	-	-	-	-	-		
SH-SY5Y	sUTR – 1	949.50	5.07	22.68	0.48	63.81	0.22	0.04	sUTR	**0.03**
SH-SY5Y	sUTR – 2	1298.25	15.28	21.78	0.10	46.41	0.34	0.03		
SH-SY5Y	mUTR – 1	1144.50	44.19	23.83	0.08	170.00	8.02	0.10	mUTR	**0.11**
SH-SY5Y	mUTR – 2	1032.25	72.41	24.32	0.06	214.19	17.58	0.13		
SH-SY5Y	lUTRG – 1	990.50	23.27	23.78	0.27	142.31	3.74	0.08	lUTRG	**0.11**
SH-SY5Y	lUTRG – 2	1198.25	21.31	24.12	0.42	219.05	4.77	0.13		
SH-SY5Y	lUTRA – 1	1056.00	44.31	25.23	0.21	414.04	20.51	0.24	lUTRA	**0.16**
SH-SY5Y	lUTRA – 2	780.00	19.41	24.04	0.08	133.31	3.42	0.08		
SH-SY5Y	NoUTR – 1	1149.50	92.59	26.80	0.66	1356.18	130.55	0.80	No UTR	**1.00**
SH-SY5Y	NoUTR – 2	1528.75	73.83	27.02	0.08	2046.62	100.01	1.20		

Avg., average; CT, quantitative polymerase chain reaction cycle threshold; lUTRA, long UTR rs6311 A allele; lUTRG, long UTR rs6311 G allele; Luc, luciferase; mRNA, messenger RNA; mUTR, medium UTR; RLU, relative light units; sUTR, short UTR; UTR, untranslated region.
[a]CT values transformed using the following formula: $(1/2^{CT})*10000000$

Table S4. Demographics, Covariates[a], Pre-Existing Conditions[b], and Side Effects[c] for STAR*D Cohort

Race (*n*)	Sex M:F	Yrs. of School	Age	Initial QIDS	Final QIDS	Final Dose	Cum. Side Effects
White (990)	406:584	14.1 ± 3.2	43.2 ± 13.4	16.1 ± 3.2	7.5 ± 5.4	20.1 ± 4.0	19.1 ± 6.0
Black (157)	65:92	13.2 ± 2.8	45.5 ± 12.8	16.8 ± 3.7	8.8 ± 5.8	20.1 ± 4.2	17.5 ± 5.8
Asian (21)	9:12	16.1 ± 3.2	32.6 ± 13.9	16.5 ± 3.0	4.9 ± 3.7	21.9 ± 8.1	20.9 ± 6.7
American Indian/Alaskan (23)	11:12	14.0 ± 2.6	37.4 ± 11.8	16.5 ± 3.0	7.4 ± 5.3	19.3 ± 3.1	19.8 ± 7.6
Hawaiian (19)	6:13	12.7 ± 3.4	37.7 ± 10.7	17.8 ± 3.2	7.4 ± 5.9	19.5 ± 2.3	18.4 ± 7.9
Mixed/Other (14)	6:8	14.5 ± 2.4	40.0 ± 10.5	15.1 ± 2.4	9.1 ± 5.6	20.0 ± 3.9	18.4 ± 6.2
All (1224)	503:721	14.0 ± 3.1	43.1 ± 13.4	16.2 ± 3.3	7.6 ± 5.4	20.2 ± 4.1	19.0 ± 6.1

Cum., cumulative; F, female; M, male; QIDS, Quick Inventory of Depressive Symptomatology.

[a]Variables for each covariate are as follows:

Current marital status: married, divorced, never married, separated, widowed, cohabiting

Highest degree obtained: none, GED, high school diploma, associate degree, college diploma, master's degree, doctoral/professional degree

Current employment status: unemployed – looking, unemployed – not looking, part-time, full-time, self-employed, retired – not working

Weight change immediately prior to study entry: Loss (≥-5lbs, >-5≥-2lbs, >-2≥-1lbs), Gain (≥5lbs, >5≥2lbs, >2≥1lbs), No change (0lbs)

Menopausal/post-hysterectomy: menopausal, post-hysterectomy, or male; premenopausal female with no hysterectomy

Treatment-emergent suicidal ideation phenotype: case, control

[b]Pre-existing conditions from the Cumulative Illness Rating Scale and severity (no problem, current mild problem or past significant problem, severe constant significant disability/"uncontrollable" chronic problems, extremely severe/immediate treatment required/end organ failure/sever impairment in function): heart, vascular, haematopoietic, respiratory, eyes/ears/nose/throat/larynx, upper gastrointestinal, lower gastrointestinal, liver, renal, genitourinary, musculoskeletal/integument, neurological, endocrine/metabolic and breast, psychiatric illness (excluding major depressive disorder).

[c]Categories and side effects self-reported on the Patient Rated Inventory of Side Effects: Gastrointestinal (diarrhea, constipation, dry mouth, nausea/vomiting), Heart (palpitations, dizziness on standing, chest pain), Skin (rash, increases perspiration, itching, dry skin), Nervous system (headache, tremors, poor coordination, dizziness), Eyes/Ears (blurred vision, ringing in ears), Genital/Urinary (difficulty urinating, painful urination, frequent urination, menstrual irregularity).

Table S5. SNPs Correlated with CpG Methylation in *HTR2A* (See also: Figure 4)

		p-value			r^2 to rs6311		
SNP[a]	**Coordinate (hg18)**	**All**	**Cauc**	**AA**	**All**	**Cauc**	**AA**
1 - rs622337	46325627	7.20×10^{-3}	1.15×10^{-2}	5.06×10^{-1}	0.007	0.008	0.015
2 - rs655854	46326201	5.29×10^{-3}	1.15×10^{-2}	4.57×10^{-1}	0.005	0.008	0.021
3 - rs2296972	46326472	3.41×10^{-3}	1.15×10^{-2}	1.58×10^{-1}	0.027	0.008	0.074
4 - rs1928042	46335217	2.93×10^{-3}	2.48×10^{-2}	4.49×10^{-2}	0.236	0.237	0.232
5 - rs6561336	46346061	6.69×10^{-7}	1.49×10^{-3}	2.57×10^{-4}	0.705	0.931	0.516
6 - rs972979	46347165	3.44×10^{-4}	3.16×10^{-3}	2.67×10^{-2}	0.526	0.484	0.574
7 - rs1928039	46351187	7.29×10^{-3}	9.52×10^{-3}	2.54×10^{-1}	0.052	0.063	0.041
8 - rs2770304	46353366	3.05×10^{-3}	5.20×10^{-4}	2.91×10^{-1}	0.438	0.411	0.469
9 - rs4942587	46360801	1.12×10^{-2}	1.36×10^{-1}	6.92×10^{-2}	0.116	0.202	0.043
10 - rs4941573	46362858	9.21×10^{-5}	1.49×10^{-3}	5.47×10^{-3}	0.670	0.931	0.439
11 - rs1328684	46364231	2.68×10^{-4}	3.00×10^{-2}	3.39×10^{-3}	0.348	0.389	0.304
12 - rs2296973	46364782	6.55×10^{-6}	1.15×10^{-3}	3.64×10^{-3}	0.227	0.290	0.170
13 - rs2070037	46365071	4.17×10^{-3}	1.36×10^{-1}	1.42×10^{-2}	0.118	0.202	0.047
14 - rs9534511	46366581	2.40×10^{-7}	1.22×10^{-3}	1.15×10^{-4}	0.441	0.596	0.311
15 - rs6313	46367941	2.79×10^{-7}	8.27×10^{-4}	8.29×10^{-5}	0.954	1.000	0.904
16 - rs6312	46368825	2.23×10^{-3}	2.16×10^{-2}	2.64×10^{-2}	0.090	0.052	0.133
17 - rs6311	46369479	6.40×10^{-7}	8.27×10^{-4}	2.87×10^{-4}	-	-	-
18 - rs732821	46370880	2.87×10^{-7}	6.40×10^{-4}	3.11×10^{-4}	0.618	0.870	0.422
19 - rs17289394	46371221	9.74×10^{-4}	7.40×10^{-2}	7.46×10^{-3}	0.227	0.411	0.092
20 - rs4142900	46371551	1.48×10^{-3}	7.89×10^{-2}	1.11×10^{-2}	0.482	0.661	0.334
21 - rs2149434	46376345	1.11×10^{-2}	1.21×10^{-2}	2.88×10^{-1}	0.379	0.469	0.284
22 - rs10507546	46381075	1.60×10^{-2}	1.25×10^{-1}	1.30×10^{-1}	0.094	0.187	0.018

AA, African American; Cauc, Caucasian; SNP, single nucleotide polymorphism.
[a]Number preceding SNP refers to annotation in Figure 4.
Value in red indicates the proposed functional SNP modulating expression of the extended 5′ untranslated region.

Table S6. Linkage Disequilibrium Between Functional SNPs and Genome-Wide Human SNP Array 5.0 Surrogate Markers

	Population			
	CEU		YRI	
Functional SNP / Surrogate	**MAF**	***D′* and r^2**	**MAF**	***D′* and r^2**
rs6311 / rs6313	0.450 / 0.450	1, 1	0.424 / 0.390	1, 0.869
rs6314 / rs7323441	0.062 / 0.063	1, 0.764	0.164 / 0.185	0.926, 0.799
rs76665058 / rs585719	0.000 / 0.183	N/A[a]	0.059 / 0.025	0.629, 0.163

CEU = CEPH (Utah Residents with Northern and Western European Ancestry); YRI = Yoruba in Ibadan, Nigeria.
MAF, minor allele frequency; SNP, single nucleotide polymorphism.
[a] Cannot be calculated because rs76665058 is not found in CEU samples

Table S7. SNP-SNP Interactions with Baseline QIDS

rs6313*SNP	*p*	r^2 with rs6314[a]	*D'* with rs6314[a]	rs7323441*SNP	*p*	r^2 with rs6311[a]	*D'* with rs6311[a]
rs7323441	**0.011**	**0.764**	**1**	rs4941573	0.003	1	1
rs2296972	0.058	0.092	0.616	**rs6313**	**0.011**	**1**	**1**
rs2025296	0.062	0.005	0.086	rs985933	0.018	0.474	1
rs1923888	0.066	0.092	0.616	rs1928040	0.02	0.711	0.857
rs1360020	0.126	0	0.05	rs985934	0.021	0.474	1
rs1923882	0.182	0.324	1	rs582854	0.028	0.545	1
rs582854	0.237	0.054	1	rs4942578	0.063	0.033	0.402
rs1928038	0.246	0.002	0.062	rs2770297	0.07	0.31	1
rs1928040	0.253	0.011	0.336	rs17289854	0.082	0.176	0.882
rs2770299	0.275	0.001	1	rs1328685	0.096	0.099	1
rs1745837	0.309	0.092	0.616	rs9567739	0.096	0.004	0.109
rs9567739	0.336	0.189	1	rs9316232	0.106	0.004	0.109
rs4942578	0.387	0.001	0.209	rs17359763	0.113	0.036	1
rs9316232	0.392	0.189	1	rs2296972	0.135	0.002	0.081
rs2016711	0.405	0.005	1	rs1360020	0.144	0.703	0.959
rs1328683	0.409	0.002	0.084	rs666693	0.16	0.174	1
rs4941573	0.445	0.002	0.142	rs1002513	0.176	0.174	1
rs2770297	0.452	0.006	0.171	rs1928038	0.178	0.036	1
rs1328685	0.564	0	0.008	rs1923888	0.21	0.002	0.081
rs582385	0.618	0.017	1	rs17289304	0.247	0.099	1
rs985934	0.656	0.001	0.094	rs1328683	0.254	0.205	1
rs985933	0.679	0.001	0.094	rs1923882	0.299	0.001	0.053
rs9595550	0.706	N.D.	N.D	rs9595550	0.353	N.D.	N.D
rs6316	0.713	N.D.	N.D	rs6316	0.365	N.D.	N.D
rs7335733	0.733	N.D.	N.D	rs7330205	0.371	N.D.	N.D
rs4942577	0.736	0.035	1	rs7334093	0.371	N.D.	N.D
rs17289854	0.755	0.013	0.776	rs2016711	0.375	0.051	1
rs9595549	0.757	N.D.	N.D	rs9595546	0.378	N.D.	N.D
rs666693	0.761	0.017	1	rs9526245	0.388	0.226	1
rs9595546	0.77	N.D.	N.D	rs9595549	0.408	N.D.	N.D
rs7330205	0.771	N.D.	N.D	rs1745837	0.416	0.002	0.081
rs7334093	0.772	N.D.	N.D	rs2025296	0.469	0.099	1
rs977003	0.858	0.09	1	rs582385	0.474	0.174	1
rs17359763	0.868	0.004	1	rs7335733	0.521	N.D.	N.D
rs17289304	0.89	0	0.008	rs977003	0.578	0.009	0.102
rs1002513	0.915	0.017	1	rs4942577	0.742	0.03	0.295
rs9526245	0.935	0	0.023	rs2770299	0.875	0.007	1

N.D., not determined; QIDS, Quick Inventory of Depressive Symptomatology; SNP, single nucleotide polymorphism.
[a]Calculated in HapMap CEU population.
Values in red indicate SNPs genotyped in STAR*D that are surrogates for proposed functional variants.

Supplemental References

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