

Functional polymorphisms and transcriptional analysis in the 5' region of the human serotonin receptor 1B gene (HTR1B) and their associations with psychiatric disorders

Xi Xia

China medical university

Mei Ding

China medical university

Jin-feng Xuan

China medical university

Jia-xin Xing

China medical university

Jun Yao

China medical university

Xue Wu

China medical university

Bao-jie Wang (✉ wangbj77@163.com)

China Medical University <https://orcid.org/0000-0003-1931-423X>

Research article

Keywords: HTR1B, polymorphism, transcriptional regulation, schizophrenia, psychiatric disorder

Posted Date: April 15th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-21296/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published on October 9th, 2020. See the published version at <https://doi.org/10.1186/s12888-020-02906-4>.

Abstract

Background

The 5-hydroxytryptamine 1B receptor (5-HT_{1B}) plays an essential role in the serotonin (5-HT) system and is widely involved in a variety of brain activities. *HTR1B* is the gene encoding 5-HT_{1B}. Genome-wide association studies have shown that *HTR1B* polymorphisms are closely related to multiple mental and behavioral disorders; however, the functional mechanisms underlying these associations are unknown. This study investigated the effect of several *HTR1B* haplotypes on reporter protein expression levels in vitro and the functional sequences in the 5' regulatory region of *HTR1B* to determine their potential association with mental and behavioral disorders.

Methods

Six haplotypes consisting of rs4140535, rs1778258, rs17273700, rs1228814, rs11568817, and rs130058 and several truncated fragments of the 5' regulatory region of *HTR1B* were transfected into SK-N-SH and HEK-293 cells. The relative fluorescence intensities of the different haplotypes and truncated fragments were detected using a dual-luciferase reporter assay system.

Results

Compared to the major haplotype T-G-T-C-T-A, the relative fluorescence intensities of haplotypes C-A-T-C-T-A, C-G-T-C-T-A, C-G-C-A-G-T, and C-G-T-A-T-A were significantly lower, and that of haplotype C-G-C-A-G-A was significantly higher. Furthermore, the effects of the rs4140535T allele, the rs17273700C-rs11568817G linkage combination, and the rs1228814A allele made their relative fluorescence intensities significantly higher than their counterparts at each locus. Conversely, the rs1778258A and rs130058T alleles decreased the relative fluorescence intensities. In addition, we found that regions from -1587 to -1371 bp (TSS, +1), -1149 to -894 bp, -39 to +130 bp, +130 to +341 bp, and +341 to +505 bp upregulated gene expression. In contrast, regions -603 to -316 bp and +130 to +341 bp downregulated gene expression. Region +341 to +505 bp contained the core promoter region and played a decisive role in gene transcription.

Conclusions

HTR1B 5' regulatory region polymorphisms have regulatory effects on gene expression and potential correlate with several pathology and physiology conditions. This study suggests that the core promoter sequence of *HTR1B* is located in region +341~+505 bp. Regions -1587 to -1371 bp, -1149 to -894 bp, -603 to -316 bp, -39 to +130 bp, and +130 to +341 bp contain functional sequences that can promote or suppress the *HTR1B* gene expression.

1. Background

Serotonin or 5-hydroxytryptamine (5-HT) is a crucial neurotransmitter that is widely involved in various physiological activities (e.g., mood, sleep, pain, feeding, and temperature regulation)[1]. A variety of mental or behavioral disorders are associated with 5-HT system dysfunction. The 5-HT receptor family includes seven categories and 14 subtypes[2]. The 5-HT 1B receptor (5-HT1B) is encoded by *HTR1B* and is closely associated with a variety of mental illnesses[3–6]. In previous behavioral studies, *HTR1B* knockout mice showed increased impulsivity and aggressiveness in certain behavioral phenotypes compared to wild-type mice[7]. Doxycycline restored *HTR1B* expression in adult mice and reversed the impulsive phenotype but not the aggression. *HTR1B* is located on chromosome 6, within 6q13-q26. Evidence suggests that there is a susceptibility gene for schizophrenia in this area[8]. HTR1B is also thought to be involved in the pathogenesis of major depression and antidepressant treatment. Selective ablation of 5-HT1B in mice caused increased extracellular serotonin levels following the administration of a selective serotonin reuptake inhibitor (SSRI) and induced antidepressant-like effects[9].

Many genetic polymorphisms are located in the *HTR1B* coding region and 5' and 3' regulatory regions, such as the missense mutations rs130060 (Cys124Phe), rs130061 (Phe219Leu), rs130063 (Ile367Val), and rs130064 (Glu374Lys) and the synonymous mutations rs6296 (Val287) and rs6298 (Ser43)[10]. However, variations in the non-coding sequences may be more likely to cause disease. T-261G and A-161T are located in the 5' regulatory region of *HTR1B* [REF]. Duan et al. demonstrated that the T-261G mutation generates a new AP2 binding site that enhances transcriptional activity while the A-161T mutation results in reduced transcriptional activity because of its different binding characteristics for AP1[11]. The rs9361233 and rs9361235 polymorphisms are located in the transcription factor binding sites (TFBSs) in *HTR1B* and are significantly associated with clinical improvement after treatment with fluoxetine[12]. The A-A-A-C haplotype of the rs6297, rs130058, rs1213366, and rs1213371 polymorphisms in the promoter region of *HTR1B* have a potential association with the gender of schizophrenic patients in the Spanish population [13]. rs13212041 is located in the 3' regulatory region of *HTR1B*. The rs13212041 G allele attenuates microRNA-directed mRNA silencing of *HTR1B* [14]. Individuals with the rs13212041 AA homozygous genotype are more aggressive than those that are G allele carriers. Conner et al. found that males with a low expression haplotype, who carry the rs13212041 A allele tend to exhibit greater anger and hostility[15]. In a previous study, our group found that in the northern Han Chinese population, there were at least six SNPs in the 5' regulatory region of *HTR1B*, including rs4140535, rs1778258, rs17273700, rs1228814, rs11568817, and rs130058. Among these SNPs, rs1778258 appears to be associated with schizophrenia. However, the effects of polymorphisms and regulatory sequences of *HTR1B* require further study.

2. Materials And Methods

2.1 Ethical compliance

The study was approved by the China Medical University Review Committee. All samples were collected in accordance with the principle of informed consent.

2.2 Samples

Based on the data of our previous study, we selected genomic DNA samples containing six haplotypes (rs4140535, rs1778258, rs17273700, rs1228814, rs11568817, and rs130058) to construct the pGL3 recombinants. (Table 1). The target gene was obtained from GenBank using the reference sequence NC_000006.12 (Homo sapiens chromosome 6, GRCh38.p7 Primary Assembly).

Table 1
The polymorphism comparison of six SNPs in the haplotype recombinants

Haplotype	rs4140535	rs1778258	rs17273700	rs1228814	rs11568817	rs130058
H1	T	G	T	C	T	A
H2	C	A	T	C	T	A
H3	C	G	T	C	T	A
H4	C	G	C	A	G	T
H5	C	G	T	A	T	A
H6	C	G	C	A	G	A

2.2 Construction of haplotype recombinants in the 5' regulatory region of HTR1B

According to data from DNA sequencing in our previous study, DNA samples containing haplotypes H1 to H6 were used as DNA templates for PCR. The target fragments spanned from - 1587 to 711 bp in the 5' regulatory region (TSS, + 1). The 5' end of the primers introduced KpnI and XhoI cleavage sites. The primer sequences were 5'-GGGGTACCCGGTTTGTGCTTTATTGCCTT-3' (sense), and 5'-CCGCTCGAGCAGAGGATAAGTTGGCTTG-3' (antisense). The target fragments were cloned into the pBM20S vector (Biomed, Beijing, China), and then re-cloned into the KpnI/XhoI site of the promoterless and enhancerless reporter vector pGL3-Basic (Promega, Madison, WI). The target haplotype of each construct was verified by DNA sequencing.

2.3 Construction of truncated sequence recombinants in the 5' regulatory region of HTR1B

The amplified targets consisted of a series of DNA fragments with a common end at + 711 bp (TSS, + 1). The longest fragment was from - 1587 to + 711 bp, and fragments of different lengths were sequentially truncated. The shortest fragment was from + 505 to + 711 bp (Fig. 1). Ten pairs of primers were used for PCR amplification of the target fragments (Table 2). KpnI and XhoI cleavage sites were introduced into the 5' end of the primers. The target gene was cloned into the KpnI/XhoI site of pGL3-Basic in the same manner as for the haplotype recombinants. The truncated sequence recombinants were verified by DNA sequencing.

Table 2
Primer sequences of the target fragments

Primer	sequences
D0 (-1587 ~ + 711)-F	5' GGGGTACCCGGTTTGTGCTTTATTGCCTT 3'
D1 (-1371 ~ + 711)-F	5' GGGGTACCAGGAATTCGTAGTGGGGATCAT 3'
D2 (-1149 ~ + 711)-F	5' GGGGTACCGCAAAGGAGACACACAAGATG 3'
D3 (-894 ~ + 711)-F	5' GGGGTACCTGTCTTTTTCCCTGTTGACTCATAG 3'
D4 (-603 ~ + 711)-F	5' GGGGTACCTAAGGACATGGGTCTCACTGG 3'
D5 (-316 ~ + 711)-F	5' GGGGTACCGGACCGGACCTGGACTCTATAT 3'
D6 (-39 ~ + 711)-F	5' GGGGTACCGTGGCCAGAGAGTGAAAAGAG 3'
D7 (+ 130 ~ + 711)-F	5' GGGGTACCCTGCAAGCTTTGGTCTCTACAC 3'
D8 (+ 341 ~ + 711)-F	5' GGGGTACCAACCCAGGTCTAAGACCCGGT 3'
D9 (+ 505 ~ + 711)-F	5' GGGGTACCGAACTATCAACTGGGGACAAAC 3'
Common end (+ 711)-R	5' CCGCTCGAGCAGAGGATAAGTTGGCTTG 3'

2.4 Cell Lines And Cell Culture

The human neuroblastoma (SK-N-SH) and embryonic kidney (HEK-293) cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China)[16, 17]. SK-N-SH and HEK-293 cells were cultured in HyClone® DMEM supplemented with 15% FBS (PAN-Biotech) and KeyGEN BioTECH® DMEM in the presence of 10% FBS, respectively. All cells were grown in a humidified 37 °C environment at 5% CO₂.

2.5 Transfection And Dual-Luciferase Reporter Assay

Cells were plated in 24-well plates (2×10^5 cells/well) and grown for 36 to 48 h to 70 to 90% confluence. The pGL3 recombinants (500 ng) or control plasmid pGL3-Control (Promega, Madison, WI) were transiently transfected using Lipofectamine®3000 reagent (Invitrogen, CA), according to the manufacturer's protocol. pRL-TK (50 ng) (Promega, Madison, WI) was co-transfected with each plasmid to normalize for transfection efficiency. Cell lysates were collected for the reporter assay 28 to 30 h post-transfection. The reporter assay was carried out using the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI). The firefly luciferase activity (LUC) was normalized to the renilla luciferase activity (TK).

2.6 Statistical Analysis

The relative fluorescence intensity was calculated by dividing the LUC value by that of TK[18]. The comparison between the different recombinants was performed using SPSS19.0 software (IBM, Armonk, NY, USA). Changes in relative fluorescence intensity between the different haplotype recombinants were compared using the Bonferroni or Dunnett's T3 test following one-way analysis of variance (ANOVA). Differences in the relative fluorescence intensities between adjacent truncated sequence recombinants (D0 versus D1, D1 versus D2, D2 versus D3, D3 versus D4, D4 versus D5, D5 versus D6, D6 versus D7, D7 versus D8, and D8 versus D9) were calculated using the t-test. $P < 0.05$ indicated statistical significance.

2.7 Transcription Factor Prediction

Transcription factor predictions for the functional fragments were carried out using JASPAR (<http://jaspar.genereg.net>)[19]. The filter was set to a threshold of 85%.

3. Results

3.1 The polymorphisms of the 5' regulatory region of HTR1B have regulatory effects on transcriptional activity.

In the previous study, we found that haplotype H1 was the major haplotype in the northern Han Chinese population[20]. In SK-N-SH cells, the less prominent haplotypes H2, H3, H4, and H5 had significantly lower relative fluorescence intensities compared to H1 ($p = 1.892E-06$, $1.370E-03$, $2.337E-05$, and $6.123E-03$, respectively). In contrast, haplotype H6 demonstrated increased transcriptional activity compared to H1 ($p = 1.266E-03$) (Fig. 2a). Haplotypes H2, H4, and H6 caused similar effects in HEK-293 cells ($p = 1.986E-06$, $3.977E-02$, and $1.017E-04$, respectively) (Fig. 2b).

We observed a variation between haplotypes H1 and H3 in rs4140535 (T/C). The relative fluorescence intensity of haplotype H1 was significantly higher than that of haplotype H3 in the SK-N-SH cells, indicating that the rs4140535T allele increased *HTR1B* gene expression. We also noticed that there was a difference between haplotypes H2 and H3 at rs1778258 (A/G). The relative fluorescence intensity of haplotype H2 was much lower than that of haplotype H3 in both cell lines, suggesting that the rs1778258A allele had a negative effect on *HTR1B* transcriptional activity. The rs1228814A allele of H5 also appeared to upregulate *HTR1B* gene expression relative to haplotype H3 in HEK-293 cells. Haplotype H6 had the highest relative fluorescence intensity. The variations between haplotypes H5 and H6 were present in rs17273700 (T/C) and rs11568817 (T/G), and there was a strong linkage between rs17273700 and rs11568817 (T-T/C-G). Thus, the C-G combination enhanced *HTR1B* gene expression. Haplotypes H4 and H6 exhibited different transcriptional effects in the two cell lines. Based on their variation at rs130058 (A/T), the rs130058T allele may inhibit *HTR1B* gene expression.

3.2 Analysis of functional sequences of 5' regulatory region of HTR1B

In SK-N-SH, the changes in the relative fluorescence intensities between the truncated fragments D0 and D1, D2 and D3, D3 and D4, D6 and D7, and D7 and D8 showed an upward trend, while the differences

between D4 and D5 and D8 and D9 fragments exhibited a downward trend (Fig. 3). The trends were similar in both cell lines, except for the comparison of D3 and D4, where a significant difference was only observed in SK-N-SH cells. Overall, our results suggested that the sequences between - 1587 and - 1371 bp (D0-D1), -1149 and - 894 bp (D2-D3), -39 and + 130 bp (D6-D7), and + 130 and + 341 bp (D7-D8) had negative effects on *HTR1B* gene expression while the sequences located between - 603 and - 316 bp (D4-D5) and + 341 and 505 bp (D8-D9) promoted *HTR1B* gene expression.

4 Discussion

Binding of transcription factors to specific sequences is important for post-transcriptional regulation. Various studies have shown that 5' regulatory region polymorphisms are closely associated with changes in gene expression. For example, rs2239630G/A is present in the promoter of the CCAT enhancer-binding protein epsilon (CEBPE) gene. The rs2239630A allele can enhance promoter activity and CEBPE expression and is associated with the risk of acute lymphoblastic leukemia[21]. The Matrix metalloproteinase (MMP-9) gene promoter polymorphism - 1562C/T regulates its transcriptional activity and affects the course of thyroid carcinoma[22].

In this study, six haplotype recombinants were transfected into SK-N-SH and HEK-293. The H2 and H4 haplotypes were associated with decreased transcriptional activity, whereas haplotype H6 increased transcriptional activity in both cell lines. In our previous study, we observed that the distribution of haplotype H6 was different between schizophrenic patients and healthy individuals in the northern Han Chinese population; however, the statistical difference was lost after performing the Bonferroni correction[20]. Schizophrenia is a complex polygenetic disease[23]. Haplotype C-G-C-A-G-A (H6), which is found in rs4140535, rs1778258, rs17273700, rs1228814, rs11568817, and rs130058, significantly increased *HTR1B* gene expression levels, which could have potential effects in schizophrenia. It should be noted that haplotype H2 caused the lowest *HTR1B* expression in both SK-N-SH and HEK-293. Although there was no evidence that this haplotype is associated with schizophrenia based on our previous studies, we cannot rule out that it may be related to other mental disorders caused by 5-HT1B dysfunction.

We also focused on the effects of *HTR1B* SNPs on gene expression. We found that the rs4140535T allele could enhance transcriptional activity. Previous studies found that 5-HT1B plays an important role in regulating feeding behavior. *HTR1B* polymorphism G861C was associated with minimum lifetime body mass indices (BMIs) in female patients with bulimia nervosa[24]. Another study showed that rs4140535 could be a genetic factor for obesity in African Americans, and the rs4140535T allele was a protective allele against excessive BMI[25]. Blocking 5-HT1B eliminates the anorexia and weight loss caused by leptin and AM 251[26], and serotonin-induced decreases in appetite require activation of 5-HT1B [27]. Our data suggest that the *HTR1B* polymorphism rs4140535T/C is likely related to weight gain. Our results also show that the rs1778258A allele has a negative regulatory effect on *HTR1B* gene expression. In our previous study, we found that the rs1778258A allele was potentially associated with schizophrenia in the northern Han Chinese population and speculated that this result might be related to the effect of the

rs1778258A/G polymorphism on gene expression[20]. The function of rs1778258A/G was confirmed in the current study.

We found that the C-G combination in rs17273700 and rs11568817 upregulated *HTR1B* expression, and rs17273700 and rs11568817 had a strong linkage relationship[20]. Duan et al. confirmed that the rs11568817G allele could generate a new TFAP2 binding site, resulting in improved transcriptional activity. The TFAP2 family, which includes TFAP2A, TFAP2B, and TFAP2C, represents a group of transcriptional activators that regulate the expression of specific genes[11]. rs11568817 is also believed to be related to other psychiatric disorders, such as drug abuse, alcohol and nicotine dependence, and anxiety[28]. It is likely related to the sexual dimorphism of the serotonergic system[29]. However, the function of rs17273700 needs further exploration. The rs1228814A allele could enhance transcriptional activity based on the results. Although there have been studies on the relationship between rs1228814 and depression and methamphetamine addiction[30, 31], there is currently no evidence associating rs1228814 with mental illness. Our study also found that the rs130058T allele could significantly downregulate gene expression. While rs130058 has been confirmed to bind to TFAP1, the rs130058A and rs130058T alleles exhibit different characteristics when binding to TFAP1, which affects the transcriptional activity of *HTR1B*[11].

Not all of the specific binding sequences for transcription factors included polymorphisms. Ten different truncated fragments were transfected into SK-N-SH and HEK-293 cells. The trends of the changes in relative fluorescence intensity between D0 and D1, D2 and D3, D6 and D7, and D7 and D8 were upward, while the trends for changes between D4 and D5 and D8 and D9 were downward in both cell lines. Therefore, the deleted fragments ranging from - 1587 to -1371 bp (D0-D1), -1149 to -894 bp (D2-D3), -39 ~ + 130 bp (D6 ~ D7), and + 130 ~ + 341 bp (D7 ~ D8) contain transcriptional suppression regions, and - 603~-316 bp (D4-D5), + 341 ~ + 505 bp (D8-D9) contain enhancing regulatory regions for gene expression. The functional regions were analyzed by JASPAR (<http://jaspar.genereg.net>)[19], and we found many transcription factors that probably bind to *HTR1B*, such as TBX2, E2F6, TFAP2, and KLF3 (Fig. 4).

The bioinformatics platform predicted that the deleted fragment from - 1587 to -1371 bp (D0-D1) contains the binding site for T-box transcription factor 2 (TBX2). This transcription factor is a transcriptional repressor of *ADAM10*. Its effects are mediated by binding to two TBX2 binding sites within the core promoter region of *ADAM10*, and substrate cleavage by ADAM10 has been implicated in pathological situations such as Alzheimer's disease.[32]. We also found that E2F6 binding sites were present in the deleted fragments from - 1149 to -894 bp (D2-D3) and - 39 to + 130 bp (D6-D7). E2F6 plays a vital role in controlling the cell cycle and contains a modular suppression domain that has inhibitory effects on transcription[33, 34]. Our analysis also predicted that the deleted fragment from + 130 to + 341 bp (D7-D8) contains a binding site for Kruppel-like factor 3 (KLF3). KLF3 belongs to the family of Sp1/Kruppel-like zinc finger transcription factors. These transcription regulators modulate the expression of a large number of genes that have GC-rich promoters and may take part in all facets of

cellular function[35]. KLF3 is widely expressed as a transcriptional repressor that functions by binding with the co-repressor protein C-terminal binding protein (CtBP)[36].

Deletion of the fragment from - 603 to -316 bp (D4-D5) caused a decrease in transcription activity. This region contained a binding site for TFAP2C. Studies have shown that TFAP2C plays a role in cerebral cortex development, it directly regulates the basal progenitor fate determinants Tbr2 and NeuroD.[37]. TFAP2C can also control hippocampal neurogenesis in adults and regulate cognitive behavior[38]. It was impressive that the luciferase activity of D8 was 7 to 8-fold higher than that of D9 in SK-N-SH cells. Therefore, the truncated fragment + 341 ~ + 505 bp (D8-D9) is likely to be the core promoter region of *HTR1B* and plays a decisive role in its transcription.

This study investigated the function of the 5' regulatory region of *HTR1B*. However, the mechanisms of the functional polymorphisms still require further exploration, and the potential functional regions must be verified with additional experiments. We attempt to perform animal experiments in the following studies to investigate the regulatory mechanism of the downstream pathways and on the correlation between the function of *HTR1B* and psychiatric disorders.

5 Conclusions

HTR1B 5' regulatory region polymorphisms have regulatory effects on gene expression and may correlate with several pathological and physiological conditions, such as weight gain and schizophrenia. The results of this study suggest that the core promoter sequence of *HTR1B* is located in the region from + 341 to + 505 bp (TSS + 1). Regions - 1587 to -1371 bp, -1149 to -894 bp, -603 to -316 bp, -39 to + 130 bp, and + 130 to + 341 bp contain functional sequences that can promote or suppress *HTR1B* expression.

Declarations

Ethics approval and consent to participate

The study was approved by the China Medical University Review Committee and performed according to the national guidelines.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

BW conceived and designed this study. XX conducted the experiment, statistical analyses, and finished the manuscript. MD, JX1, JX2, JY, and XW played important roles in interpreting the results and providing relevant assistance in conducting study.

Consent for publication

Not applicable.

Funding

This work was supported by China Medical University.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We thank all the members of the Forensic Genetics and Biology Department of China Medical University for assistance with the experimental design, technical guidance, and financial support.

References

1. Liy-Salmeron G, Meneses A. Role of 5-HT₁₋₇ receptors in short- and long-term memory for an autoshaping task: intrahippocampal manipulations. *BRAIN RES.* 2007;1147:140–7.
2. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *NEUROPHARMACOLOGY.* 1999;38(8):1083–152.
3. Moul C, Dobson-Stone C, Brennan J, Hawes DJ, Dadds MR. Serotonin 1B Receptor Gene (HTR1B) Methylation as a Risk Factor for Callous-Unemotional Traits in Antisocial Boys. *PLOS ONE.* 2015;10(5):e126903.
4. Lopez-Figueroa AL, Norton CS, Lopez-Figueroa MO, Armellini-Dodel D, Burke S, Akil H, Lopez JF, Watson SJ. Serotonin 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. *Biol Psychiatry.* 2004;55(3):225–33.
5. Hakulinen C, Jokela M, Hintsanen M, Merjonen P, Pulkki-Raback L, Seppala I, Lyytikainen LP, Lehtimaki T, Kahonen M, Viikari J, et al. Serotonin receptor 1B genotype and hostility, anger and

- aggressive behavior through the lifespan: the Young Finns study. *J BEHAV MED*. 2013;36(6):583–90.
6. Svob SD, Nedic EG, Nikolac PM, Nenadic-Sviglin K, Konjevod M, Grubor M, Pivac N. The association between HTR1B gene rs13212041 polymorphism and onset of alcohol abuse. *Neuropsychiatr Dis Treat*. 2019;15:339–47.
 7. Nautiyal KM, Tanaka KF, Barr MM, Tritschler L, Le Dantec Y, David DJ, Gardier AM, Blanco C, Hen R, Ahmari SE: **Distinct Circuits Underlie the Effects of 5-HT1B Receptors on Aggression and Impulsivity**. *NEURON* 2015, **86**(3):813–826.
 8. Duan J, Sanders AR, Molen JE, Martinolich L, Mowry BJ, Levinson DF, Crowe RR, Silverman JM, Gejman PV. Polymorphisms in the 5'-untranslated region of the human serotonin receptor 1B (HTR1B) gene affect gene expression. *Mol Psychiatry*. 2003;8(11):901–10.
 9. Nautiyal KM, Tritschler L, Ahmari SE, David DJ, Gardier AM, Hen R. A Lack of Serotonin 1B Autoreceptors Results in Decreased Anxiety and Depression-Related Behaviors. *NEUROPSYCHOPHARMACOL*. 2016;41(12):2941–50.
 10. Sanders AR, Cao Q, Taylor J, Levin TE, Badner JA, Cravchik A, Comeron JM, Naruya S, Del RA, Salvi DA, et al. Genetic diversity of the human serotonin receptor 1B (HTR1B) gene. *GENOMICS*. 2001;72(1):1–14.
 11. Duan J, Sanders AR, Molen JE, Martinolich L, Mowry BJ, Levinson DF, Crowe RR, Silverman JM, Gejman PV. Polymorphisms in the 5'-untranslated region of the human serotonin receptor 1B (HTR1B) gene affect gene expression. *Mol Psychiatry*. 2003;8(11):901–10.
 12. Gasso P, Rodriguez N, Blazquez A, Monteagudo A, Boloc D, Plana MT, Lafuente A, Lazaro L, Arnaiz JA, Mas S. Epigenetic and genetic variants in the HTR1B gene and clinical improvement in children and adolescents treated with fluoxetine. *Prog Neuropsychopharmacol Biol Psychiatry*. 2017;75:28–34.
 13. Gilabert-Juan J, Ivorra JL, Tolosa A, Gratacos M, Costas J, Sanjuan J, Molto MD. Potential involvement of serotonin receptor genes with age of onset and gender in schizophrenia: a preliminary study in a Spanish sample. *Psychiatry Res*. 2011;186(1):153–4.
 14. Jensen KP, Covault J, Conner TS, Tennen H, Kranzler HR, Furneaux HM. A common polymorphism in serotonin receptor 1B mRNA moderates regulation by miR-96 and associates with aggressive human behaviors. *Mol Psychiatry*. 2009;14(4):381–9.
 15. Conner TS, Jensen KP, Tennen H, Furneaux HM, Kranzler HR, Covault J. Functional polymorphisms in the serotonin 1B receptor gene (HTR1B) predict self-reported anger and hostility among young men. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B(1):67–78.
 16. Kaushik G, Xia Y, Pfau JC, Thomas MA. Dysregulation of autism-associated synaptic proteins by psychoactive pharmaceuticals at environmental concentrations. *NEUROSCI LETT*. 2017;661:143–8.
 17. Salim K, Fenton T, Bacha J, Urien-Rodriguez H, Bonnert T, Skynner HA, Watts E, Kerby J, Heald A, Beer M, et al. Oligomerization of G-protein-coupled receptors shown by selective co-immunoprecipitation. *J BIOL CHEM*. 2002;277(18):15482–5.

18. Wu X, Yao J, Ding M, Shi ZS, Xu FL, Zhang JJ, Wang BJ. 5-HT_{1A} receptor (HTR1A) 5' region haplotypes significantly affect protein expression in vitro. *NEUROSCI LETT*. 2017;638:51–4.
19. Fornes O, Castro-Mondragon JA, Khan A, van der Lee R, Zhang X, Richmond PA, Modi BP, Correard S, Gheorghe M, Baranasic D, et al. JASPAR 2020: update of the open-access database of transcription factor binding profiles. *NUCLEIC ACIDS RES*. 2020;48(D1):D87–92.
20. Xia X, Ding M, Xuan JF, Xing JX, Pang H, Wang BJ, Yao J. Polymorphisms in the human serotonin receptor 1B (HTR1B) gene are associated with schizophrenia: a case control study. *BMC PSYCHIATRY*. 2018;18(1):303.
21. Studd JB, Yang M, Li Z, Vijayakrishnan J, Lu Y, Yeoh AE, Paulsson K, Houlston RS. Genetic predisposition to B-cell acute lymphoblastic leukemia at 14q11.2 is mediated by a CEBPE promoter polymorphism. *LEUKEMIA*. 2019;33(1):1–14.
22. Roncevic J, Djoric I, Selemetjev S, Jankovic J, Dencic TI, Bozic V, Cvejic D. MMP-9-1562 C/T single nucleotide polymorphism associates with increased MMP-9 level and activity during papillary thyroid carcinoma progression. *PATHOLOGY*. 2019;51(1):55–61.
23. Harrison PJ. Recent genetic findings in schizophrenia and their therapeutic relevance. *J PSYCHOPHARMACOL*. 2015;29(2):85–96.
24. Levitan RD, Kaplan AS, Masellis M, Basile VS, Walker ML, Lipson N, Siegel GI, Woodside DB, Macciardi FM, Kennedy SH, et al. Polymorphism of the serotonin 5-HT_{1B} receptor gene (HTR1B) associated with minimum lifetime body mass index in women with bulimia nervosa. *Biol Psychiatry*. 2001;50(8):640–3.
25. Edwards TL, Velez ED, Villegas R, Cohen SS, Buchowski MS, Fowke JH, Schlundt D, Long J, Cai Q, Zheng W, et al. ADIPOR1, PPARGC1A, and CYP19A1 and obesity in a cohort of Caucasians and African Americans: an evaluation of gene-environment interactions and candidate genes. *AM J EPIDEMIOL*. 2012;HTR1B(1):11–21., , **175**.
26. Wierucka-Rybak M, Wolak M, Juszczak M, Drobnik J, Bojanowska E. The inhibitory effect of combination treatment with leptin and cannabinoid CB₁ receptor agonist on food intake and body weight gain is mediated by serotonin 1B and 2C receptors. *J PHYSIOL PHARMACOL*. 2016;67(3):457–63.
27. Mancilla-Diaz JM, Escartin-Perez RE, Lopez-Alonso VE, Floran-Garduno B, Romano-Camacho JB. Role of 5-HT_{1A} and 5-HT_{1B} receptors in the hypophagic effect of 5-HT on the structure of feeding behavior. *Med Sci Monit*. 2005;11(3):R74–9.
28. Cao J, LaRocque E, Li D. Associations of the 5-hydroxytryptamine (serotonin) receptor 1B gene (HTR1B) with alcohol, cocaine, and heroin abuse. *Am J Med Genet B Neuropsychiatr Genet*. 2013;162B(2):169–76.
29. Muller D, Grevet EH, Panzenhagen AC, Cupertino RB, Da SB, Kappel DB, Mota NR, Blaya-Rocha P, Teche SP, Vitola ES, et al. Evidence of sexual dimorphism of HTR1B gene on major adult ADHD comorbidities. *J PSYCHIATR RES*. 2017;95:269–75.

30. Ujike H, Kishimoto M, Okahisa Y, Kodama M, Takaki M, Inada T, Uchimura N, Yamada M, Iwata N, Iyo M, et al. Association Between 5HT1b Receptor Gene and Methamphetamine Dependence. *CURR NEUROPHARMACOL*. 2011;9(1):163–8.
31. Wang P, Lv Q, Mao Y, Zhang C, Bao C, Sun H, Chen H, Yi Z, Cai W, Fang Y. HTR1A/1B DNA methylation may predict escitalopram treatment response in depressed Chinese Han patients. *J Affect Disord*. 2018;228:222–8.
32. Reinhardt S, Schuck F, Stoye N, Hartmann T, Grimm M, Pflugfelder G, Endres K. Transcriptional repression of the ectodomain sheddase ADAM10 by TBX2 and potential implication for Alzheimer's disease. *CELL MOL LIFE SCI*. 2019;76(5):1005–25.
33. Cheng F, Lin HY, Hwang TW, Chen YC, Huang RL, Chang CB, Yang W, Lin RI, Lin CW, Chen G, et al. E2F6 functions as a competing endogenous RNA, and transcriptional repressor, to promote ovarian cancer stemness. *CANCER SCI*. 2019;110(3):1085–95.
34. Wan Z, Zhi N, Wong S, Keyvanfar K, Liu D, Raghavachari N, Munson PJ, Su S, Malide D, Kajigaya S, et al. Human parvovirus B19 causes cell cycle arrest of human erythroid progenitors via deregulation of the E2F family of transcription factors. *J CLIN INVEST*. 2010;120(10):3530–44.
35. Kaczynski J, Cook T, Urrutia R. Sp1- and Kruppel-like transcription factors. *GENOME BIOL*. 2003;4(2):206.
36. van Vliet J, Turner J, Crossley M. Human Kruppel-like factor 8: a CACCC-box binding protein that associates with CtBP and represses transcription. *NUCLEIC ACIDS RES*. 2000;28(9):1955–62.
37. Pinto L, Drechsel D, Schmid MT, Ninkovic J, Irmeler M, Brill MS, Restani L, Gianfranceschi L, Cerri C, Weber SN, et al. AP2gamma regulates basal progenitor fate in a region- and layer-specific manner in the developing cortex. *NAT NEUROSCI*. 2009;12(10):1229–37.
38. Mateus-Pinheiro A, Alves ND, Patricio P, Machado-Santos AR, Loureiro-Campos E, Silva JM, Sardinha VM, Reis J, Schorle H, Oliveira JF, et al. AP2gamma controls adult hippocampal neurogenesis and modulates cognitive, but not anxiety or depressive-like behavior. *Mol Psychiatry*. 2017;22(12):1725–34.

Figures

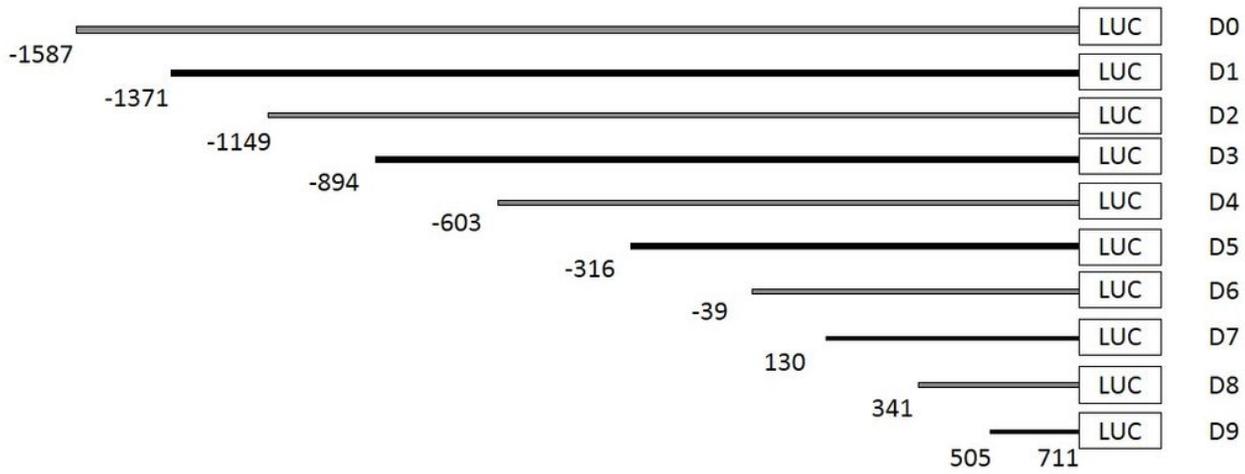


Figure 1

Ten truncated sequence recombinants in the 5' regulatory region of HTR1B The target gene was obtained using the GenBank reference sequence NC_000006.12 (Homo sapiens chromosome 6, GRCh38.p7 Primary Assembly). The ten DNA fragments shared a common 3' end at +711 bp (TSS, +1). The longest fragment was from -1587 to +711 bp, and the shortest fragment was from +505 to +711 bp.

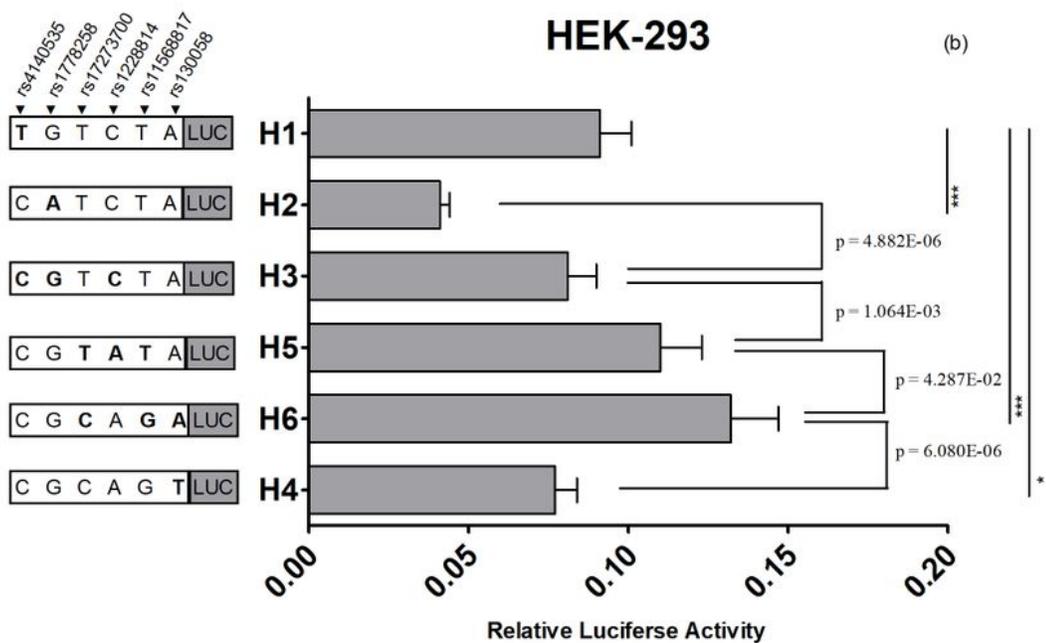
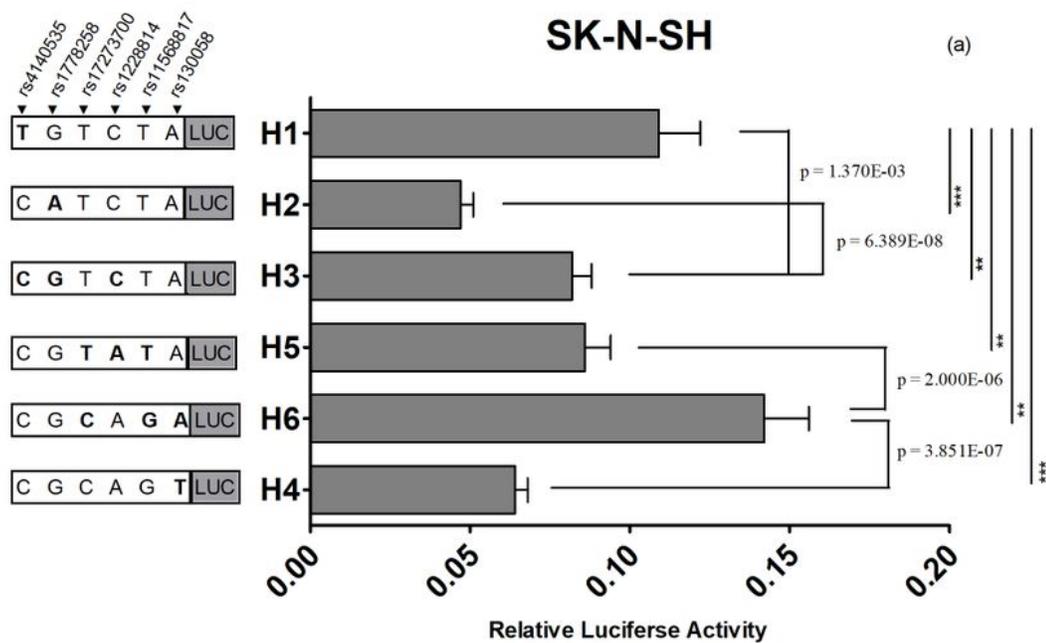


Figure 2

Relative fluorescence intensities of the haplotype recombinants in SK-N-SH and HEK-293 cells. The haplotypes H2, H3, H4, H5, and H6 showed significantly different luciferase activity compared to the major haplotype H1 in SK-N-SH cell (Figure 2a), the haplotype H2, H4 and H6 also showed significantly different luciferase activity compared to the major haplotype H1 in HEK-293 cell (Figure 2b). *** $p < 0.001$; ** $p < 0.01$, * $p < 0.05$. The relative fluorescence intensity of haplotype H1 (with rs4140535T allele) was

significantly higher than that of haplotype H3 (with rs4140535C allele) in the SK-N-SH cells, The relative fluorescence intensity of haplotype H2 (with rs1778258A allele) was much lower than that of haplotype H3 (with rs1778258G allele) in both cell lines, The haplotype H5 (with rs1228814A allele) appeared to upregulate the luciferase activity relative to haplotype H3 (with rs1228814C) in HEK-293 cells, the haplotype H6 (with rs17273700C-rs11568817G) had higher relative luciferase intensity than haplotype H5 (with rs17273700T-rs11568817T), and haplotype H4 (with rs130058T allele) had lower relative luciferase intensity than haplotype H6 (with rs130058A allele) in both SK-N-SH and HEK-293 cell lines (Figure 2a,2b).

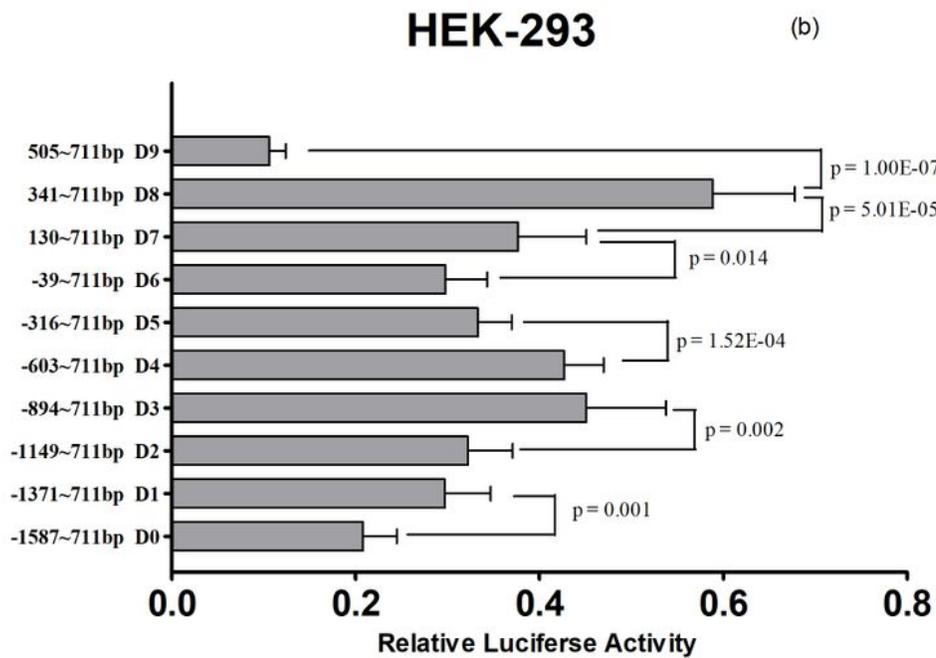
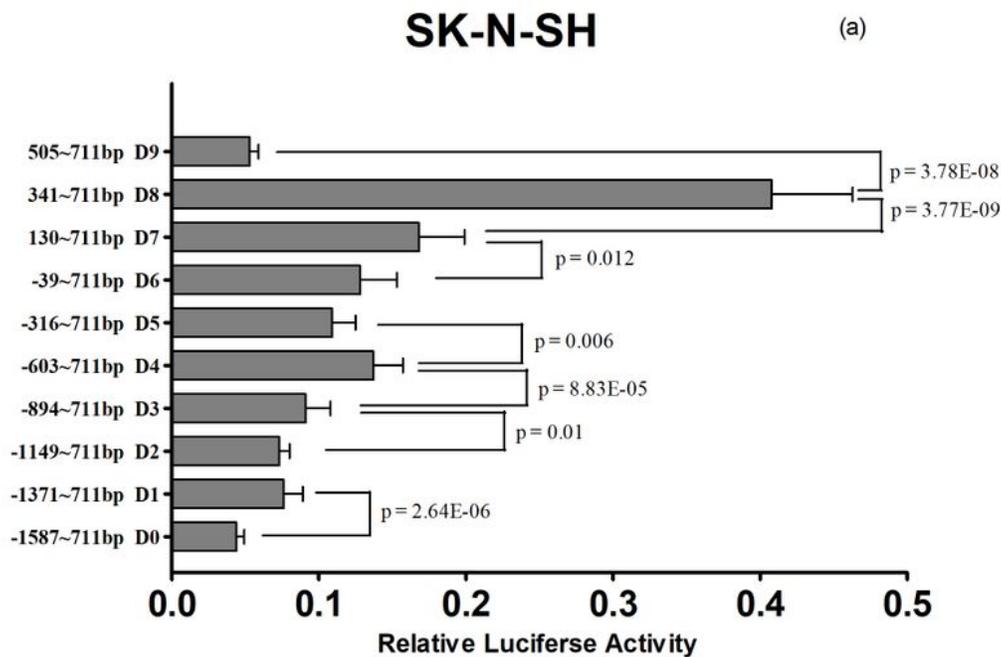


Figure 3

Relative fluorescence intensities for ten truncated sequence recombinants in SK-N-SH and HEK-293 cells. There was a significant difference in the relative fluorescence intensities between D0 versus D1, D2 versus D3, D2 versus D3, D3 versus D4 (SK-N-SH only), D4 versus D5, D6, and D7, D7 versus D8, and D8 versus D9. The data are presented as the mean \pm SD.

D0-D1	-1406	TTTTATTAGATACACTCT <u>AGGTGCCAAACATGGATCTAGG</u>	-1367
		TBX2	
D2-D3	-1117	CTGATGATGGTTCCAGTCCTCCCCGCCCCACCATCCCCCT	-1078
		E2F6	
D4-D5	-487	GGCTGGGAGGAT <u>GGCCCAGGGTGGGAAGCGGGGAGATAGG</u>	-448
		TFAP2C	
D6-D7	+83	TGCCCCAGCTTGGGGCG <u>AGGGGTGGGAATGCAAGATCTCG</u>	+122
		E2F6	
D7-D8	+304	TCCAGGGAGCTGGAGCGGAGC <u>AGGGCTCGGTGGGCCAGCT</u>	+343
		KLF3	

Figure 4

The prediction of transcription factor The predictive results for transcription factor binding in the functional fragments are shown. The numbers in the figure annotate the position of the fragment (TSS, +1).