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Original Article

Functional tag SNPs inside the DRD2 gene as a genetic risk factor for major depressive disorder in the Chinese Han population

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Abstract: Background: The D2 dopamine receptor (DRD2) has been extensively investigated and has been associated with the occurrence of neuropsychiatric disorders. Polymorphisms in the DRD2 gene have also been determined as a possible predisposing component for major depressive disorders (MDD). The present study focused on evaluating the connection of polymorphisms inside the whole DRD2 gene in MDD patients as well as in non-MDD participants in a group selected from the Chinese Han population. Materials and methods: In total, 831 unrelated Chinese adults from the Han population were sampled, including 497 non-MDD participants and 334 MDD patients for this evaluation. After the haplotype bins were built, 14 tag single-nucleotide polymorphisms (tSNPs) and the two most investigated SNP were chosen for the whole DRD2 gene. An improved multiplex ligation detection reaction (iMLDR) technique was used to choose the genotypes. Following this, the allelic frequencies and clinical features were contrasted between the two independent Chinese Han populations. Transcriptional enhancer activities were measured to assess the functionality of the rs7131056 polymorphism. Results: Sixteen SNPs were identified, including the two most examined in the Chinese Han population, and all were recurrent SNPs. Of the 16 SNPs, two (rs4648317 and rs7131056) were significantly connected to MDD. Patients with MDD were more apt to carry the rs4648317G and rs7131056A allele in contrast to the non-MDD controls ($P < 0.05$). The genetic risk effect on MDD occurrence was associated with the haplotype GTGATCGCGCAGGC of fourteen tag SNPs (OR = 1.52, 95% CI: 1.06 to 2.18, P = 0.02). Moreover, the rs7131056 polymorphism contained intronic silencer activities. Conclusions: This case-control evaluation involving the Chinese Han population suggests that the rs4648317 and rs7131056 polymorphisms and the haplotype GTGATCGCGCAGGC inside the DRD2 gene could be possible markers to forecast vulnerability to MDD.

Keywords: Dopamine D2 receptor, tag single nucleotide polymorphisms, intronic silencer, major depressive disorder

Introduction

Mood disorders are a primary cause of disability and the second-leading cause of disease burden. Major depressive disorder (MDD) is a psychiatric disorder that occurs across the globe and is distinguished by unhappiness, loss of interest and delight, or other mood disorders, cognitive dysfunction, and even psychotic symptoms. The lifetime prevalence of MDD is 5-10% based on epidemiological and familial studies. The World Health Organization (WHO) shows that the annual prevalence of MDD in the world is about 11%. Worldwide 322 million people have depression. About 50% of these people reside in Southeast Asia or the Western Pacific area, demonstrating the large populations in the area, which includes India and China, who are affected by the condition. As for disease burden, depression has become the world's fourth largest disease, and is predicted to be the second leading cause of disability by 2020 [1]. Hence, MDD is a very serious public health problem. With advancements in our knowledge of molecular genetics within the last couple of decades, our understanding

of inherited disorders has increased. Despite these advancements, the pathogenesis of MDD remains unclear. Several potential factors may be putative risks for MDD, such as genetics, personality, psychopathology and psychosocial functioning, which lead the prevalence of MDD to vary by country and area, from 2.6% of men in the Western Pacific area to 5.9% of women in the African area [2]. The underlying reason for these variations in occurrence is not fully clear, but it likely pertains to the heterogeneity in the populations examined that establish their vulnerability.

Unlike many monogenic diseases, psychiatric disorders do not manifest with obvious Mendelian traits, and the mode of transmission has not been clearly established. Family and twin studies suggest that psychiatric disorders such as MDD aggregate in families. The heritability of MDD is estimated at approximately 37% [3]. Many genes have been identified as being connected to an elevated risk for MDD by candidate gene association studies. Over 393 polymorphisms in 102 genes were examined for a potential relationship with depression, e.g., the 9/10 variable number tandem repeat (VNTR) in the 3'-untranslated region (UTR) of the dopamine transporter (DAT) gene (DAT1/SLC6A3) was determined to be connected to MDD [4]. The A1 allele of the DRD2/ANKK1 Taq1A polymorphism (rs1800497) predisposes individuals to depression and addiction through its impact on the post-synaptic dopamine D2 receptor [5, 6]. A positive association with the MTHFR C766T polymorphism and the risk of depression has been reported by a meta-analysis [7].

Dopamine (DA) is a plentiful catecholaminergic neurotransmitter in the brain, and it has a pivotal part in the control of feelings, inspiration, reward, and reinforcement behavior through the mesocorticolimbic pathway. The interruption or impairment of the DA pathway has been connected to numerous pathological conditions, such as schizophrenia, Parkinsonism, attention deficit hyperactivity disorder, posttraumatic stress disorder (PTSD), MDD, and drug addiction [8, 9]. In humans, the DRD2 gene is found on chromosome 11q22-23 and covers a minimum of 270 kilobases with 8 exons [10]. Some evaluations have suggested that DRD2 gene variants could modulate neuropsychiatric symptom severity and the reactivity of pharmacotherapy by affecting mRNA stability and synthesis, impacting the density and affinity of the D2 receptor, and lowering D2 receptor binding. According to these discoveries, the DRD2 gene is an appropriate candidate for a substantial genetic risk factor contributing to MDD.

Numerous single-nucleotide polymorphisms (SNPs) have been determined inside the whole DRD2 gene over the last several decades (www.hapmap.org). Some of the SNPs, including rs1800497, rs6277, rs1076560, and rs1799732, are most often the ones that are examined and detailed. One of the earliest polymorphisms to be investigated and described in the DRD2 gene is the rs1800497, also known as ANKK1/DRD2 Taq1A SNP. Several studies have examined the connection between this polymorphism and depression; however, the findings did not entirely agree [5, 11]. Another well-examined polymorphism in the DRD2 gene is a synonymous polymorphism called C957T (rs6277), which impacts the functions of D2 dopamine receptors (DRD2) that are primarily expressed in the indirect pathway of the basal ganglia, and they have been discovered to impact suppression and punishment learning [12]. Notwithstanding these evaluations, just a few of the DRD2 gene polymorphisms have been fully examined. In addition, there is an obvious lack of knowledge of the worldwide biosignificance of the genetic variants inside the entire DRD2 gene.

In this evaluation, using a SNP haplotype tagging technique, a vast endeavor has been made to determine the possible biologic significance of every one of the established genetic variants inside the whole DRD2 gene (www.hapmap.org). In addition, a set of tSNPs, which possibly typify the biologic significance of every one of the established genetic variations inside the whole DRD2 gene, was chosen by constructing haplotype blocks. Finally, tSNPs associated with MDD susceptibility within a large sample of the Chinese Han population with MDD were investigated.

Experimental procedures

Study design and data collection

Unrelated Chinese adults (831 in total) from the Han population, including 497 non-MDD

participants and 334 patients who had been diagnosed with MDD, were enlisted from the Chongqing District for this evaluation. The Third Military Medical University Ethical and Protocol Review Committee and the local committee approved the protocol for data collection and sampling of individuals. Each individual subject was made aware of the possible advantages and risks of inclusion in this evaluation and written informed consent was acquired from the participants or their nearest relative.

Non-MDD group

Non-MDD participants (the control group) made up the identical group as noted by Duan et al. [8]. The group was made up of 298 men and 199 women (median age of 34.5 ± 5.3 years, age range 18-58). The volunteers were members of the nursing staff, medical staff, university staff, and students at the Third Military Medical University. Each of the volunteers completed a formal screening for psychological disorders, and they were enlisted in the Chongqing area and they were part of the Han population.

MDD participants

There were 334 unrelated Chongqing Han patients (241 women and 93 men) who met the Structured Clinical Interview for DSM-IV criteria for MDD and had a 17-item Hamilton Depression Rating Scale (HAMD) score of at least 18 [13] who were enlisted. The diagnosis of every patient was verified during a psychiatric evaluation conducted by an experienced and board-certified psychiatrist. Volunteers were eliminated for alcohol/substance abuse or dependence during the last six months, social phobia, PTSD, bipolar I or bipolar II, obsessive-compulsive disorder, a history of psychosis, and any learning disabilities that might prevent them from carrying out a cognitive task. The group of patients had a mean age of 40.5 years ± 6.2. Each of the patients had a substantial understanding of Chinese and they were able to comprehend the administered questionnaires. The patients were admitted to the Clinical Psychology Department of Southwest Hospital of Chongqing, Department of Psychiatry of the Mental Health Center of Chongqing, and outpatients in the Department of Psychiatry of the Third Military Medical University between June 1, 2012 and December 31, 2016.

Chosen SNPs

The full sequence of the human *DRD2* gene (Accession Number: NC-000011.10 Reference GRCh37.p5 Primary Assembly) examined in this evaluation encompassed 10 kb upstream of the transcription start site, each of the exons and introns, and 10 kb downstream of the stop codon (87.65 kb in total), which was narrowed down to chromosome 11, position 113270317-113356001 (data were obtained from Genbank on the NCBI web site). Data on genetic variations for the full *DRD2* gene were acquired from the HapMap Project for 45 healthy Chinese Han Beijing (CHB) adults (www.hapmap.org). Overall, 149 SNPs were determined from this database; of these, 79 SNPs with the minor allele frequency (MAF) of at least 0.10 were chosen for the evaluation of haplotype-tagging SNPs (htSNPs). Haploview (v. 4.0, Broad Institute of MIT and Harvard, Cambridge, MA) was utilized in the building of haplotype blocks in the whole DRD2 gene [8]. These haplotype blocks represent the areas that are inherited without large recombinations in the predecessors of the current population. The past recombination among a pair of SNPs can be approximated utilizing the normalized measure of allelic association D' (the value of D prime between the two loci). The criteria for choosing SNPs to build a haplotype block was that each of the SNPs in an area must be strong LD with D' > 0.98 for the upper 95% confidence bound and > 0.7 for the lower bound. The Tagger software program (http://www.broad.mit.edu/mpg/haploview) was then used to choose a maximally informative htSNP from every block as described previously [8].

Genomic DNA extraction

Peripheral blood mononuclear cells (PBMC) were used for the extraction of genomic DNA, followed by purification with a Wizard genomic DNA purification kit (Promega, Madison, Wisconsin, USA) based on the manufacturer's instructions. The DNA concentration in each of the samples was modified to a concentration of 50 μg/mL with sterile distilled water following initial concentration determination by UV spectrophotometry and stored at -80°C until further use.

SNP Genotyping

Genotyping of the 16 chosen SNPs in *DRD2* was performed utilizing polymerase chain reac-

Table 1. Demographic and clinical variables of the non-MDD controls and patients with MDD

Variable	Non-MDD participants	MDD	Total	P
Age (y)	34.50 ± 5.30	40.55 ± 6.23	37.33 ± 5.82	0.20
Gender (F/M)	298/199	93/241	391/440	0.00
Education (< High school)	92 (18.51)	53 (15.87)	145 (17.45)	0.35
Employed (yes/no)	426/71	274/60	700/131	0.17
Married (yes/no)	381/116	257/77	638/193	0.93

tion (PCR)-ligase detection reaction (LDR) on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), along with technical assistance from the Shanghai Genesky Biotechnology Company as described previously [8]. The alleles for every SNP were determined by various fluorescent labels of allele-specific oligonucleotide probe pairs (the primers and probes sequences will be provided upon request). GeneMapper Software v 4.0 (Applied Biosystems) was used to determine SNPs by extended lengths at the 3' end. A blinded protocol was utilized for genotyping, and about 10% of the samples were genotyped in duplicate to establish genotyping quality, producing a reproducibility of 100% as described previously [8].

Intronic reporter constructs

A reported gene assay system was used to investigate the possible effect of rs7131056 polymorphisms located in intron 2 of the *DRD2* gene on the transcription activity. *Sac*I and *Xho*I restriction sites were targeted with specific primer pairs (provided upon request), which were utilized to amplify fragments covering the intron 2 region of *DRD2* from +16434 bp to +16886 bp relative to the transcription start site from human genomic DNA. The PCR products, namely, the different alleles, were acquired. The PCR products had no sequence differences at any site, except for rs7131056 C/A. The MiniBEST Agarose Gel DNA Extraction Kit Ver.4.2 (TaKaRa, Dalian, China) was used to purify each allele from the PCR products in the gel, following which they were severed with *Sac*I and *Xho*I restriction enzymes. Two products were immediately added into the *Sac*I and *Xho*I sites of the pmirGlo du-luciferase reporter gene vector (Promega, Madison, WI, USA) utilizing T4 DNA ligase (TaKaRa, Dalian, China). The inserts with various alleles were verified by PCR-direct sequencing. Plasmids with varying alleles were transfected into MES23.5 cells using Lipofectamine 2000™ (Life Technologies, Carlsbad, CA, USA) and incubated for 48 h. Luciferase activity was established with the Dual-Glo Luciferase Assay system (Promega, Madison, WI, USA) and a Luminoskan Ascent luminometer (Thermo Labsystems, Helsinki, Finland) utilizing Renilla luciferase activity to quantify transcriptional activity with normalization by Firefly luciferase activity in every assay (i.e., in the identical well). The outcomes are shown as a fold increase in the relative luciferase activity of Firefly and Renilla (F/R). Three replications were conducted for the experiment with quintuplicate assays within each replicate.

Statistical analyses

Gene counting was employed to determine allele frequencies for each tSNP. Deviations from the Hardy-Weinberg equilibrium in the genotype distribution of every tSNP were analyzed using χ^2 analyses. Haploview software version 4.2 was utilized to establish the extent of pairwise LD (r^2-value) among polymorphisms. An independent-sample T-test was employed for comparing luciferase expression. A χ^2 test (Pearson's) was conducted to establish statistical connections among the allele and disease status. Multivariate logistic regression with ORs with 95% confidence intervals was used to approximate the relative risk of MDD in three genetic models (allele-dose, dominant, and recessive). Every one of the ORs was modified for age, gender, education, employment, marital status, and drinking and smoking status. JLIN was utilized to produce haplotypes and they were examined for linkage disequilibrium (LD) measures (D' and r^2) [14]. The P-values were two-sided, and $P < 0.05$ after Bonferroni's correction for multiple testing was established as being statistically significant. Statistical analyses were conducted with SPSS version 20.0 statistical software (SPSS, Inc., Chicago, IL, USA).

Table 2. Frequencies of DRD2 gene polymorphisms in non-MDD controls and patients with MDD

NCBI rs#	Location	Position	Variant	MAF (Hapmap-HCB)	Non-MDD controls					Patients with MDD				
					Genotype, N (497)			MAF	P for HWE	Genotype, N (334)			MAF	P for HWE
rs10891556	5'flanking	-6760	G/T	0.50	GG 169	GT 228	TT 100	0.43	0.15	GG 120	GT 161	TT 53	0.40	0.93
rs1799978	5'flanking	-350	T/A	0.18	TT 315	TC 156	CC 26	0.21	0.25	TT 201	TC122	CC 11	0.22	0.14
rs4648317	Intron2	14469	G/A	0.43	GG 177	GA 229	AA 91	0.41	0.26	GG 127	GA 168	AA 39	0.37	0.14
rs7131056	Intron2	16627	C/A	0.42	CC 185	CA 238	AA 74	0.39	0.86	CC 96	CA 189	AA 49	0.43	0.17
rs4460839	Intron2	24205	T/C	0.22	TT 381	TC 110	CC 6	0.12	0.54	TT 254	TC 75	CC 5	0.13	084
rs12574471	Intron2	29765	C/T	0.19	CC 343	CT 138	TT 16	0.17	0.64	CC 229	CT 98	TT 7	0.17	0.35
rs4648319	Intron2	31638	G/A	0.36	GG 212	GA 221	AA 64	0.35	0.59	GG 141	GA 155	AA 38	0.35	0.64
rs4648318	Intron2	32612	C/T	0.40	CC 180	CT 224	TT 93	0.41	0.11	CC 122	CT 161	TT 51	0.39	0.86
rs4586205	Intron2	38872	G/T	0.50	GG 166	GT 230	TT 101	0.43	0.19	GG 112	GT 170	TT 52	0.41	0.34
rs4436578	Intron2	39236	T/C	0.45	TT 178	TC 225	CC 94	0.42	0.13	TT 106	TC 165	CC 63	0.44	0.93
rs2075652	Intron3	51103	G/A	0.32	GG 206	GA 212	AA 43	0.37	0.06	GG 132	GA 153	AA 49	0.38	0.67
rs2734833	Intron3	53086	G/A	0.11	GG 446	GA 48	AA 2	0.05	0.56	GG 305	GA 28	AA 1	0.04	0.68
rs1076560	Intron6	62313	C/A	0.43	CC 79	CA 117	AA 46	0.43	0.82	CC 62	CA 106	AA 37	0.44	0.48
rs6279	exon8 (3'UTR)	64928	G/C	0.47	GG 146	GC 247	CC 104	0.46	0.98	GG 100	GC 171	CC 60	0.44	0.380
rs2242591	3'flanking	66080	C/T	0.46	CC 173	CT 246	TT 78	0.40	0.54	CC 119	CT 167	TT 48	0.39	0.39
rs1800479	3'flanking	75173	G/A	0.41	GG 174	GA 246	AA 77	0.40	0.52	GG 116	GA 169	AA 49	0.40	0.32

MAF indicates minor allele frequency; HWE indicates Hardy-Weinberg Equilibrium.

Results

Demographic and clinical measures

The demographics and clinical variables of non-MDD controls and MDD patients are shown in **Table 1**. There were twice as many female subjects with MDD compared to males (P = 0.00). It is fundamental to note that there were no significant differences in demographic variables with the exception of gender among the non-MDD controls and MDD patients.

Allele frequencies and genotype dispersal of the DRD2 gene polymorphisms in MDD participants

Sixteen SNPs with a minor allele frequency ≥ 10% were discovered in the Chinese Han population in Beijing (CHB), which led to the four haplotype bins being built. Utilizing the iMLDR technique improved the genotyping success rates of the 16 SNPs (99.6-100%). The minor allele frequencies (MAFs) between the 497 non-MDD controls and the 334 MDD patients are included in **Table 2**. These MAFs were very close to those noted in the 45 unrelated CHB cohorts in the HapMap database. An evaluation of the genotype distribution of each of the 16 SNPs demonstrated that they were consistent with the Hardy-Weinberg equilibrium (P > 0.05, **Table 2**).

Association of DRD2 gene polymorphisms with development of clinical symptoms of MDD

The MDD patient cohort met the Structured Clinical Interview for DSM-IV criteria for MDD and consisted of 334 consecutive Chinese Han patients, 93 male and 241 female, with a mean ± SD age of 40.5 ± 6.2 years. No significant differences in age or sex ratio among the patients were observed when stratified according to the different genotypes of each tSNP. Polymorphisms between patients with MDD and the non-MDD controls was compared. Among the 14 tSNPs of the *DRD2* gene, only rs4648317 and ra7131056 were significantly connected with the risk of MDD in each of the allele dose analysis models (P = 0.04 for rs4648317, and P = 0.01 for rs7131056, **Table 3**). Stratification analyses for the dominant or recessive effects and for the interactions among genotypes and MDD for every single SNP were then performed. For the recessive effect, when contrasted with rs4648317 AA genotypes, the blended genotypes rs4648317 GA/GG had a significantly reduced impact on MDD risk (OR = 0.59, 95% CI: 0.39 to 0.88, P = 0.01), while for the dominant effect, in contrast to rs7131056 CC genotypes, the blended genotypes rs7131056 AA/AC had a significantly elevated MDD risk (OR = 1.47, 95% CI: 1.09 to 1.98, P = 0.01 **Table 3**).

Combination effects of the 14 tSNPs

We then investigated whether the ultimate function of a conserved haplotype is the result of interactions among polymorphisms within the block. The LD coefficient between polymorphisms was calculated, which revealed that all genotyped polymorphisms were in strong LD. Fourteen tSNPs in the LD block were selected to reconstructed haplotypes, and haplotype frequencies were compared between the MDD patients and non-MDD controls. The haplotype analysis showed that the haplotype of fourteen tSNPs, ht2 (GTGATCGCGCAGGC), was significantly higher in patients than non-MDD controls, which suggested that ht2 had a genetic risk effect on MDD development (OR = 1.52, 95% CI: 1.06 to 2.18, P = 0.02, **Table 4**).

Intronic silencer activity of the DRD2 gene

Based on the location of the rs7131056 in intron 2 of the *DRD2* gene, we hypothesized that there may be potential silencer activity in these introns. The silencer activity of these regions was thus examined using luciferase reporter gene constructs containing different variants in these regions in MES23.5 cells. Relative luciferase activity (RLA) was significantly higher in cells transfected with the wild C allele of rs7131056 than it was in those transfected with the variant A allele (0.408 ± 0.005 vs. 0.394 ± 0.005, P = 0.028) (**Figure 1**). These results suggest the presence of a negative regulatory element between +16434 bp and +16866 bp from the transcriptional start site.

Discussion

Sixteen SNPs were selected in this study to conduct the genetic association analyses of MDD in 831 members of the Chinese Han population, which included 497 non-MDD participants and 334 MDD patients. Due to their strong LD with un-assayed variants, these

Table 3. Association between SNPs in DRD2 and MDD using the additive, dominant, and recessive models

SNP	Genotype	MDD (n = 337, freq)	Control (n = 497, freq)	χ^2	P	OR (95% CI)
rs10891556	GG	120 (35.90)	169 (34.00)	1.41	0.30	1.14 (0.93-1.39)
	GT	161 (48.20)	228 (45.88)			
	TT	53 (15.90)	100 (20.12)			
	TT+GT vs GG			0.33	0.60	0.92 (0.69-1.23)
	TT vs GT+GG			2.41	0.14	0.75 (0.52-1.08
rs12574471	CC	229 (68.60)	343 (69.01)	1.09	0.58	1.02 (0.79-1.33)
	CT	98 (29.30)	138 (27.77)			
	TT	7 (2.10)	16 (3.22)			
	TT+CT vs CC			0.02	0.94	1.02 (0.76-1.38)
	TT vs CT+TT			0.94	0.39	0.64 (0.26-1.58)
rs1799978	TT	201 (60.20)	315 (63.38)	3.59	0.17	1.04 (0.82-1.32)
	TC	122 (36.50)	156 (31.39)			
	CC	11 (3.30)	26 (5.23)			
	CC+CT vs TT			0.87	0.38	1.15 (0.86-1.52)
	CC vs CT+TT			1.76	0.23	0.62 (0.30-1.27)
rs1800479	GG	116 (34.70)	174 (35.01)	0.14	0.93	1.00 (0.81-1.21)
	GA	169 (50.60)	246 (49.50)			
	AA	49 (14.70)	77 (15.49)			
	AA+GA vs GG			0.01	0.94	1.01 (0.76-1.35)
	AA vs GA+GG			0.11	0.77	0.94 (0.64-1.38)
rs2075652	GG	132 (39.50)	206 (41.45)	0.83	0.66	1.02 (0.83-1.24)
	GA	153 (45.80)	212 (42.66)			
	AA	49 (14.70)	79 (15.91)			
	AA+GA vs GG			0.31	0.61	1.08 (0.82-1.44)
	AA vs GA+GG			0.23	0.70	0.91 (0.62-1.34)
rs2242591	CC	119 (35.60)	173 (34.81)	0.28	0.87	1.46 (0.86-1.28)
	CT	167 (50.00)	246 (49.50)			
	TT	48 (14.40)	78 (15.69)			
	TT+CT vs CC			0.06	0.82	0.97 (0.72-1.29)
	TT vs CT+TT			0.27	0.62	0.90 (0.61-1.33)
rs2734833	GG	305 (91.30)	446 (89.92)	0.47	0.79	0.85 (0.54-1.35)
	GA	28 (8.40)	48 (9.68)			
	AA	1 (0.30)	2 (0.40)			
	AA+GA vs GG			0.45	0.55	0.85 (0.53-1.37)
	AA vs GA+GG			0.06	1.00	0.74 (0.07-8.21)
rs4436578	TT	106 (31.70)	178 (35.82)	1.70	0.43	1.09 (0.89-1.32)
	TC	165 (49.90)	225 (45.27)			
	CC	63 (18.90)	94 (18.91)			
	CC+CT vs TT			1.48	0.23	1.20 (0.89-1.61)
	CC vs CT+TT			0.00	1.00	1.00 (0.70-1.42)
rs4460839	TT	254 (76.00)	381 (76.66)	0.15	0.93	1.04 (0.78-1.40)
	TC	75 (22.50)	110 (22.13)			
	CC	5 (1.50)	6 (1.21)			
	CC+CT vs TT			0.04	0.87	1.03 (0.75-1.43)
	CC vs CT+TT			0.13	0.76	1.24 (0.38-4.10)

rs4586205	GG	112 (35.50)	166 (33.40)	3.34	0.19	1.10 (0.91-1.35)
	GT	170 (59.90)	230 (46.28)			
	TT	52 (15.60)	101 (20.32)			
	TT+GT vs GG			0.00	1.00	1.00 (0.74-1.33)
	TT vs GT+GG			3.00	0.10	0.72 (0.50-1.04)
rs4648317	GG	127 (38.00)	177 (35.61)	**6.68**	**0.04**	**0.83 (0.68-1.01)**
	GA	168 (50.30)	229 (46.08)			
	AA	39 (11.70)	91 (18.31)			
	AA+GA vs GG			0.24	0.66	0.93 (0.70-1.24)
	AA vs GA+GG			**6.66**	**0.01**	**0.59 (0.39-0.88)**
rs4648318	CC	122 (36.50)	180 (36.22)	1.79	0.41	1.08 (0.89-1.32)
	CT	161 (48.20)	224 (45.07)			
	TT	51 (15.30)	93 (18.71)			
	TT+CT vs CC			0.008	0.94	0.99 (0.74-1.32)
	TT vs CT+TT			1.65	0.22	0.78 (0.54-1.14)
rs4648319	GG	141 (42.20)	212 (42.65)	0.54	0.76	0.98 (0.80-1.20)
	GA	155 (46.40)	221 (44.47)			
	AA	38 (11.41)	64 (12.88)			
	AA+GA vs GG			0.02	0.94	1.02 (0.77-1.35)
	AA vs GA+GG			0.42	0.59	0.87 (0.57-1.33)
rs6279	GG	110 (30.20)	146 (29.38)	0.98	0.61	0.93 (0.76-1.13)
	GC	168 (51.70)	247 (49.70)			
	CC	60(18.10)	104 (20.92)			
	CC+GC vs GG			0.07	0.82	0.96 (0.71-1.30)
	CC vs GC+GG			1.28	0.29	0.82 (0.57-1.16)
rs7131056	CC	96 (28.70)	185 (37.22)	**7.20**	**0.03**	**1.19 (0.97-1.45)**
	CA	189 (56.60)	238 (47.89)			
	AA	49 (14.70)	74 (14.89)			
	AA+CA vs CC			**6.42**	**0.01**	**1.47 (1.09-1.98)**
	AA vs CA+CC			0.008	1.00	0.98 (0.67-1.45)
rs1076560	CC	62 (30.24)	79 (32.64)	0.05	0.83	1.03 (0.79-1.34)
	CA	106 (51.71)	117 (48.35)			
	AA	37 (18.05)	46 (19.01)			
	AA+CA vs CC			0.50	0.54	1.15 (0.77-1.71)
	AA vs CA+CC			0.83	0.90	0.95 (0.59-1.53)

Note: recessive effect = variant homozygotes versus heterozygotes + wild-type homozygotes; dominant effect = variant homozygotes + heterozygotes versus wild-type homozygotes. Bold data: *P* value < 0.05.

SNPs theoretically suggest the biological significance of each of the genetic variations throughout the whole *DRD2* gene. Two tSNPs inside the *DRD2* gene, rs7131056, and rs4648317, were discovered to have a strong connection with an elevated risk to MDD vulnerability in two independent samples. MDD development was significantly connected to the A allele of the rs7131056 and rs4648317 polymorphisms. In addition, MDD patients more frequently carried the A allele in contrast to the non-MDD controls. This suggests that the rs7131056 and rs4648317 polymorphisms add to the genetic vulnerability to MDD. A haplotype examination also indicated that haplotype GTGATCGCGCAGGC of 14 tSNPs had a risk impact on MDD. There were no significant differences in demographic variables except gender among the non-MDD controls and MDD patients, which is in agreement with prior evaluations [15, 16], suggesting that MDD is more prevalent among women, particularly in the younger age group.

The dopaminergic system is involved in emotional conditioning and behavior, and thus is

Table 4. Analyses of the association of DRD2 gene 14 tag SNPs haplotypes with the risk of MDD

Loci	Genotype	Frequency		Chi^2	Pearson's p	Odds Ratio (95% CI)
		MDD (freq)	Control (freq)			
ht1	G T G A T C A T T T G G C T	85.81 (0.13)	119.28 (0.12)	0.29	0.59	1.09 (0.79-1.50)
ht2	G T G A T C G C G C A G G C	70.94 (0.11)	74.63 (0.08)	5.30	0.02	1.52 (1.06-2.18)
ht3	T T A C T C A T T T G G C T	59.71 (0.09)	108.53 (0.11)	1.96	0.16	0.80 (0.55-1.11)
ht4	T T A C T C G C G C A G G C	123.70 (0.19)	203.13 (0.21)	1.25	0.26	0.85 (0.64-1.13)

Note: Haplotypes were ignored if the haplotype frequency was less than 5%.

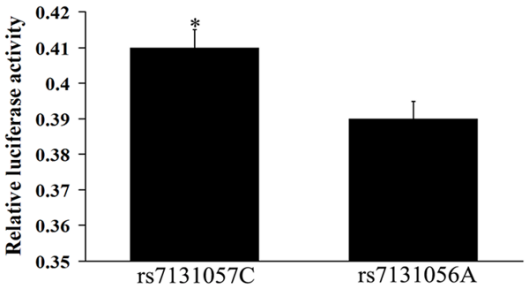

Figure 1. Impact of the rs7131056C/A polymorphism of the *DRD2* gene on transcriptional activity. Relative luciferase activity (RLA) was quantified in MES23.5 cells transfected with rs7131056C or rs7131056A plasmid constructs. The RLA was significantly greater in cells transfected with the wild C allele compared to that transfected with the variant A allele (*P = 0.028).

thought to be involved in MDD. The DRD2 receptor is central to the dopaminergic system, and a flaw in the DRD2 receptor could cause elevated generation of DA, which in turn escalates the risk of psychopathology. Our outcomes are in agreement with prior evaluations [8], indicating that DRD2 could be connected to the development of a depression disorder.

The *Taq*IA SNP (rs1800497), the most commonly studied genetic variation in different psychiatric disorders, has consistently been reported to be a risk factor for MDD, possibly via its impact on the reduced D2 receptor binding. The association of the A1 allele with surfacing manifestations of anxiety and depression in addition to parent-child experiences typified by negative feelings has been reported previously [17]. Comparably, children between the ages of 10-12 years with the A1 allele were more responsive to negative feedback throughout a probabilistic learning task. In a longitudinal evaluation of 2,347 adult men, the A1 allele was connected to an escalated risk of experiencing depressive manifestations. In keeping with these data, veterans with PTSD who carried the A1 allele had more manifestations of anxiety, depression, and social dysfunction compared to A2/A2 homozygotes. However, several studies have failed to replicate this significant association, especially in the Chinese population [18, 19], which is consistent with the results of our research. We also found no main association between *Taq*IA and MDD (P = 0.93).

Another commonly studied genetic variation of *DRD2* in recent years is rs1076560, which was reported to alter *DRD2* RNA alternative splicing and the ratio of D2S and D2L isoforms and subsequently impact pre- and post-synaptic dopamine signaling. Further, rs1076560 is also found to increase the risk for psychiatric disorders such as schizophrenia and the risk for developing substance dependence [20]. Currently, no study has shown an association of rs1076560 with MDD. Moreover, our study failed to find a significant association between rs1076560 and MDD (P = 0.83).

The other well-known SNP located by prior genetic association studies in the *DRD2* gene is rs6277, which could be depicted by rs6279 in our evaluation. The rs6277 polymorphism is found in exon 8 of the human *DRD2* gene, which leads to associated coding C-T transition at position 957. This SNP alters the receptor's affinity and controls DRD2 availability *in vivo*, but its impact varies based on the brain area being evaluated. An elevated amount of 957C alleles is connected to lowered suppression in an attentional blink task, and 957C homozygotes have a tougher time regulating the contents of working memory compared to the carriers of the 957T allele. Other studies suggest that the C957T genetic polymorphism might be associated with a risk of schizophrenia, and may also affect explicit memory, specifically for rewarded stimuli [21]. However, our results

failed to demonstrate the allelic association between the 957C/T polymorphism of the *DRD2* gene and MDD (P = 0.29), which is consistent with our previous research using a small sample size (n = 144).

These disparate results could be attributed to the differences in distribution of *HLA* class II alleles based on the ethnic distribution of the study populations. Together with previous data [8], this study further supports that ethnic differences might be an important reason for the disparate results with respect to rs1800497, rs6277 and rs1076560 associations with MDD. The second cause might be the nature of the control and MDD populations. Some research used random control populations, and the MDD populations do not exclude people who may have drug or alcohol dependency or other psychiatric disorders as comorbidities. Nevertheless, in our evaluation, the MDD populations did not include the patients with different major Axis I diagnoses, including bipolar disorder, schizophrenia, or additional psychotic disorders, and alcohol or substance abuse that occurred within the last six months. Patients were also eliminated if they had other medical or psychiatric conditions, such as pregnancy or lactation, which could have health risks throughout the evaluation. Further, we restricted the patients to the Chinese Han population to prevent an artifact as a result of different populations. In the context of a biologically pertinent phenotype and a racially consistent population, this could increase the likelihood of locating a strong genetic association.

Considering the clinical pertinence of the tSNPs, just rs7131056 and rs4648317 polymorphisms and haplotype GTGATCGCGCAGGC had a risk impact on MDD. We focused on functional polymorphisms that could be responsible for the connection signals between MDD and rs7131056 in D2-like dopamine receptors. In general, SNPs found in functional genomic areas, including mRNA splicing or regulatory areas, often lead to functional defects and diseases. Backing this, researchers have discovered that the T allele of rs1076560 was partial to the inclusion of exon 6 of *DRD2*, which led to the reduced expression of the *D2S* splice variant in prefrontal cortex autopsy tissues. Thus, we evaluated whether the SNPs in these areas may be in functional genomic areas, including those connected to mRNA splicing and the potential of intronic silencers. Association studies have been utilized to focus on markers in protein-coding areas, while current evaluations suggest that regulatory polymorphisms in non-coding areas happen more often [22]. However, since we were examining live humans, we could not quantify the expression levels of *DRD2S* splice variants in the brain. Therefore, we focused on the potential of intronic silencers. The outcomes of the intronic silencer activity reveal that the variant A allele of rs7131056 has greater transcriptional silencer activity compared to the wild C allele.

These outcomes suggest that there could be a negative regulatory element between +16434 bp and +16886 bp from the transcriptional start site, and the core area of this intronic silencer could be between +50917 bp and +51316 bp from the transcriptional start site. The *DRD2* intronic silencer determined in this evaluation is not a typical silencer; however, this kind of silencer can be found in numerous human genes, e. g., there is a functional intronic promoter found in *MDM2*, an established oncogene [23]. Of note, a regulatory SNP (rs2279744) in this intronic promoter could control the expression of *MDM2*, diminish the P53 tumor suppressor pathway, and add to the genetic vulnerability of several types of cancer. In our evaluation, only rs7131056 had an intronic silencer; thus, we speculate that the *DRD2* intronic silencer may operate in a comparable manner as the intronic *MDM2* promoter.

In conclusion, substantial evidence supports the important function of *DRD2* in MDD. This study investigated individual variability in susceptibility to MDD as it relates to the genetic variations within the entire *DRD2* gene by means of constructing haplotype bins in patients with MDD. We have demonstrated that among the 16 SNPs, only rs7131056, rs4648317, and haplotype GTGATCGCGCAGGC of 14 tSNPs had clinical relevance in the development of MDD. Our data suggests that the rs7131056 and rs4648317 polymorphisms and the GTGATCGCGCAGGC haplotype within the *DRD2* gene might be used as a relevant risk estimate for susceptibility to MDD, in which rs7131056 might be a novel intronic silencer and functional susceptibility SNP in Chinese Han populations. In an *in vitro* model, we found

that the rs7131056 C > A SNP found in the silencer region changes the transcription activity, which could control the expression of *DRD2*, and add to the genetic vulnerability of *DRD2*. The outcomes additionally verify that genetic variations in the dopaminergic pathway could have pivotal parts in the human reaction to stress, and lead to more precise knowledge, risk evaluation, early diagnosis, and the targeted treatment of MDD.

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Disclosure of conflict of interest

None.

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