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Genetic association of dopamine beta-hydroxylase (*DBH*) rs72393728 and D-amino acid oxidase activator (*DAOA*) rs3918342 polymorphisms with bipolar disorder in northeastern Algeria

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Abstract: Genes involved in neurotransmitter alteration are implicated in the pathophysiology of bipolar disorder (BD), particularly those belonging to dopaminergic and glutamatergic pathways. This study aimed to investigate the putative association between dopamine beta-hydroxylase (*DBH*) 19bp insertion/deletion rs72393728 and D-amino acid oxidase activator (*DAOA*) M23 rs3918342 polymorphisms with bipolar disorder in a population from northeastern Algeria. The case-control research included 95 patients and 148 controls. The salting out method was applied for the extraction of DNA. PCR was performed to genotype *DBH* rs72393728; genotyping of *DAOA* rs3918342 was conducted by PCR-RFLP. The findings demonstrated significant variations in genotype (P=0.025) and allele (P=0.0088) frequencies of *DBH* between cases and controls. However, there was no significant correlation between the two groups for *DAOA* either in genotype or allele distributions (P=0.54, and P= 0.26, respectively), although a significant association was detected for grandiose delusion and M23 (P=0.0015). The *DBH* rs72393728 polymorphism is reported here for the first time to be associated with the risk of bipolar disorder, both in the Algerian population and in the scientific literature. Despite there being no direct association for *DAOA* rs3918342 polymorphism and bipolar disorder, the two polymorphisms showed significant correlations when combined.

Keywords: dopamine, glutamate, *DBH*, *DAOA*, bipolar disorder

INTRODUCTION

Bipolar disorder (BD) is a severe, lifelong psychiatric disorder characterized by the recurrent alternation of manic and depressive states separated by an euthymic period [1,2]. It is mainly classified into two distinct subtypes: type 1, which typically occurs in mania and depression, and type 2, which presents hypomanic and depressive phases [1,3]. The estimated prevalence of type 1 and type 2 BD is 0.6% and 0.4%, respectively [4,5]. About 1% of the general population suffers from BD [6,7].

BD can manifest through various clinical signs that affect emotion, cognition, and behavior [1]. These manifestations may be explained by the involvement of distinct biological pathways in their pathophysiology. Although the exact cause of BD remains unknown, multiple studies have pointed to a strong genetic component in addition to environmental influences [8]. Genetic susceptibility to BD appears to involve several candidate genes, each contributing a minor effect [9]. Many of these participate in functional pathways [10] and are related to neurotransmitter alteration [11]. Two of the most robustly investigated systems for

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their potential role in the pathophysiology of BD are the dopamine and glutamate systems [12,13]. Multiple candidate genes are involved in these pathways, among them dopamine beta-hydroxylase (*DBH*) and D-amino acid oxidase activator (*DAOA*).

DBH is a neurotransmitter-synthesizing enzyme that converts dopamine (DA) into noradrenaline (NA), playing a key role in maintaining the NA/DA balance in the brain [14-16]. The *DBH* gene is mapped to chromosome 9q34 and composed of 12 exons spanning about 23 kb [17]. It contains several potential pathogenic variants, one of which is the 19 bp InsDel rs72393728, which is located 4.5 kb upstream of the transcriptional start site [18]. Increased levels of DBH plasmatic activity are associated with manic episodes, while decreased activity is related to depressive episodes in BD patients [15]. DBH's plasmatic activity is genetically controlled, and the *DBH* rs72393728 polymorphism was proven to be strongly associated with it. The Del allele was correlated with reduced promoter activity, causing lower DBH levels and enzymatic activity in plasma and cerebrospinal fluid (CSF) [18,19]. A genome-wide association study confirmed the implication of the *DBH* gene as a major determinant of DBH plasmatic activity by identifying three variants in the *DBH* promoter region, attributing the strongest association to the rs1611115 polymorphism. Additionally, the study highlighted two other loci (*SARDH* and *LOC338797*) as potential contributors to the regulation of DBH expression [20].

The DAOA protein is comprised of 153 amino acids and is located in various regions of the central nervous system (CNS). It activates D-amino acid oxidase (DAO), an enzyme crucial for synaptic plasticity and neuronal migration in the brain, and responsible for the degradation of D-serine, a potent activator of N-methyl-D-aspartate (NMDA) type glutamate receptors [21]. Glutamate is an excitatory neurotransmitter implicated in several brain processes [22]. Alterations in the glutamate system have been associated with bipolar disorder (BD), as evidenced by elevated plasma glutamate levels observed in patients with BD [23]. *DAOA* is located on chromosome 13q34, contains 7 exons, and spans 29 kb [21]. The *DAOA* rs3918342, a single-nucleotide polymorphism C>T, also called M23, is situated upstream of the 5'UTR [22], an area that has been found to be positively associated with BD [24]. It was demonstrated that the T allele of the M23 polymorphism was implicated in both the disturbance of the glutamatergic transmission in BD and dopamine turnover [25]. These findings led to the hypothesis of an eventual connection between glutamatergic and dopaminergic systems, highlighted by Andreou et al. [21], who showed that *DAOA* and *DBH* are linked, despite belonging to distinct pathways. Indeed, the DAO enzyme, after activation by DAOA, is involved in the conversion of D-DOPA to L-DOPA, which is the direct precursor of DA; this in turn is converted to NA by the DBH enzyme.

Multiple studies have investigated genetic susceptibility to psychiatric disorders. According to Turkish research, the rs6271 variant of *DBH* is associated with BD [26]. However, findings of a recent case-control study conducted in eastern Algeria found no association between the *DBH* rs72393928 polymorphism and schizophrenia (SCZ) [27]. In addition, several single nucleotide polymorphisms (SNPs), including rs5907577 in the intragenic region at Xq27.1 and rs2111504 in the DPY19L3 gene at 19q13.11, have been linked to bipolar disorder (BD) in a genome-wide association study conducted on European and African American populations, which also confirmed the association of the *ankyrin 3* (*ANK3*) region with BD [28].

The *DAOA* gene (also known as *G72*) and the neighboring region containing the *DAO* (also referred to as *G30*) gene have been extensively analyzed for their genetic association with BD and SCZ in multiple studies. The M23 variant of *DAOA* has been substantially linked to both diseases in a British population [29]. According to family-based research, the *DAOA* rs1935062 polymorphism is specifically correlated to BD [30]. In addition, meta-analyses revealed contradictory findings about the genetic association between *DAOA* and BD [31]. Similarly, *DAO* (*G30*) showed conflicting results regarding its association with BD and SCZ; although several studies have connected the *DAO* gene to SCZ, this correlation could not be confirmed in a British sample [29]. Based on a German investigation, only *DAOA* was linked to BD, whereas both *DAOA* and *DAO* have been related to SCZ [32]. These findings suggest that the *DAOA* (*G72*) gene contributes to susceptibility to both SCZ and BD; however, the considerable variability in results across studies indicates that specific polymorphisms may help differentiate between the two disorders [30].

The main purpose of our study was to explore the implication of two specific genetic polymorphisms: the 19bp InsDel in the *DBH* gene and the M23 single-nucleotide polymorphism in the *DAOA* gene, as potential risk factors for susceptibility to BD in a community of northeastern Algeria. These genes were selected due to their involvement in the dopaminergic and glutamatergic pathways, both of which are strongly implicated in the neurobiology of BD. To the best of our knowledge, this is the first study investigating the association of *DBH* and *DAOA* with bipolar pathology in the Algerian population, thereby providing novel insights to psychiatric genetics research in North Africa.

MATERIALS AND METHODS

Ethics statement

All participants signed a written informed consent to be part of this study. The research protocol was approved by the Ethics Committee of the Dr. Benbadis University Hospital Center of Constantine (Reference Number: CE/CHUC/12/12/2024) and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Study population

This study follows a case-control design. The patient group comprised 95 individuals diagnosed with BD, all of whom met the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [33] criteria for BD and were evaluated through structured interviews conducted by experienced psychiatrists. The patients were recruited from the Psychiatric Hospital of Constantine and El-Athmania, and several private medical practices in the region of Constantine, according to the following inclusion criteria: confirmed diagnosis of BD, consenting patients, age ≥18 years, and the feasibility of the blood sampling. Medical data, including medical history and clinical features of patients, were collected for analysis. The control group, consisting of 148 healthy individuals, was matched to the patient group by age and gender, and recruited from the same geographical area. None had a personal or family history of psychiatric disorders.

DNA extraction

The genomic DNA was extracted from leukocytes of whole blood samples collected in EDTA-containing tubes using the salting-out method [34]. The purity and concentration of DNA samples were assessed using a Nanodrop spectrophotometer and then stored until use at -20°C.

Genotyping of the *DBH* rs72393728 polymorphism

A PCR (polymerase chain reaction) assay was conducted to genotype the polymorphism rs72393728 of the *DBH* gene using the following primer pair: forward: 5`-GCAAAAGTCAGGCACATGCACC-3`; reverse: 5`-CAATAATTTGGCCTCAATCTTGG-3` [18]. The reaction protocol was carried out as described previously [27]. The obtained PCR products were resolved on a 3% agarose gel (NIPPON Genetics EUROPE, Germany), stained with GelRed (Roth, Germany), and visualized using the Gel Doc XR+ Gel Documentation System (Bio-Rad, USA).

Genotyping of the *DAOA* rs3918342 polymorphism

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was performed to genotype the polymorphism rs3918342 of the *DAOA* gene employing the following primer pair: forward: 5`-GGAAACCAGAAGGTGAAA-3`; reverse: 5`- GAATCAGAAAGGAAAAGTGT-3`[22]. The PCR reaction was prepared in a final volume of 25 µL containing 2.5 µL of 1 × PCR buffer, 0.25 µL of each primer (0.2 µM), 1 µL of dNTP mix (0.2 mM), 2 µL of $MgCl_2$ (1.5 mM), 0.5 U Taq DNA polymerase (Solis-Biodyne, Estonia), and 50 ng of genomic DNA. The thermocycling program included an initial denaturation at 94˚C for 5 min, followed by 30 cycles at 94˚C for 30 s (denaturation), 30 s at 58˚C (annealing), and 30 s at 72˚C (elongation), then a final elongation step at 72˚C for 7 min. The amplified PCR products were resolved on a 2% agarose gel (NIPPON Genetics EUROPE, Germany), stained with GelRed (Roth, Germany), and visualized under UV light. The successfully amplified fragments obtained were digested with the *BsaAI* restriction enzyme (New England Biolabs,

Table 1. Clinical characteristics of cases and controls

Demographic and clinical features	Cases (N=95)	Controls (N=148)	P
Age (years)			
Mean (SD)	42.64 (13.14)	43.4 (13.09)	0.67
Age of onset (years)			
Mean (SD)	26.46 (9.14)	-	
Gender			
Male N (%)	37 (38.95)	58 (39.19)	0.97
Female N (%)	58 (61.05)	90 (60.81)	
Subtype of BD			
Type 1 N (%)	73 (76.84)	-	
Type 2 N (%)	22 (23.16)	-	
Polarity of first episode			
Depression N (%)	46 (48.42)	-	
Mania or hypomania N (%)	46 (48.42)	-	
Most recurrent episode			
Depression N (%)	42 (44.21)	-	
Mania or hypomania N (%)	50 (52.63)	-	
Hospitalization			
Yes N (%)	51 (53.68)	-	
No N (%)	44 (46.32)	-	
Family history of psychiatric disorders	58 (61.05)	-	
Yes N (%)	37 (38.95)	-	
No N (%)			
Positive Suicidal history			
Suicidal attempt N (%)	18 (18.95)	-	
Suicidal ideation N (%)	35 (36.84)	-	
Positive Psychotic Symptoms			
Delusions N (%)	60 (63.16)	-	
Hallucinations N (%)	24 (25.26)	-	
Tobacco			
Smokers N (%)	27 (28.42)	20 (13.51)	**0.0142**
Nonsmokers N (%)	65 (68.42)	120 (81.08)	
NA N (%)	3 (3.16)	8 (5.41)	
HTA			
Yes N (%)	13 (13.68)	14 (9.46)	0.376
No N (%)	75 (78.95)	127 (85.81)	
NA N (%)	7 (7.37)	7 (4.73)	
Diabetes			
Yes N (%)	13 (13.68)	6 (4.05)	**0.0138**
No N (%)	75 (78.95)	135 (91.22)	
NA N (%)	7 (7.37)	7 (4.73)	

N – Number of subjects, SD – standard deviation, NA – not available

USA) for genotyping. To validate the RFLP results, randomly selected PCR products were sequenced directly using a BigDye Terminator and an ABI3500xl genetic analyzer (Applied Biosystems, USA).

Statistical analysis

Statistical analyses were performed using the SPSS software version 26.0 (SPSS Inc., Chicago, Illinois, USA). The Hardy-Weinberg equilibrium (HWE) was assessed separately in the patient and control groups using the chi-square goodness-of-fit test. Association analyses between the patient and control groups for qualitative variables were evaluated using the chi-square test. Logistic regression was applied to analyze genotypic frequencies and to compare type 1 and type 2 BD, genetic models, and the clinical variables within patients. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to measure the significance of the associations. The ANOVA test was used for quantitative variables. Statistical significance was defined as a P value of less than 0.05.

RESULTS

We investigated the potential association of *DBH* 19bp InsDel rs72393728 and *DAOA* M23 rs3918342 polymorphisms, which are involved in dopamine and glutamate systems, respectively, with BD in a population of northeastern Algeria.

Demographic and clinical characteristics

A total of 95 BD patients and 148 healthy controls were included (76.84% with type 1 BD and 23.16% with type 2 BD). The mean age was 42.64±13.14 years for the patients and 43.4±13.09 years for the controls. Both groups had a female predominance, with females comprising 61.05% of the patient group and 60.81% of the control group, resulting in an M/F ratio of 0.64 in both groups. No significant differences were observed between the two groups in terms of age (P=0.67) or gender distribution (P=0.97). However, comparison of demographic and clinical features between bipolar patients and healthy subjects revealed significant differences in the prevalence of tobacco use (P=0.0142) and diabetes (P=0.0138) (Table 1).

Table 2. Genotypic and allelic distributions and genetic models of *DBH* rs72393728 and *DAOA* rs3918342 in cases and controls

Polymorphism		Cases N (%)	Controls N (%)	P	OR (95% CI)	P (adjusted)	OR (95% CI) (adjusted)
DBH rs 72393728	**Genotype**						
	InsIns	19 (20)	52 (35.14)	**0.0298**	1.00	**0.025**	1.00
	InsDel	48 (50.53)	66 (44.59)		1.99 (1.04-3.79)		2.00 (1.05-3.81)
	DelDel	28 (29.47)	30 (20.27)		2.55 (1.22-5.33)		2.60 (1.24-5.44)
	Allele						
	Ins	86 (45.26)	170 (57.43)	**0.0088**	0.61 (0.42-0.88)	-	-
	Del	104 (54.74)	126 (42.57)				
	Genetic Models						
	Dominant						
	InsIns	19 (20)	52 (35.1)	**0.0115**	1.00	**0.0095**	1.00
	InsDel+DelDel	76 (80)	96 (64.9)		2.17 (1.18-3.97)		2.18 (1.19-4.00)
	Recessive						
	InsIns+InsDel	67 (70.5)	118 (79.7)	0.1012	1.00	0.096	1.00
	DelDel	28 (29.5)	30 (20.3)		1.64 (0.90-2.98)		1.67 (0.92-3.04)
	Log-additive	-	-	-	-	**0.0095**	1.61 (1.12-2.32)
DAOA rs3918342	**Genotype**						
	CC	22 (23.16)	44 (29.73)	0.5267	1.00	0.54	1.00
	CT	47 (49.47)	68 (45.95)		1.38 (0.73-2.60)		1.37 (0.73-2.59)
	TT	26 (27.37)	36 (24.32)		1.44 (0.74-2.96)		1.43 (0.70-2.95)
	Allele						
	C	91 (47.89)	156 (52.70)	0.3014	0.82 (0.57-1.19)	-	-
	T	99 (52.11)	140 (47.30)				
	Genetic Models						
	Dominant						
	CC	22 (23.2)	44 (29.7)	0.2620	1.00	0.27	1.00
	CT+TT	73 (76.8)	104 (70.3)		1.40 (0.78-2.54)		1.39 (0.77-2.52)
	Recessive						
	CC +CT	69 (72.6)	112 (75.7)		1.00	0.6	1.00
	TT	26 (27.4	36 (24.3)	0.5961	1.17 (0.65-2.10)		1.15 (0.65-1.94)
	Log-additive	-	-	-		0.33	1.20 (0.84-1.71)

N – number of subjects, Ins – insertion, Del – deletion, OR – odds ratio, CI – confidence interval

Genotyping of the *DBH* rs72393728 polymorphism

The genotype frequencies of the *DBH* rs72393728 polymorphism adhered to Hardy-Weinberg equilibrium in both patient and control groups (P=0.85 and 0.33, respectively), indicating no significant deviation from the normal distribution. Supplementary Fig. S1 illustrates the electrophoresis profile of this polymorphism, showing three different genotypes: a single band of 163pb for homozygous subjects carrying the insertion allele (InsIns) and a single band of 144pb for homozygous individuals with the deletion allele (DelDel). While heterozygous individuals (InsDel) are characterized by both bands (163 and 144bp).

The association analysis between this polymorphism and BD is presented in Table 2. The results revealed significant differences in both genotype and allele distributions between cases and controls. The genotypic frequency analysis indicates a significant association before and after adjusting for gender and age (P=0.03 and 0.025, respectively), where the DelDel

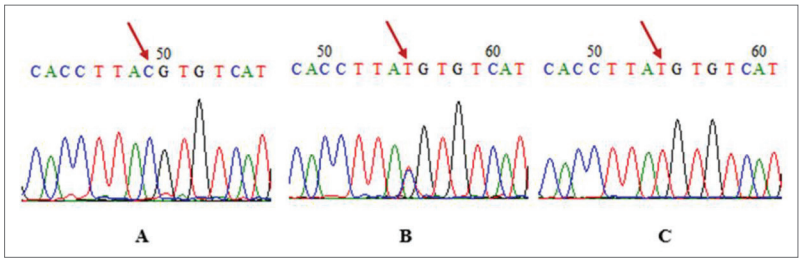

Fig. 1. Direct DNA sequencing results of DAOA rs3918342 polymorphism. **A** – Direct DNA sequencing indicates the presence of 2 copies of the C allele; this patient is homozygote CC. **B** – Direct sequencing indicates the presence of the C allele and the T allele; this patient is heterozygote CT. **C** – Direct DNA sequencing indicates the presence of 2 copies of the T allele; this patient is homozygote TT.

genotype was notably more frequent among cases (29.47%) compared to controls (20.27%), conferring a 2.6-fold increased risk to develop BD compared to the InsIns carriers. Conversely, the frequency of the InsIns genotype was more prevalent in the healthy group (35.14%) than in patients (20%). Allelic frequency analysis also revealed a significant difference between the two groups, with the Del allele being more frequent in cases (54.74%) than in controls (42.57%), supported by a P value of 0.0088.

Results of the genetic model analysis of *DBH* rs72393728 polymorphism are shown in Table 2. In the dominant model, individuals carrying at least one Del allele (InsDel+DelDel) showed a 2.18-fold increased risk of developing BD compared to those with the InsIns genotype, with strong statistical significance (P=0.0095). The log-additive model also demonstrated a highly significant association (P=0.0095), indicating that each additional copy of the Del allele further increased the risk of BD. In contrast, the recessive model did not reach statistical significance (P=0.096), although individuals with the DelDel genotype still showed a 1.61-fold elevated risk.

Genotyping of the *DAOA* rs3918342 polymorphism

The *DAOA* rs3918342 polymorphism displayed genotype frequencies consistent with Hardy-Weinberg equilibrium in both patients and controls (P=0.93 and 0.39, respectively), indicating no significant deviation from the expected distribution. Supplementary Fig. S2 presents the electrophoresis profile illustrating the different genotypes of this polymorphism after applying RFLP. Homozygous (CC) individuals present two bands (356 and 82pb) while homozygous (TT) subjects display a single 440pb band, and heterozygous (CT) individuals are distinguished by the presence of the three bands (440, 356, and 82pb). These results have been confirmed by sequencing for some patients (Fig. 1).

Association analysis results for the *DAOA* rs3918342 polymorphism (Table 2) globally showed no evidence for any statistically significant differences between patients and healthy participants. The genotypic frequency analysis indicated that the CC genotype was higher in controls (29.73%) than in cases (23.16%). However, it did not achieve statistical significance. Similarly, the allele frequencies did not differ between the two groups (P=0.3). Moreover, when we applied genetic model analyses, no significant association was found under the dominant (P=0.27), recessive (P=0.6), or log-additive (P=0.33) models.

Comparison between type 1 and type 2 BD

Table 3 summarizes the comparison of the association analysis between the two types of BD in patients and controls. Results are presented after adjustment for gender and age. Analysis of *DBH* rs72393728 polymorphism revealed a significant association with type 1 BD (P=0.015). Particularly, the DelDel genotype was found to be more frequent among type 1 BD patients (31.5%) than controls (20.3%), with a 3.1-fold higher risk of developing BD. Conversely, the InsIns genotype showed a significantly lower frequency in type 1 BD patients (17.8%) when compared to healthy subjects (35.1%). We did not observe a significant difference between type 2 BD patients and controls (P=0.79). Despite the higher frequency of the InsIns genotype that was observed in controls (35.1%) compared to type 2 BD patients (27.3%), there was no statistical significance obtained. *DBH* rs72393728 polymorphism showed a significant association with BD, and more interestingly, it was found to be associated with type 1 BD but not with type 2 BD.

There were notable differences in the genotype distributions of the *DAOA* rs3918342 polymorphism. The CT genotype was more frequent in controls (46%)

Table 3. Genotypic distribution of *DBH* rs72393728 and *DAOA* rs3918342 in type 1 and type 2 BD

Polymorphism	Genotype	Controls N (%)	BD Type1 N (%)	OR (95% CI)	P Type1 VS Controls	BD Type2 N (%)	OR (95% CI)	P Type2 VS Controls	BD N (%)	OR (95% CI)	P BD VS Controls
***DBH* rs72393728**	InsIns	52 (35.1)	13 (17.8)	1.00		6 (27.3)	1.00		19 (20)	1.00	
	InsDel	66 (44.6)	37 (50.7)	2.25 (1.08-4.66)	**0.015**	11 (50)	1.44 (0.550-4.17)	0.79	48 (50.5)	2.00 (1.05-3.81)	**0.025**
	DelDel	30 (20.3)	23 (31.5)	3.10 (1.37-7.01)		5 (22.7)	1.34 (0.37-4.81)		28 (29.5)	2.60 (1.24-5.44)	
***DAOA* rs3918342**	CC	44 (29.7)	14 (19.2)	1.00		8 (36.4)	1.00		22 (23.2)	1.00	
	CT	68 (46)	41 (56.2)	1.88 (0.92-3.85)	0.21	6 (27.3)	0.48 (0.16-1.49)	0.24	47 (49.5)	1.37 (0.73-2.59)	0.54
	TT	36 (24.3)	18 (24.7)	1.61 (0.70-3.70)		8 (36.4)	1.17 (0.40-3.46)		26 (27.4)	1.43 (0.70-2.95)	

N – number of subjects, OR – odds ratio, CI – confidence interval

than in type 2 BD patients (27.3%), while it was more prevalent in type 1 BD patients (56.2%) than in controls (27.3%). Nevertheless, there was no significant association found between patients and healthy individuals in both types of BD (P=0.21 for type 1 and P=0.24 for type 2).

Association analysis of *DBH* rs72393728 and *DAOA* rs3918342 polymorphisms with clinical variables in patients

Associations between clinical features and the two polymorphisms in the patient group are described in Table 4. Comparison of the genotypic distributions of *DBH* rs72393728 polymorphism with the clinical symptoms of BD did not reveal any substantial associations with most of the clinical variables (P>0.05), except for suicidal ideation, which showed a significant association (P=0.034). Specifically, the DelDel genotype was notably less frequent among patients with suicidal thoughts (14.3%) compared to those without (38.3%).

Regarding *DAOA* rs3918342 polymorphism, we observed a significant association with gender (P=0.016). The CT genotype was more prevalent in males (67.6%) than in females (37.9%), whereas the TT genotype was more frequent in females (34.5%) than in males (16.2%). We also found that grandiose delusions presented a strong significant difference (P=0.0015), with the CT genotype being notably more frequent among patients with this symptom (70%) versus those without (34.5%). For the other clinical variables, no findings of significance were observed.

Association of combined genotypes of *DBH* rs72393728 and *DAOA* rs3918342 polymorphisms with BD

Combined genotype analyses of *DBH* and *DAOA* polymorphisms were performed to assess potential interactions between them. The results shown in Table 5 revealed two important combinations associated with BD. The combination InsIns (*DBH*) – CC (*DAOA*) was significantly more frequent in controls (32.4%) than in BD patients (21.5%), with a P value of 0.0095, indicating a potential protective effect. Conversely, the combination DelDel (*DBH*) and CT (*DAOA*) was more prevalent in patients (39.4%) than in healthy subjects (33.1%), with a P value of 0.0291, showing a significant association with increased risk.

Our case-control study revealed a significant association between *DBH* rs72393728 and BD, with the Del allele as a risk factor. When analyzing each subtype of BD separately, a statistically significant association was detected with only type 1 BD, suggesting a subtype-specific genetic contribution. Additionally, this polymorphism exhibited a significant correlation with suicidal thoughts in BD. However, the *DAOA* rs3918342 polymorphism showed no direct association

Table 4. Association of *DBH* rs72393728 and *DAOA* rs3918342 genotypes with clinical variables in patients

Polymorphism	*DBH* rs72393728			P	*DAOA* rs3918342			P
Genotype	**InsIns**	**InsDel**	**DelDel**		**CC**	**CT**	**TT**	
Gender								
Male N (%)	6 (16.2)	20 (54)	11 (29.7)	0.75	6 (16.2)	25 (67.6)	6 (16.2)	**0.016**
Female N (%)	13 (22.4)	28 (48.3)	17(29.3)		16 (27.6)	22 (37.9)	20 (34.5)	
Age								
Mean	39.74	43.69	42.82	0.529	48.14	41.47	40.12	0.076
(SD)	(11.03)	(13.98)	(13.11)		(11.95)	(13.53)	(12.53)	
Age of onset								
Mean	24.44	27.06	26.71	0.713	26.95	26.02	26.8	0.987
(SD)	(7.54)	(9.66)	(9.29)		(9.83)	(8.73)	(9.64)	
Family history of psychotic disorders				0.1				0.38
Yes N (%)	11 (19)	34 (58.6)	13 (22.4)		15 (25.9)	25 (43.1)	18 (31)	
No N (%)	8 (21.6)	14 (37.8)	15 (40.5)		7 (18.9)	22 (59.5)	8 (21.6)	
Subtype of BD								
Type 1 N (%)	13 (17.3)	37 (50.7)	23 (31.5)	0.61	14 (19.2)	41 (56.2)	18 (24.7)	0.072
Type 2 N (%)	6 (27.3)	11 (50)	5 (22.7)		8 (36.4)	6 (27.3)	8 (36.4)	
Polarity of first episode								
Depression N (%)	8 (17.4)	26 (56.5)	12 (26.1)	0.14	9 (19.6)	19 (41.3)	18 (39.1)	0.25
Mania or hypomania N (%)	11 (23.9)	20 (43.5)	15 (32.6)		11 (23.9)	27 (58.7)	8 (17.4)	
Most recurrent episode								
Depression N (%)	8 (19.1)	22 (52.4)	12 (28.6)	0.76	10 (23.8)	18 (42.9)	14 (33.3)	0.83
Mania or hypomania N (%)	11 (22)	24 (48)	15 (30)		10 (20)	28 (56)	12 (24)	
Grandiose delusions								
Yes N (%)	11 (27.5)	21 (52.5)	8 (20)	0.1	7 (17.5)	28 (70)	5 (12.5)	**0.0015**
No N (%)	8 (14.6)	27 (49.1)	20 (36.4)		15 (27.3)	19 (34.5)	21 (38.2)	
Persecutory delusions								
Yes N (%)	7 (17.9)	20 (51.2)	12 (30.7)	0.91	7 (17.9)	21 (53.8)	11 (28.2)	0.59
No (%)	12 (21.4)	28 (50)	16 (28.6)		15 (26.8)	26 (46.4)	15 (26.8)	
Hallucinations								
Yes N (%)	5 (20.8)	11 (45.8)	8 (33.3)	0.85	3 (12.5)	14 (58.3)	7 (29.2)	0.49
No N (%)	14 (19.7)	37 (52.1)	20 (28.2)		19 (26.8)	33 (46.5)	19 (26.8)	
Suicidal ideation								
Yes N (%)	9 (25.7)	21 (60)	5 (14.3)	**0.034**	6 (17.1)	20 (57.1)	9 (25.7)	0.55
No N (%)	10 (16.7)	27 (45)	23 (38.3)		16 (26.7)	27 (45)	17 (28.3)	
Suicidal attempt								
Yes N (%)	5 (27.8)	11 (61.1)	2 (11.1)	0.11	4 (22.2)	10 (55.6)	4 (22.2)	0.49
No N (%)	14 (18.2)	37 (48)	26 (33.8)		18 (23.4)	37 (48)	22 (28.6)	
Tobacco								
Smokers N (%)	14 (20.6)	34 (50)	20 (29.4)	0.97	18 (26.5)	29 (42.6)	21 30.9)	0.74
Nonsmokers N (%)	5 (18.5)	15 (51.9)	8 (29.6)		4 (14.8)	18 (66.7)	5 (18.5)	

N – number of subjects, SD – standard deviation

with bipolar pathology, even though it was found to be strongly related to grandiose delusions in BD. On the other hand, the combined genotypes InsIns (*DBH*) and CC (*DAOA*), and DelDel (*DBH*) and CT (*DAOA*) are associated with bipolar illness.

DISCUSSION

BD is a chronic psychiatric illness defined by the presence of recurrent episodes of mania and depression [1]. Being a complex and multifactorial pathology, the implication of genetic factors alongside environmental ones in its etiology is well established [1]. Multiple potential candidate genes were related to neurotransmitter

Table 5. Combined genotypes of *DBH* rs72393728 and *DAOA* rs3918342 in cases and controls

Genotypes	Patients (N)	Patients (frequency)	Controls (N)	Controls (frequency)	X^2(df)	P
InsIns(DBH) CC(DAOA)	41	0.215	96	0.324	6.72(1)	**0.0095**
InsIns(DBH) CT(DAOA)	66	0.347	120	0.405	1.65(1)	0.1994
InsIns(DBH) TT(DAOA)	45	0.236	88	0.297	2.12(1)	0.1451
InsDel(DBH) CC(DAOA)	70	0.368	110	0.371	0.0051(1)	0.9432
InsDel(DBH) CT(DAOA)	95	0.5	134	0.452	1.04(1)	0.3086
InsDel(DBH) TT(DAOA)	74	0.389	102	0.344	1.0069(1)	0.3156
DelDel(DBH) CC(DAOA)	50	0.263	74	0.25	0.10(1)	0.7457
DelDel(DBH) CT(DAOA)	75	0.394	98	0.331	4.74(1)	**0.0291**
DelDel(DBH) TT(DAOA)	54	0.284	66	0.222	2.33(1)	0.127

N – number of subjects, X2 – chi-square statistic, df – degrees of freedom

alterations [11]. Specifically, dopamine and glutamate neurotransmission have been identified as key factors in the pathophysiology of BD [3,23], and both systems have been extensively studied [12,13]. In the present study, we aimed to assess a potential association of *DBH* rs72393728 and *DAOA* rs3918342 genes, which are members of the dopaminergic and glutamatergic systems, respectively, with BD in an Algerian population.

Various *DBH* polymorphisms have been explored for their association with BD. In this study, we investigated the implication of the 19bp InsDel variant of *DBH* rs72393728. Our results demonstrated a significant association of this polymorphism with BD risk. The Del allele, which was more common among patients, showed a substantial correlation with the pathology. This association suggests that this allele may serve as a susceptibility factor influencing the risk of developing BD in our cohort. Additionally, the genetic models revealed a significant effect of the Del allele under both dominant and log-additive models, indicating that carrying a single copy of this allele increases the risk of BD, with each additional copy further elevating the risk. However, this association was observed only in type 1 BD and not in type 2. To the best of our knowledge, this is the first study to examine the *DBH* 19 bp InsDel variant in the Algerian population and the first to identify an association between this polymorphism and susceptibility to bipolar disorder in the literature. A single study by Zebarjad et al. on the Iranian population explored the implication of the *DBH* 19 pb InsDel polymorphism rs72393728 in the risk of type 1 BD [6]. In contrast to our findings, their results did not support any association between the studied polymorphism and the risk of type 1 BD; in fact, allele frequencies were similar between cases and unaffected controls [6]. Other DBH variants have been examined for their association with BD in British, German, and Turkish populations [35,36,26]; among these, only the Turkish study identified a significant association with rs6271 [26], while no associations were found for the other polymorphisms in the British and German cohorts [35,36]. BD shares overlapping clinical symptoms with other psychiatric disorders, such as major depressive disorder (MDD) and schizophrenia (SCZ), especially with MDD during depressive episodes [15,16]. Due to these symptomatic similarities, it is hypothesized that these three pathologies may share common genetic predisposition variants [2]. Thus, the involvement of *DBH* has been investigated through association studies not only with BD, but also with MDD and SCZ. An association of *DBH* rs72393728 polymorphism with MDD was detected in Danish and Chinese populations [37,38], highlighting the effect of the Del allele as a risk factor. Nevertheless, no association was registered with SCZ [18,39,40], including a recent study involving the Algerian population [27].

From a neurobiological perspective, the balance between dopamine (DA) and noradrenaline (NA) plays a crucial role in both emotion regulation and cognitive function, mediated by the DBH enzyme that catalyzes the conversion of DA to NA [14,15]. The rate of DBH protein production is tightly genetically controlled [19]. Indeed, variants of the *DBH* gene, including the 19 pb InsDel, are known to be significantly related to DBH enzymatic activity [16,19,41]. Specifically, the Ins allele of *DBH* rs72393728 is associated with higher plasma activity [14], whereas the Del allele is associated with lower plasma activity of DBH [41,42]. In fact, the Del allele has been linked to reduced promoter activity, leading to decreased enzymatic activity of DBH in the brain, resulting in less efficient conversion of DA to

NA [19]. The disruption in the DA/NA ratio caused by the Del allele effect is involved with depression [37].

Research has shown a strong correlation between dysregulation of DBH enzyme activity and the mood phases of bipolar disorder, with elevated plasma DBH activity observed in manic episodes and reduced activity during depressive episodes [16,23]. It has also been reported that plasmatic variations in DBH activity may be associated with the psychotic symptoms in SCZ [43]. Consequently, an altered enzymatic activity of DBH was related to mental disorders, and a low plasma DBH activity is considered a risk factor [16,37,44]. In this context, the *DBH* gene may serve as a marker for common psychiatric symptoms [19,37].

Herein, the *DBH* rs72393728 polymorphism was found to be associated with suicidal ideations in BD patients. Indeed, we registered that patients who reported suicidal thoughts differed significantly from those who did not in terms of their genotype. However, suicidal attempts did not show any statistical relevance. Very few studies have considered analyzing the relationship between the *DBH* gene and suicidal behavior in BD and other psychiatric disorders. One study examined the 19 bp InsDel polymorphism of *DBH* rs72393728 in relation to suicide attempts among schizophrenic patients. The findings reported no correlation [42].

The *DAOA* gene has elicited considerable scientific interest, not only due to its implication in the glutamate system, which has a crucial role in excitatory neurotransmission in the brain [13,23], but also due to its location on chromosome 13q34, a region strongly associated with psychiatric disorders (13q34), particularly BD and SCZ [23,39]. The *DAOA* rs3918342 polymorphism is one of the most commonly investigated for its involvement in the pathophysiology of BD [46]. However, results are conflicting across studies [22,46].

Our results showed no significant association between the *DAOA* M23 polymorphism and BD, as neither genotype nor allele distributions differed significantly between patients and healthy controls. The same results were obtained when applying genetic models (all P values >0.05). Consistent with our findings, two studies conducted in Chinese populations [5,46], as well as studies in Canadian [47] and German [48] populations, reported no association between the *DAOA* (rs3918342) polymorphism and BD, suggesting that this variant does not contribute to susceptibility to the illness. Conversely, the results of other studies carried out on British [29] and Iranian [22] populations demonstrated the presence of a significant association of M23 with BD, with the T allele being frequently more common in patients than in controls [22,29], suggesting that it may be a risk factor for susceptibility to BD.

In another exploratory study of the German population [45], M23 polymorphism was related to BD patients with psychotic symptoms, particularly those with persecutory delusions. The C allele was more prevalent in these patients, while patients with no psychotic signs showed a similar genotypic distribution as the control group. In the same study conducted in a Polish population, identical results were obtained [45].

In addition to its individual role, *DAOA* has also been studied in combination with the adjacent *G30* gene in haplotype analyses for their association with BD and SCZ [49,50]. *DAOA*/*G30* haplotypes have been reported to be associated with psychotic features and mood alterations involving delusions in Romanian patients with BD [51,52], suggesting that these haplotypes may influence affective psychosis without directly contributing to the pathogenesis of BD [52]. Our findings align with these results regarding M23 and psychotic features. In our cohort, the association involved the subgroup with grandiose delusions compared to patients without this psychotic symptom. Accordingly, based on the findings from our study and the aforementioned research, the *DAOA* rs3918342 polymorphism may not be directly associated with susceptibility to developing bipolar disorder. However, it may be linked to psychotic features of the illness.

Neurobiological evidence may offer a clearer understanding of the involvement of the *DAOA* gene and the glutamatergic system in the pathophysiology of bipolar disorder. Glutamate signaling dysfunction plays a critical role in the etiopathogenesis of BD [10], as reflected by elevated glutamate levels observed in both the plasma of BD patients [23] and in postmortem brain tissue samples [23,53]. A meta-analysis also reported that brain levels of glutamate and glutamine were increased in bipolar patients [54]. Alves and colleagues, in their review, reported that elevated glutamatergic function was related to depression [52]. Imaging genetics approaches have potentially related

genetics to structural brain phenotypes. Indeed, alterations in the *DAOA* gene have been associated with reductions in gray matter volume [11]. Another study conducted by Zuliani et al. revealed that M23 polymorphism of the *DAOA* gene may influence structural brain abnormality, where the TT genotype was found to be associated with temporal pole and amygdala gray matter reduction in BD patients [55].

A possible interaction between *DBH* and *DAOA* genes was revealed in our findings of combined genotypes. The genotype InsIns (*DBH*) and CC (*DAOA*) was more frequent among unaffected subjects, which indicates a potential protective effect against BD for individuals carrying this combination, while the DelDel (*DBH*) and CT (*DAOA*) genotypes were more common in patients, suggesting its involvement in BD susceptibility as a risk factor. It has been demonstrated that psychosis may be caused by an interaction between dopamine and glutamate systems, where increased dopamine function may be linked to glutamatergic dysfunction in psychotic disorders [56]. These findings have substantiated this hypothesis, although further research on this interaction is necessary to elucidate the mechanism of action between these two systems.

Our study had certain limitations that should be acknowledged. The primary limitation was the relatively small sample size, which, although sufficient to detect meaningful associations, may increase the risk of both false-positive and false-negative results. Secondly, the scarcity of prior research on *DBH* and BD limited the comparison of our results with existing data. Therefore, to corroborate the observed findings, the reproducibility of the results must be validated in further research involving larger samples and diverse demographics. It is also recommended to expand the genetic analysis by investigating a wider range of polymorphisms within the dopaminergic and glutamatergic pathways.

In conclusion, this study provides novel insights into the involvement of the *DBH* rs72393728 polymorphism in bipolar disorder, identifying the Del allele as a risk factor for susceptibility in the northeastern Algerian population. Conversely, the *DAOA* rs3918342 polymorphism did not show a direct association with BD, while findings revealed a significant association with grandiose delusions in our cohort. Nonetheless, our results suggest a potential interaction between these two polymorphisms. This study highlights the genetic diversity of bipolar disorder and the distinct pathways that may contribute to its etiology, underscoring the need for further research to elucidate the precise mechanisms underlying these associations.

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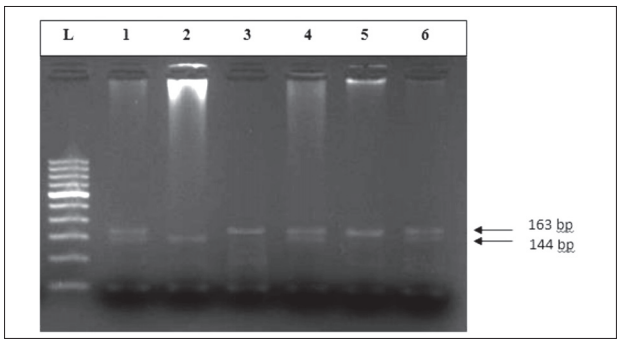
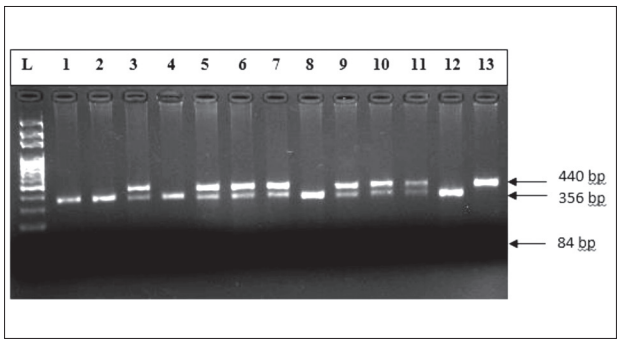
SUPPLEMENTARY MATERIALS



Supplementary Fig. S1. Electrophoresis profile of *DBH* rs72393728 by PCR. Lane L – ladder (50 bp); lanes 1, 4, and 6 are heterozygotes (InsDel); lane 2 is homozygote (DelDel); lanes 3 and 5 are homozygotes (InsIns).



Supplementary Fig. S2. Electrophoresis profile of *DAOA* rs3918342 by PCR-RFLP. Lane L – ladder 100 bp; lanes 1, 2, 4, 8, and 12 are homozygotes (CC); lanes 3, 5, 6, 7, 9, 10, and 11 are heterozygotes (CT); lane 13 is homozygote (TT).

ONLINE SUPPLEMENTARY RESEARCH DATASET

The raw data underlying this article is available as an online supplementary research dataset:
https://www.serbiosoc.org.rs/NewUploads/Uploads/Bernou%20et%20al_11120-Dataset.pdf