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New insights into susceptibility to major depression in a Colombian population

Short Running Title: Genetic Insights into Depression in Colombia

Jesús Montoya[1*], Laura I. Rojas-Serrano[1*], Daniel A. Ruiz-Torres[1], Nicolas Caicedo[1], Isabella Van Londoño[1], Caroll Tamayo[1], Juan C. Rojas-Lopez[1], Marcela Galvez[1], Nora Contreras[1], Rafael Tierradentro[1], Laura Castro[1], Natalia Fonseca[1], Dora J. Fonseca-Mendoza[1]

1. Universidad Del Rosario, School of Medicine and Health Sciences, Center for Research in Genetics and Genomics (CIGGUR), Institute of Translational Medicine (IMT), Bogotá D.C., Colombia.

1* These authors contributed equally, both are first authors

Corresponding Author:
Dora Janeth Fonseca-Mendoza.
Telephone number: 571 3222132264
e-mail: dora.fonseca@urosario.edu.co
Address: Cra 24 # 63 C 69. Universidad del Rosario, Bogotá-Colombia
Postal Address: 111221

Abstract

Objective: Major Depressive Disorder (MDD) is a multifactorial psychiatric disease influenced by a combination of genetic and environmental factors. Among the genes linked to MDD, the Melanocortin 1 Receptor (*MC1R*), Catechol-O-Methyltransferase (*COMT*), Brain-Derived Neurotrophic Factor (*BDNF*), and the serotonin transporter (*5-HTT*) are of particular interest due to their critical roles in stress regulation and

neural function. Despite their biological significance, the contribution of specific polymorphisms within these genes to MDD risk remains understudied.

Methods: This retrospective observational case-control study included 87 Colombian patients diagnosed with MDD according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The control group comprised Latino/admixed individuals without, sourced from the gnomAD v2.1.1 database. The complete coding region of the *MC1R* gene and three polymorphisms: *5-HTTLPR* Insertion/Deletion 44 bp, *BDNF*-c.196G>A, and *COMT*-c.472G>A were genotyped using PCR and Sanger sequencing.

Results: The polymorphisms rs885479 and rs4680 were identified as protective factors against MDD, while the polymorphisms rs796296176, rs779504604, rs1805005 were associated with an increased risk of developing MDD (OR:22.87, OR:51.26, OR: 1.97, respectively).

Conclusions: Several of the analyzed polymorphisms (rs796296176, rs779504604, rs1805005) increase the risk for MDD. Notably, we provide novel evidence of these polymorphisms in MC1R as a risk to MDD.

Keywords: Major depression, single nucleotide polymorphism, sanger sequencing, genetic association, Melanocortin 1 receptor.

Introduction

Major Depressive Disorder (MDD) is considered the third leading cause of global disease burden, and the World Health Organization (WHO) has estimated that it will become the leading cause by 2030.[1] MDD is characterized by persistently low or depressed mood, anhedonia or loss of interest in pleasurable activities, feelings of guilt, low energy, poor concentration, and suicidal thoughts, among other symptoms.[2] Recent studies have demonstrated an association between MDD and higher mortality rates, which has a global impact, especially considering that as of 2023, the WHO estimated that nearly 280 million people worldwide suffer from the disorder.[3] Some authors have pointed out that the COVID-19 pandemic was associated with a significant increase in the global prevalence of MDD.[4] Interestingly, it has been established that Latin American populations exhibit a high rate of mental

disorders, with higher prevalence rates of depression compared to other regions of the world (12.58%). This could be explained by region-specific factors such as socioeconomic characteristics, income inequality, education, unemployment opportunities, and disparities in the healthcare system, among many others.[5–7] A recent meta-analysis by Errazuriz et al., 2023, analyzed prevalence data for Colombia, reporting a rate of 10.26% based on eight different studies.[7]

Previous studies have analyzed the association of single nucleotide polymorphisms (SNPs) in genes such as *SLC6A4* (serotonin transporter), *BDNF* (brain-derived neurotrophic factor), and *COMT* (catechol-O-methyltransferase), which are key components in the molecular neurobiology of depression, anxiety, and other affective disorders. The susceptibility attributed to specific SNPs within these genes is related to their regulatory roles in molecular processes such as serotonergic and dopaminergic neurotransmission, as well as synaptic plasticity .[8]

In addition, our analysis incorporated the evaluation of the complete coding region of *MC1R* (melanocortin 1 receptor), a gene located at the intersection of melanocortin signaling, stress hormones, and immune modulation, providing plausible biological pathways through which *MC1R* variation could influence mood and stress responses.[9,10]

This effect may be explained by several mechanisms, including *MC1R* activation by α-MSH and ACTH (POMC-derived peptides), molecules that increase under stress and interact with the hypothalamic–pituitary–adrenal axis and peripheral immune cells; the relationship between *MC1R* activity and inflammatory pathways mediated by cytokine release implicated in depression; neurotrophic and neurogenic effects; and finally, *MC1R* expression in immune cells and peripheral tissues, suggesting that gene variants could alter systemic inflammation or endocrine signaling that influences brain function.[9,11]

Given the molecular complexity underlying genetic susceptibility associated with MDD, analyzing new candidate genes such as *MC1R* or genetic polymorphisms previously related to depression is essential to establish genomic risk profiles in understudied populations, such as the Latin American population. Interestingly, our results demonstrated a statistically significant association with several of the

identified polymorphisms. To our knowledge, this is the first report associating some of these SNPs with susceptibility to MDD in a Latin American population. Our findings highlight the previously unrecognized relevance of the *MC1R* gene in the genetic architecture of MDD.

Patients and Methods

Sample and Data Collection

The study population consisted of 87 patients over 18 years of age who met the diagnostic criteria for major depressive disorder as outlined in the DSM-IV. The patients were recruited from specialized psychiatric centers in the city of Bogota during the year 2013 and were surveyed using the Mini-International Neuropsychiatric Interview (MINI) instrument.[12] Information on age, gender, suicide risk, previous hospitalizations, recurrent episodes, personal history of psychiatric illness, panic disorders, anxiety disorders, use of 2 or more medications, and other psychiatric pathologies was obtained and organized into databases. The age at diagnosis for most patients was between 19 and 40 years (60.5%), followed by middle adulthood (41-45 years), which accounted for 34.9% of the cases.

The control group was constituted by data from Latino/admixed individuals obtained from the gnomAD v2.1.1 database (https://gnomad.broadinstitute.org/), a single age range could not be established, as the description of the population varies according to the molecular variant analyzed.

Since this data corresponded to whole exome sequencing analysis, we considered that it might have inadequate coverage for the identification of the 5-HTTLPR Insertion/Deletion 44 bp promoter polymorphism. Therefore, only for this SNP, the control group consisted of 100 individuals from the Colombian population without clinical evidence of MDD which was determined as outlined in theDSM-IV. evidence of MDD which was determined as outlined in the DSM-IV. The age groups that conformed this control sample consisted of 19 and 40 years (64%), followed by middle adulthood (41-45 years), which accounted for 36% of the cases.

All individuals signed an informed consent form before sample collection and data collection. The experimental procedures conducted in this study were approved by

the Ethics Committee of Universidad del Rosario (CEI-AMH002-0000331) and followed the principles of the Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study.

Molecular Analysis

Blood samples from the analyzed cases were collected in EDTA tubes for subsequent DNA extraction using the Zymoresearch extraction kit (D3020, https://zymoresearch.eu/pages/dna). Genomic regions adjacent to the 5-*HTTLPR* Insertion/Deletion 44 bp, *BDNF*-c.196G>A (p.Val66Met), and *COMT*- c.472G>A (p.Val158Met) polymorphisms were amplified using polymerase chain reaction (PCR), reaction mixture was prepared using the GoTaq Green Master Mix kit (Promega- M7122). For each sample, 12.5 µL of Master Mix, 1 µL of each primer (10 pmol/µL), 200 ng of DNA, and nuclease-free water were used to reach a final reaction volume of 25 µL. The PCR was performed under the following conditions: initial denaturation at 95°C for 10 min, denaturation at 94°C for 40 sec, annealing at 60°C (BDNF)/61°C (COMT)/64°C (5-HTT) for 40 sec, extension at 72°C for 40 sec, and final extension at 72°C for 10 min (30 cycles). For the *MC1R* gene, the complete coding region was amplified using polymerase chain reaction (PCR) followed by Sanger sequencing. PCR conditions were: initial denaturation at initial denaturation at 95°C for 10 min, denaturation at 94°C for 40 sec, annealing at 60°C for 40 sec, extension at 72°C for 1 min, and final extension at 72°C for 10 min (30 cycles).

The amplification verification for all analyzed genes was performed on 1% agarose gels stained with ethidium bromide. The amplification primers used for the analysis of 5-*HTTLPR* were previously described by Owens et al.[13], while primers for the other polymorphisms and the *MC1R* gene were manually designed using the Primer 3 bioinformatics software (Supplementary Table 1). Genotype determination for the 5-*HTTLPR* Insertion/Deletion 44 bp polymorphism was performed by identifying the presence of the insertion allele or deletion allele on 1% agarose gels stained with ethidium bromide. For the *BDNF*-c.196G>A (p.Val66Met), *COMT*- c.472G>A (p.Val158Met) polymorphisms, and the coding region of *MC1R*, the amplified products were sequenced using the Sanger method. The sequences were analyzed

using FinchTV v1.5.0 software (Geospiza Inc.) and compared to the reference sequence using Clustal W v2.1 (https://www.genome.jp/tools-bin/clustalw).

Statistical Analysis

To determine allelic frequencies, genotypic frequencies, and Hardy-Weinberg equilibrium, the SNP-Stats software (https://www.snpstats.net/start.htm) was used. Deviation from Hardy-Weinberg equilibrium was assessed using a χ^2 goodness-of-fit test with 1 degree of freedom. The χ^2 test was also applied to compare allele frequencies between cases and controls. For the association analysis, codominant, dominant, and recessive models were used, and the best-fitting genetic model was selected based on Akaike's Information Criterion (AIC) using the SNPassoc v2.02 R package. The significance level (p-value) was set at <0.05 following Yates' correction. Yates' correction was applied to reduce potential false positives by adjusting the chi-square value downward, thereby preventing overestimation of statistical significance.

Results

Demographic characteristics of the analyzed population

The clinical and demographic characteristics of the study population are presented in Table 1. The predominantly affected sex with MDD was female (81.4%). A significant proportion of cases (58.1%) had a suicide risk and a family history of mental disorders (53.5%), notably, 76% (65/86) of the patients reported a psychiatric condition, with the most common being panic disorder (30.2%), generalized anxiety disorder (15.1%), and agoraphobia (10.5%). Relapse was documented in 47.7% of cases. Pharmacological treatment with more than one medication was observed in 20.9% of cases. It is important to note that demographic and clinical information for one patient was unavailable; however, this patient was included in subsequent genetic analyses.

Table 1. Demographic and clinical characteristics of the cohort.

Variable	Characteristic	Number (n = 86)	Percentage (%)
Sex	Male	16	18.6
	Female	70	81.4
Age *	Young adults (19-40)	52	60.5
	Middle Adulthood (41-65)	30	34.9
	Older adulthood (65+)	4	4.7
Risk of Suicide	Present	50	58.1
Previous Hospitalization	Yes	20	23.3
Family History of depression	Present	46	53.5
Panic Disorder	Yes	26	30.2
Generalized Anxiety Disorder	Yes	13	15.1
Eating Disorders	Yes	6	7.0
Bipolar Disorder	Yes	5	5.8
Agoraphobia	Yes	9	10.5
Social Anxiety Disorder	Yes	7	8.1
Obsessive Compulsive disorder	Yes	6	7.0
Alcohol Use Disorder	Yes	4	4.7
Psychotic Disorders	Yes	1	1.2
Relapse	Yes	41	47.7
More than 1 Medication	Yes	18	20.9

*Age classification proposed By Sacco et al, 2013.[14]

Allelic and Genotypic Frequencies of BDNF, COMT, HTTLPR, and MC1R Genes Polymorphisms

After PCR amplification and Sanger sequencing analysis, genotypes were assigned for the SNPs *BDNF*-c.196G>A (GG/GA/AA) and *COMT*-c.472G>A (GG/GA/AA). For the *HTTLPR*-44 bp Insertion/Deletion polymorphism, genotypes were determined through direct analysis on agarose gel, with the L allele corresponding to the 44 bp insertion and the S allele to the 44 bp deletion (LL/LS/SS). The *MCR1* gene, which is monoexonic, was fully sequenced, allowing the identification of eight molecular variants, all of which had been previously reported: c.488G>A, p.R163Q, rs885479; c.86 dup, p.N29Kfs*14, rs796296176; c.104 G>T, p.C35F, rs779504604; c.178G>T, p.V60L, rs1805005; c.274G>A, p.V92M, rs2228479; c.425G>A, p.R124H, rs11547464; c.451C>T, p.R151C, rs1805007; c.464T>C, p.I155T, rs1110400; and c.942A>G, p.T314T, rs2228478 (Table 2).

Allelic and genotypic frequencies for controls were extracted from the gnomAD database, v2.1.1 (https://gnomad.broadinstitute.org/). For the case-control association study, data corresponding to the Latin American population were considered. For the HTTLPR-44 bp Insertion/Deletion SNP, allelic and genotypic frequencies were determined from the 100 controls analyzed for this polymorphism, as described in the methodology section. Table 2 presents the allelic frequencies, genotypic frequencies, and Hardy-Weinberg Equilibrium (HWE) for all analyzed variants and data extracted from gnomAD database.

Table 2. Allelic and genotypic frequencies for cases and controls.

Gene	Geno type	Cases count (n=87)	Frequency (cases)	Allele (cases)	Count (cases)	Frequency (cases)	HWE P-value	Controls count	Frequency (controls)	Allele (controls)	Count (controls)	Frequency (controls)	HWE P-value
MC1R	**R163Q (c.488G>A)**												
	GG	54	0,62	G	136	0,78	0,59	6764	0,38	G	21374	0,61	7,47e-21
	GA	28	0,32	A	38	0,22		7846	0,44	A	13950	0,39	
	AA	5	0,06					3052	0,17				
	N29Kfs*14 (c.86dup)												
	No Ins/No ins	86	0,99	No ins	173	0,99	0,96	17952	1,00	No Ins	35914	0,9997	0,97
	Ins/No Ins	1	0,01	Ins	1	0,01		10	0,00	Ins	10	0,0003	
	Ins/Ins	0	0,00					0	0,00				
	C35F (c.104G>T)												
	GG	86	0,99	G	173	0,99	0,96	17634	1,00	C	35272	0,9999	0,99
	GT	1	0,01	T	1	0,01		4	0,00	F	4	0.0001	
	TT	0	0,00					0	0,00				
	V60L (c.178 G>T)												
	GG	69	0,79	G	156	0,90	0,28	15534	0,88	G	33107	0,94	2,06e-9
	GT	18	0,21	T	18	0,10		2039	0,12	T	2073	0,06	
	TT	0	0,00					17	0,00				
	V92M (c.274G>A)												
	GG	82	0,94	G	169	0,97	0,78	16214	0,91	G	33927	0,95	0,01
	GA	5	0,06	A	5	0,03		1499	0,08	A	1603	0,05	
	AA	0	0,00					52	0,00				
	R142H (c.425G>A)												

	GG	85	0,98	G	172	0,99	0,91	17433	0,99	G	35101	0,99	0,18
	GA	2	0,02	A	2	0,01		235	0,01	A	239	0,01	
	AA	0	0,00					2	0,00				
	R151C (c.451C>T)												
	CC	85	0,98	C	172	0,99	0,91	17330	0,98	C	34992	0,99	0,27
	CT	2	0,02	T	2	0,01		332	0,02	T	338	0,01	
	TT	0	0,00					3	0,00				
	I155T (c.464T>C)												
	TT	86	0,99	T	173	0,99	0,96	17515	0,99	T	35178	0,996	0,58
	TC	1	0,01	C	1	0,01		148	0,01	C	148	0,004	
	CC	0	0,00					0	0,00				
	T314T (c.942A>G)												
	AA	76	0,87	A	163	0,94	0,53	14884	0,84	A	32427	0,92	0,85
	AG	11	0,13	G	11	0,06		2659	0,15	G	2901	0,08	
	GG	0	0,00					121	0,01				
COMT	**V158M (c.472G>A)**												
	GG	46	0,53	G	125	0,72	0,56	6259	0,36	G	20981	0,60	0,70
	GA	33	0,38	A	49	0,28		8463	0,48	A	14255	0,40	
	AA	8	0,09					2896	0,16				
BDNF	**V66M(G196A)**												
	GG	66	0,76	G	148	0,85	0,01	12751	0,72	G	30050	0,85	0,52
	GA	16	0,18	A	26	0,15		4548	0,26	A	5390	0,15	
	AA	5	0,06					421	0,02				
5HTT	LL	24	0,28	L	91	0,52	0,93	36	0,29	L	123	0,50	0,06
	LS	43	0,49	S	83	0,48		51	0,41	S	123	0,50	
	SS	20	0,23					36	0,29				

Major Depressive Disorder Has a Positive Association with *MC1R* and *COMT* Genes

The association analysis was conducted under different genetic models, including codominant, dominant, and recessive models. Although statistically significant associations ($p<0.05$) were identified under several models (Supplementary Table 2), the best model was the one with the lowest AIC value (Table 3). In all cases, a $p<0.05$ was considered significant after Yates' correction.

Innovatively, our results demonstrated that under the dominant model, two *MC1R* molecular variants confer risk for MDD: c.104 G>T, rs779504604 ($p<0.01$; OR: 51.26; 95% CI: 2.6-351) and c.178G>T, rs1805005 ($p=0.014871$; OR: 1.97; 95% CI: 1.1-3.2). In this context, individuals carrying the GG and GT genotypes for *MC1R* rs779504604 and the GG and GT genotypes for *MC1R* rs1805005 have a high risk of MDD (OR: 51.26 and 1.97, respectively). It is noteworthy that the *MC1R* rs796296176 variant shows a trend towards significance after Yates' correction ($p=0.051604$; OR: 20.87; 95% CI: 1.13-111) and represents a significant risk allele for the disease (Table 3).

Our findings indicated that carriers of the GA and AA genotypes for *MC1R* rs885479 had a lower risk of MDD (dominant model: OR: 0.38; 95% CI: 0.24-0.58; $p>0.01$). Similarly, it was identified that carriers of the GA or AA genotypes for *COMT* rs4680 had a lower risk of MDD (dominant model: OR: 0.49; 95% CI: 0.32-0.75; $p<0.01$) (Table 3).

No statistically significant associations were found for the other evaluated polymorphisms ($p>0.05$) (Supplementary Table 2).

Table 3. Genetic association analysis for MDD

SNP	Model	Control	%	Cases	%	OR	Lower	Upper	P-value	Yates p-values	AIC
MC1R	Codominant										
R163Q	RR	6764	38.3	54	62.1				<0.01	<0.00001	1081.95
	RQ	7846	44.4	28	32.2	0.45	0.28	0.70			
	QQ	3052	17.3	5	5.7	0.21	0.07	0.47			
MC1R	Dominant										
Q30TfsTer13	QQ	17952	99.9	86	98.9	NA	NA	NA	<0.01	0.051604	1101.78
	QT + TT	10	0.1	1	1.1	20.87	1.13	1.11e2			
MC1R	Dominant										
C35F	CC	17634	99.9	86	98.9				<0.01	<0.00001	1097.02
	CF+FF	4	0.02	1	1.1	51.26	2.61	3.51e2			
MC1R	Dominant										
V60L	VV	15534	88.3	69	79.3				0.0107	0.014871	1096.54
	VL+LL	2056	11.7	18	20.7	1.97	1.14	3.24			
COMT	Dominant										
V158M	VV	6259	35.5	46	52.9				<0.01	0.0012	1091.68
	VM+MM	11359	64.5	41	47.1	0.49	0.32	0.75			
BDNF	Recessive										
V66M	VV+VM	17299	97.6	82	94.3				0.0474	0.088923*	1100.46
	MM	421	2.4	5	5.8	2.51	0.88	5.61			

*Value out of statistical significance with Yates correction of p-value.

DISCUSSION

MDD is the leading cause of disability worldwide, with a significant annual increase in its prevalence. It is undoubtedly a complex disorder associated with multiple pathological factors, but the estimated heritability of 30 to 50% suggests a crucial role of genetic factors in the occurrence of MDD.[15] This context, along with the polygenic nature of the disorder, explains the increasing and active field of research focused on identifying genes and variants associated with MDD.[16] In this study, we determined the association of MDD with SNPs in well-studied genes such as *COMT* and *BDNF*. However, our findings fundamentally provide new insights into the involvement of the *MC1R* gene, a potential candidate gene for MDD that has received little attention to date.[9] In this context, our findings demonstrated a statistically significant association, suggesting an increased risk (under the dominant genetic model) for patients carrying the SNPs *MC1R* c.104 G>T, rs779504604 ($p < 0.01$; OR: 51.26; 95% CI: 2.6-351) and c.178 G>T, rs1805005 ($p = 0.014871$; OR: 1.97; 95% CI: 1.1-3.2). A third SNP, rs796296176, showed a trend toward significance and is of relevance ($p = 0.051604$; OR: 20.87; 95% CI: 1.13-111). To our knowledge, these *MC1R* SNPs have not previously been reported in any population as being associated with MDD. However, a literature report studying a Mexican population identified the association of other MC1R SNPs with MDD-affected patients.[9]

The association of MC1R with depression could be explained by its functional role in anti-inflammatory mechanisms[17] and the pathways underlying increased cortisol levels.[18] An altered binding activity of MC1R to its ligands, such as α-MSH, would decrease cAMP production, impairing effective activation of Protein Kinase A (PKA). If PKA is not activated, this would trigger a cascade of events resulting in a reduction in anti-inflammatory cytokines and continuous transcription of inflammatory genes through NF-kB, leading to prolonged inflammation.[17] MDD is characterized by elevated levels of pro-inflammatory cytokines such as IL-6, TNF, IL-1RA, and sIL2R, among others, indicating that patients with this disorder experience a chronic state of inflammation.[19] Increased levels of α-MSH due to improper binding with MC1R

could result in elevated cortisol levels via a hyperactivation of the HPA axis, seen in patients with depression, who present with hypercortisolemia.[20–23] This suggests that polymorphisms impairing MC1R function could contribute to the inflammatory theory of depression.[24]

The molecular variants in *MC1R* identified in our study as significantly associated with MDD could potentially alter receptor functionality in various contexts. Specifically, the MC1R p.C35F variant was found to be associated with a higher risk of developing MDD ($p < 0.01$; OR: 51.26; 95% CI: 2.6-351). This variant has been predicted to be pathogenic by approximately 75% of the commonly applied in silico predictors for missense mutations, including PolyPhen-2, Provean, MutationTaster, and SIFT, among others.[25] This is a conserved cysteine residue involved in the formation of disulfide bonds, which is crucial for proper protein folding. The absence of disulfide bond formation may hinder the formation of the α-helix structure, preventing the correct insertion of the G-protein coupled receptor (GPCR) into the cell membrane. Consequently, this polymorphism could potentially impair MC1R insertion, leading to a diminished response of the cAMP cascade due to defective receptor-ligand binding.[26] Interestingly, another mutation at the same position, MC1R p.C35Y, has been identified and shown to impair receptor function by affecting agonist binding, further supporting the critical role of this residue in receptor functionality.[27]

The MC1R p.V60L variant (rs1805005) also showed a statistically significant association with MDD in the patient group analyzed ($p = 0.014871$; OR: 1.97; 95% CI: 1.1-3.2), and, similar to p.C35F, our study is the first to identify it as a risk factor for the disorder. This variant is located in the intracellular domain of the first transmembrane region (TM1) of MC1R and has been associated with a reduction in the receptor's basal activity, affecting its ability to elevate intracellular cAMP levels.[28,29] Functional validation studies have demonstrated that the V60L variant shows a smaller reduction in plasma membrane receptor levels, reaching 55% of the levels observed with the consensus allele, supporting the hypothesis that the variant alters normal receptor function.[27] To date, this variant has been linked to several phenotypic and clinical outcomes, such as nevus count, increased risk of melanoma,

and non-melanoma skin cancers.[30–32] This supports the observation of underlying genetic susceptibility related to the presence of this molecular change. Although the phenotypes associated with this variant have thus far been linked to skin pigmentation and melanoma risk, our association study provides novel evidence of its relationship with MDD.

Finally, of great interest is the *MC1R* p.N29Kfs*14 variant, which, despite showing a trend toward significance (after Yates correction), indicated a notable association with an increased risk of MDD (p = 0.051604; OR: 20.87; 95% CI: 1.13-111). This variant alters the amino acid sequence from codon 29 and leads to a premature stop codon, resulting in an estimated 86.5% loss of the receptor. The alteration predicts a truncated or absent protein due to nonsense-mediated decay (NMD) activation, ultimately leading to a loss of function. We propose that frameshift mutations in this receptor can lead to functional changes that potentially affect its expression and downstream signaling pathways, such as those involving α-MSH and cAMP production.

The only previous study investigating the relationship between MC1R variants and MDD identified 23 molecular variants and reported that only the nonsynonymous SNP R163Q (rs885479) was associated with a diagnosis of depression in 181 Mexican-American patients (p = 0.04).[13] This result contrasts with our findings, as in our population, this molecular variant was found to be a protective factor (dominant model: OR: 0.38; 95% CI: 0.24-0.58; p < 0.01). It is well recognized that discrepancies in case-control studies across different populations can be attributed to factors such as selection bias, population substructure, and differences in control group selection.[33–35] In this context, we estimate that ethnic differences between the Colombian and Mexican populations may have impacted the results. This is supported by the statistically significant difference in allele frequencies of *MC1R* 163R and 163Q identified in the case groups from both populations (p = 0.000493), with a lower frequency of the mutant allele MC1R p.163Q in the Colombian population compared to the Mexican population (0.218 vs 0.373, respectively). This reflects the complex genetic admixture present in Latin American populations.[36]

Concerning the *COMT* gene, we found an association between MDD and the p.Val158Met variant according to both dominant and recessive models. However, the lower AIC value inferred for the dominant model allowed us to determine that this is the best model to predict MDD outcomes in our population (OR: 0.49; 95% CI: 0.32-0.75; $p < 0.01$). Interestingly, Wang et al. [37] conducted a Meta-Analysis of the *COMT* Val158Met polymorphism in nearly 3.000 MDD cases and this report highlighted the effect of ethnicity on the association between MDD and polymorphism, indicating that the allele associated with the disease was not necessarily the same in populations of European and East Asian ancestry. This observation is relevant as it potentially explains the discrepancies reported in studies associating this variant with MDD and underscores the need for conducting studies in underrepresented populations, such as Latin Americans.[38,39]

The sociodemographic analysis of the study population showed that 81% of individuals diagnosed with MDD are women, while only 18% are men. This gender disparity has been attributed to both biological and social factors. Biologically, hormone fluctuations, such as those related to the menstrual cycle, pregnancy, and menopause, may influence stress responses and mood regulation, increasing susceptibility to depression in women.[40] Additionally, psychological factors, including higher rates of anxiety and emotional sensitivity, may contribute to this increased risk. Socially, gender roles and expectations may play a significant role, with women often facing greater pressures related to caregiving, employment, and social responsibilities, which may increase their vulnerability to mental health challenges.[41] Moreover, it is crucial to note that the disorder predominantly affects younger individuals, who represent 60.5% of total cases. The transitional period from childhood to adulthood, marked by significant biological changes, may reflect the consequences of accumulating cortisol during childhood, which could predict the subsequent onset of MDD in young adulthood.[42] It is important to acknowledge that our study sample does not include childhood and adolescent patients, which limits the extent of our analysis. Additionally, a significant finding from the data is that 58% of all patients are at high risk of suicide, emphasizing the critical need for comprehensive mental health support and suicide prevention strategies within this

vulnerable group. Considering that the heritability of MDD has been estimated between 30% and 50%, it is suggested that genetic factors significantly contribute to the risk of developing MDD. However, factors such as gender and age must also be considered, highlighting the complex architecture of MDD.[43]

The analyses conducted in this study identified molecular variants associated with MDD in the studied population, contributing to the understanding of the potential molecular etiopathology of a disorder that significantly impacts global health due to its increasing incidence and its profound effect on patients' quality of life.

Our study has several limitations that should be considered: a) The analyzed sample comprised individuals who attended the centers during the project's designated timeframe, meaning no specific sample size was predetermined, which may affect the statistical significance, b) although the control population represents a large number of individuals, the public database gnomAD, from which the control data were obtained, does not completely rule out the possibility that these individuals may have MDD, c) since controls were obtained from curated public databases, it was not possible to match case and control groups, as this information is not available, d) The lack of detailed family history data did not allow for an exhaustive analysis of the generational component and e) it was not possible to perform population stratification analysis within this study.

Conclusions

Our results identified a statistically significant association between molecular variants in *MC1R* and MDD, with susceptibility alleles conferring a high risk for the development of the disease: *MC1R* c.104 G>T, rs779504604 ($p < 0.01$; OR: 51.26; 95% CI: 2.6-351) and c.178 G>T, rs1805005 ($p = 0.014871$; OR: 1.97; 95% CI: 1.1-3.2). A third SNP, rs796296176, also showed a trend toward significance and is of relevance ($p = 0.051604$; OR: 20.87; 95% CI: 1.13-111). These findings suggest that *MC1R* could be considered a potential candidate gene for the development of MDD. To our knowledge, this is the first time these variants have been linked to MDD, which is significant as it proposes genetic susceptibility profiles that could aid in identifying individuals at risk. This, in turn, could enable the prediction and implementation of

preventive measures, contributing to the advancement of preventive and personalized medicine.

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Disclosure

The authors declare that they have no conflict of interest

Data Availability Statement

The authors consider that the datasets presented in this article are not readily available because the nature of this research contains information that could compromise the participants' privacy, they did not agree to share their data publicly. Requests to access the datasets should be directed to DJ-FM.

Author Contributions

DJ-FM, M-G. DA-R and J-M contributed to conception and design of the study. N-C, LI-R, C-T, J-R, R-T and L-C performed DNA extraction and genetic analysis. M-G,DJ-FM, N-F, J-M collected clinical data and organized the database. DJ-FM, N-C,J-M, LI-R performed the statistical analysis.. All authors contributed to manuscript revision, read, and approved the submitted version.

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Supplementary material

Supplementary Table 1 Primer sequences of *MC1R*, *COMT*, *BDNF* and *5HTT*

Primer sequences			
Gene	**Primer sequences**	**Location**	**Amplicon Size bp**
MC1R	F 5`- GGTTGTGAGAATCCCTGAGC-3` R 5`-GTTCCTTACCTCCCTGGTCCC-3	Exon 1	1271pb
5HTT	F 5'-GGCGTTGCCGCTCTGAATGC-3' R 5'-GAGGGACTGAGCTGGACAACCAC-3'	Promotor	490 pb
COMT	F 5´-GAGCATAGAGGCTAAGGGACCAT-3' R 5'- ACACACCCATACAAGCATTCATCA-3'	Exon 4	740 pb
BDNF	F 5'-ATCTTGGGGGAAACACTGC-3' R 5'-TCCTTATGAATCGCCAGCC-3'	Exon 2	939 pb

Supplementary Table 2. Association between *MC1R, COMT, BDNF* and *5HTT* polymorphisms and Major Depressive disorder

SNP	Model	Control	%	Cases	%	OR	Lower	Upper	P value	AIC
MC1R R163Q c.488G>A rs885479	**Codominant**									
	RR	6764	38.3	54	62.1				<0.01	1081.95
	RQ	7846	44.4	28	32.2	0.45	0.28	0.70		
	QQ	3052	17.3	5	5.7	0.21	0.07	0.47		
	Dominant									
	RR	6764	38.3	54	62.1				<0.01	1083.00
	RQ+QQ	10898	61.7	33	37.9	0.38	0.24	0.58		
	Recessive									
	RR+RQ	14610	82.7	82	94.3				<0.01	1092.58
	QQ	3052	17.3	5	5.7	0.29	0.10	0.65		
MC1R c.86dup rs796296176 I: Insertion N: No insertion	**Codominant**									
	II	17952	99.9	86	98.9	NA	NA	NA	<0.01	1101.78
	IN	10	0.1	1	1.1	20.87	1.13	1.11e2		
	NN	0	0	0	0					
	Dominant									
	II	17952	99.9	86	98.9				<0.01	1103.86
	IN+NN	10	0.1	1	1.1	20.87	1.13	1.11e2		
	Recessive									
	II+IN	17962	100	87	100	0.05	0.01	0.88		1101.78
	NN	0	0	0	0				NA	
MC1R C35F c.104G>T rs779504604	**Codominant**									
	CC	17634	99.9	86	98.9					1097.02
	CF	4	0.02	1	1.1	51.26	2.61	3.51e2	<0.01	
	FF	0	0	0	0	NA	NA	NA		
	Dominant									
	CC	17634	99.9	86	98.9				<0.01	1097.02
	CF+FF	4	0.02	1	1.1	51.26	2.61	3.51e2		
	Recessive									
	CC+CF	17638	100	87	100				<0.01	1100.70

	FF	0	0	0	0	NA	NA	NA		
MC1R V60L c.178 G>T rs1805005	**Codominant**									
	VV VL LL	15534 2039 17	88.3 11.6 0.1	69 18 0	79.3 20.7 0	1.99 3.78e-5	1.15 NA	3.27 1.73e5	<0.01	1098.24
	Dominant									
	VV VL+LL	15534 2056	88.3 11.7	69 18	79.3 20.7	1.97	1.14	3.24	<0.05	1096.54
	Recessive									
	VV+VL LL	17573 17	99.9 0.09	87 0	100 0	3.91e-5	NA	1.56e5	>0.05	1102.06
MC1R V92M c.274G>A rs2228479	**Codominant**									
	VV VM MM	16214 1499 52	91.3 8.4 0.3	82 5 0	94.3 5.7 0	0.66	0.23	1.47	>0.05	1104.52
	Dominant									
	VV VM+MM	16214 1551	91.3 8.7	82 5	94.3 5.7	0.64	0.22	1.42	>0.05	1102.86
	Recessive									
	VV+VM MM	1771 352	99.7 0.3	87 0	100 0	0.000012	1.52e-57	17.76	>0.05	1103.44
MC1R R142H c.425G>A rs11547464	**Codominant**									
	RR RH HH	17433 235 2	98.7 1.3 0.01	85 2 0	97.7 2.3 0	1.75	0.29	5.57	>0.05	1104.49
	Dominant									
	RR RH+HH	17433 237	98.7 1.3	85 2	97.7 2.3	1.73	0.28	5.52	>0.05	1102.52
	Recessive									
	RR+RH HH	17668 2	99.9 0.01	87 0	100 0	0.00019	NA	6.45e+26	>0.05	1102.99

MC1R R151C c. 451C>T rs1805007	**Codominant**									
	RR RC CC	17330 332 3	98.1 1.9 0.02	85 2 0	97.7 2.3 0	1.23	0.20	3.91	>0.05	1104.86
	Dominant									
	RR RC+CC	17330 335	98.1 1.9	85 2	97.7 2.3	1.22	0.20	3.87	>0.05	1102.89
	Recessive									
	RR+RC CC	17662 3	99.9 0.02	87 0	100 0	0.00014	NA	5.28e+19	>0.05	1102.94
MC1R I155T c.464T>C rs1110400	**Codominant**									
	II IT TT	17515 148 0	99.2 0.8 0	86 1 0	98.9 1.1 0	1.38	0.08	6.25	>0.05	1102.86
	Dominant									
	II IT+TT	17515 148	99.2 0.8	86 1	98.9 1.1	1.38	0.08	6.25	>0.05	1102.86
	Recessive									
	II+IT TT	17663 0	100 0	87 0	100 0	NA	NA	NA	NA	1100.95
MC1R T314T c.942A>G rs2228478	**Codominant**									
	T1T1 T1T2 T2T2	14884 2659 121	84.3 15.1 0.7	76 11 0	87.4 12.6 0	0.81	0.41	1.46	>0.05	1103.32
	Dominant									
	T1T1 T1T2+T2T2	14884 2780	84.3 15.7	76 11	87.4 12.6	0.77	0.39	1.40	>0.05	1102.29
	Recessive									
	T1T1+T1T2 T2T2	17543 121	99.3 0.7	87 0	100 0	0.0000051	7.13e-61	1.72	>0.05	1101.76

COMT V158M c.472G>A rs4680	**Codominant**									
	VV VM MM	6259 8463 2896	35.5 48.0 16.4	46 33 8	52.9 37.9 9.2	0.53 0.38	0.34 0.16	0.83 0.75	<0.01	1092.86
	Dominant									
	VV VM+MM	6259 11359	35.5 64.5	46 41	52.9 47.1	0.49	0.32	0.75	<0.01	1091.68
	Recessive									
	VV+VM MM	14722 2896	83.6 16.4	79 8	90.8 9.2	0.51	0.23	1.00	>0.05	1098.68
BDNF V66M G196A rs 6265	**Codominant**									
	VV VM MM	12751 4548 421	72.0 25.7 2.4	66 16 5	75.9 18.4 5.7	0.68 2.29	0.38 0.80	1.14 5.18	>0.05	1100.39
	Dominant									
	VV VM+MM	12751 4969	71.9 28.1	66 21	75.9 24.1	0.82	0.49	1.31	>0.05	1102.83
	Recessive									
	VV+VM MM	17299 421	97.6 2.4	82 5	94.3 5.8	2.51	0.88	5.61	<0.05	1100.46
5HTT	**Codominant**									
	LL LS SS	36 51 36	29.3 41.5 29.3	24 43 20	27.6 49.4 23.0	1.26 0.83	0.66 0.39	2.46 1.77	>0.05	289.39
	Dominant									
	LL LS+SS	36 87	29.3 70.7	24 63	27.6 72.4	1.09	0.59	2.01	>0.05	288.85
	Recessive									
	LL+LS	87	70.7	67	77.0				>0.05	287.88

	SS	36	29.3	20	23.0	0.72	0.38	1.35		