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Association of dopamine receptor D2 -141C insertion/deletion and dopamine beta-hydroxylase 19 bp insertion/deletion polymorphisms with schizophrenia: A case-control study in the eastern Algerian population

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Abstract: Numerous studies emphasize genetic contributions to schizophrenia, particularly focusing on genes coding for proteins in the dopaminergic pathway, which are extensively studied for their involvement in the disorder's pathophysiology. This investigation aimed to examine the potential association between the dopamine receptor D2 (*DRD2*) -141C insertion/deletion (rs1799732) and the dopamine beta-hydroxylase (*DBH*) 19 bp insertion/deletion (rs72393728) polymorphisms with schizophrenia in an eastern Algerian population. A case-control study was conducted, involving 145 patients and 146 healthy controls. DNA samples were extracted from peripheral blood cells using the salting out technique. Genotyping for the *DRD2* rs1799732 polymorphism was performed using the PCR-RFLP method, while the *DBH* rs72393728 polymorphism was analyzed using the PCR method. The results revealed a significant association between the *DRD2* rs1799732 polymorphism and schizophrenia, evidenced by significant differences in genotypic and allelic distributions between patients and controls (P=0.001 and P=0.001, respectively). However, no statistical differences were found for the *DBH* rs72393728 polymorphism between patients and controls for genotype (P=0.46) or allele frequencies (P=0.73). This study supports an association between *DRD2* rs1799732 polymorphism and schizophrenia in this population while finding no such association with *DBH* rs72393728 polymorphism. However, there may be a potential interaction between both polymorphisms.

Keywords: dopamine, genetic polymorphisms, *DRD2*, *DBH*, schizophrenia

INTRODUCTION

Schizophrenia (SCZ) is a chronic and recurrent psychiatric disorder characterized by multiple symptoms, including psychosis, cognitive deficits, and impairments in social and emotional functioning. The likelihood of developing schizophrenia is about 1% in the general population [1,2]. It has been shown that in Algeria there are more than 400,000 people affected by schizophrenia, with a prevalence of 1.83% [3].

Although the precise etiology of this disorder remains unclear, research indicates that its inheritance mechanism is likely polygenic and multifactorial. Numerous studies have highlighted the key role of genetics in the genesis of SCZ [4]. A prominent focus of these studies was on genes encoding proteins within the dopaminergic pathway due to their implication in the disorder's pathophysiology [5]. Clinical trials demonstrated that dopaminergic agonists and stimulants, such as cocaine and amphetamine, could induce psychotic symptoms in healthy subjects and exacerbate psychosis in patients with SCZ. Furthermore, the potency of antipsychotic drugs has been directly correlated to their affinity for dopamine receptors, connecting molecular activity to clinical manifestation [6]. Notably, the dopamine receptor D2 (*DRD2*)

and dopamine beta-hydroxylase (*DBH*) genes have emerged as potential candidates for understanding the genetic basis of schizophrenia.

The DRD2 is a seven trans-membrane G protein-linked receptor that functions as an autoreceptor on dopaminergic cell bodies and is a primary target for antipsychotic drugs[5,7]. Studies have revealed elevated binding density of the D2 receptor in the brains of patients with SCZ [8]. Located on chromosome 11q22-23, the *DRD2* gene contains eight exons spanning approximately 270 kb [9]. Due to its functional and positional attributes, this gene has emerged as a promising candidate risk gene for schizophrenia, a notion supported by a meta-analysis conducted by Jonsson et al. [10]. The involvement of this gene may be directly linked to functional polymorphisms that influence receptor protein expression. One of these is the insertion/deletion of a cytosine at nucleotide position -141 of the 5' promoter region of the gene (-141C Ins/Del, rs1799732) [11,12]. Some *in vitro* studies show that this molecular aberration alters gene expression by decreasing promoter activity [13].

Many investigations have attempted to establish correlations between *DRD2* polymorphisms and schizophrenia. Despite these efforts, the outcomes of association studies have shown a lack of uniformity within ethnic groups. Studies involving various populations have indicated a connection between the *DRD2* rs1799732 variant and SCZ [6,13-16]; however, this relationship has not demonstrated consistent replication across all studies and diverse population samples [17-19].

DBH is an intracellular enzyme that catalyzes the conversion of dopamine to noradrenaline [20]. The *DBH* gene is located on chromosome 9p34 and spans 23 kb containing 12 exons [21]. The protein level of DBH is strongly genetically controlled and is significantly correlated with its activity in the plasma and cerebrospinal fluid (CSF) [22,23]. Low DBH activity has been suggested as a biological marker of psychiatric diseases, including schizophrenia [21]. Cubells et al., followed by others, demonstrated that a 19 pb insertion/deletion polymorphism (rs72393728) located 4.5 upstream of the transcriptional start site of the *DBH* gene was highly associated with DBH enzymatic activity [21,24]. It has also been reported that the minor allele (Del allele) is linked to diminished promoter activity, leading to lower DBH levels and enzyme activity in the plasma and CSF [21,25].

This case-control study aimed to investigate the functional polymorphisms in *DRD2* and *DBH* as possible risk factors for schizophrenia in an eastern Algerian population. To the best of our knowledge, this is the first study to be conducted in our population.

MATERIALS AND METHODS

Ethics statement

All procedures performed in this study followed the declaration of Helsinki. The Ethics Committee of the Dr. Benbadis University Hospital Center of Constantine approved this study (Reference Number: CE/CHUC/05/11-2023). All patients and control subjects provided written informed consent for participation in the study.

Study population

The patients diagnosed with schizophrenia were recruited from the Psychiatric Hospital of Constantine, Algeria. A clinical interview conducted by a psychiatrist, according to the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders IV) criteria [26], led to the diagnosis of 145 individuals with schizophrenia. Comprehensive medical records, detailing each patient's medical history, age, gender, and age of onset, were collected for analysis. The severity of symptoms was evaluated using the positive and negative syndrome scale (PANSS) [27]. Patients with other psychiatric disorders or mental retardation were excluded from the study. The control group comprising 146 healthy volunteers was from the same geographical region as the patients. The control subjects matched the patients in terms of age and gender and did not have a positive personal or familial history of major psychiatric disorders or psychotic medication.

DNA extraction

Blood samples (8 mL) were collected into EDTA-containing tubes, and each tube was designated with a unique identification code. Genomic DNA was

extracted by the salting-out technique [28], and quality was checked with Nanodrop (Thermo Scientific, NanoDrop 8000).

Genotyping of the *DRD2* rs1799732 polymorphism

Genotyping of *DRD2* rs1799732 was determined with the PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method. The following pair of primers was used for amplification: Forward: 5'-ACTGGCGAGCAGACGGTGAGGACCC-3' and reverse: 5'-TGCGCGCGTGAGGCTGCCGGTTCGG-3' [13,14]. The reaction volume was 25 μL containing 50 ng genomic DNA, 0.2 μM of each primer, 1x PCR buffer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase (Solis BioDyne, Estonia), and 5% DMSO. The PCR cycling conditions were set as follows: 94˚C for 5 min, 35 cycles of 94˚C for 30 s, 68˚C for 40 s, and 72˚C for 40 s, with a final extension step at 72˚C for 7 min. The PCR products were subjected to electrophoresis on a 3% agarose gel (Sigma-Aldrich, USA) and then visualized with the Gel Doc XR+ Gel Documentation System (Bio-Rad, USA) after staining with GelRed (GelRed Merck, Germany). Then, after confirmed amplification, PCR products of 303 bp were digested with the *BstN*I restriction enzyme (New England Biolabs, USA). The RFLP was performed two times to confirm the *DRD2* rs1799732 polymorphism results in both the homozygous and heterozygous samples. Subsequently, a random selection of PCR products was subjected to direct sequencing using an ABI3500xl genetic analyzer and a BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, USA).

Genotyping of the *DBH* rs72393728 polymorphism

Genotyping of *DBH* rs72393728 was performed by PCR. The primers: forward – 5'-GCAAAAGTCAGGCACATGCACC-3', and reverse – 5'-CAATAATTTGGCCTCAATCTTGG-3' [29] were used for amplification. The reaction volume was 25 μL containing 50 ng genomic DNA, 0.2 μM of each primer, 1×PCR buffer, 0.2 mM dNTP,1.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (Solis-BioDyne, Estonia). The PCR cycling conditions were set as follows: 94˚C for 5 min, 30 cycles of 94˚C for 30 s, 55˚C for 45 s, and 72˚C for 30 s, with a final extension step at 72˚C for 7 min. The PCR products were subjected to electrophoresis on a 3% agarose gel (Sigma-Aldrich, USA) and then visualized with the Gel Doc XR+ Gel Documentation System (Bio-Rad, USA) after staining with GelRed (GelRed Merck, Germany).

Statistical analysis

Allele and genotype frequencies were calculated and compared between the patients and healthy subjects using the standard Chi-square test and logistic regression analysis. $P<0.05$ was considered statistically significant. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to estimate the effect of different alleles. The Hardy-Weinberg equilibrium was tested using the Chi-square goodness of fit test. The normal distribution was verified, and continuous variables were compared using ANOVA. All statistical analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, Illinois, USA). Pairwise Fst (fixation index) statistics were calculated using PLINK 1.9 software [30].

RESULTS

Characteristics of the study population

The demographic features of each group are provided in Table 1. A cohort of 145 patients and 146 control subjects had an average age of 40.07±10.43 and 37.9±10.72 years, respectively. The patient group exhibited a predominant male representation, accounting for 77.93% (n=113), with a male-to-female (M/F) sex ratio of 3.53. Likewise, the control group had a male frequency of 79.45% (n=116), with a M/F sex ratio of 3.86. There was no significant difference between patients and control subjects regarding gender ($P=0.75$) or age ($P=0.08$). Compared to the control group, schizophrenic patients showed a significantly higher frequency of smoking habits ($P<0.001$).

Genotype and allele frequencies of *DRD2* rs1799732 polymorphism

The genotype frequencies of the *DRD2* rs1799732 were in Hardy-Weinberg equilibrium in both patient and control groups ($P>0.05$), indicating no deviation

Table 1. Clinical features of patients and the control group

Demographic features	Patients (N=145)	Controls (N=146)	P
Gender Male N (%) Female N (%)	113 (77.93) 32 (22.07)	116 (79.45) 30 (20.55)	0.75
Age (years) Mean (SD)	40.07 (10.43)	37.9 (10.72)	0.08
Age of onset (years) Mean (SD)	24.55 (6.89)		
Positive family history N (%)	53(39.26)		
Tobacco Smokers N (%) Nonsmokers N (%)	83 (57.24) 55 (37.93)	51 (34.93) 87 (59.89)	**0.0001**
Positive symptoms Mean (SD)	31.26 (9.25)		
Delusion Mean (SD)	5.06 (1.85)		
Conceptual disorganization Mean (SD)	4.63 (1.73)		
Hallucinatory Behavior Mean (SD)	5.11 (1.48)		
Negative symptoms Mean (SD)	23.11 (8.92)		
General psychopathology score Mean (SD)	44.55 (10.87)		

N – Number of subjects, SD – standard deviation

from expected distribution. Fig. 1 shows the electrophoresis profile corresponding to different genotypes of the *DRD2* rs1799732. Homozygous individuals with the insertion genotype (InsIns) exhibit two distinct bands at 160 and 144 bp, whereas those with the deletion genotype (DelDel) exhibit a single band at 303 bp. Heterozygous subjects are characterized by the presence of three bands at 303, 160, and 144 bp. The sequencing results are shown in Fig. 2.

The genotype and allele frequencies of both the patient and healthy groups are presented in Table 2. A comparison of genotype frequencies between patients and controls revealed a highly significant difference (P=0.001). Specifically, the frequency of the DelDel genotype was significantly elevated in patients (12.41%) compared to controls (2.05%), whereas the frequency of the InsIns genotype was higher in the control group (58.22%) than in patients (42.76%). Moreover, the distribution of alleles between patient and control groups showed a significant difference (P=0.001), with

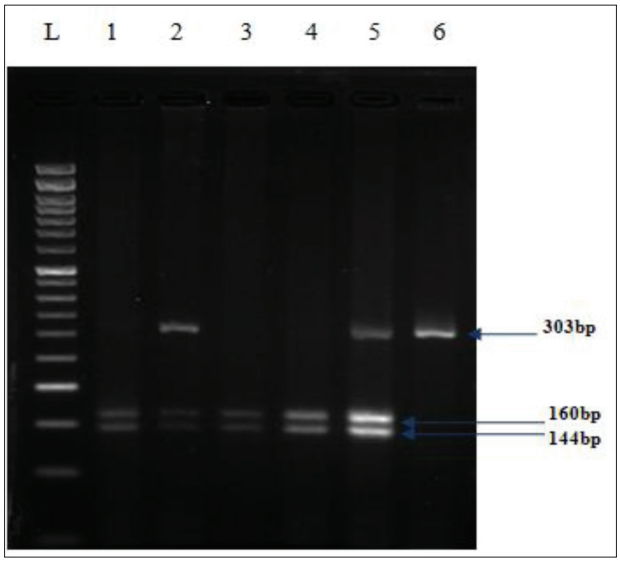

Fig. 1 Electrophoretic profile of DNA fragments resulting from PCR-RFLP for the *DRD2* rs1799732 polymorphism. The fragments amplified by PCR were digested with *Bst*NI; Lane L – Ladder (50 bp); lanes 1, 3, and 4 are homozygotes (InsIns); lanes 2 and 5 are heterozygotes (InsDel); lane 6 is homozygote (DelDel).

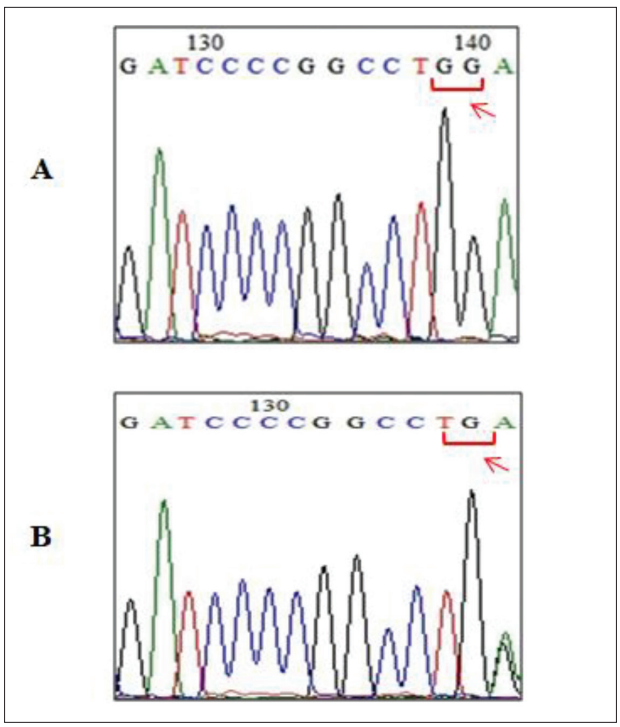

Fig. 2. Results of direct DNA sequence analysis of the *DRD2* rs1799732 polymorphism using the reverse PCR primer. **A** – Direct DNA sequencing revealed that this patient is homozygote (InsIns,) with processing a copy of the G nucleotide at the position -141 of the promoter region. **B** – Direct DNA sequencing revealed that this patient has a homozygous deletion (DelDel), characterized by the absence of the G nucleotide at position -141 of the promoter region.

Table 2. Genotype, allele frequencies, and genetic models of *DRD2* rs1799732 in patient and control groups

DRD2 rs1799732	Patients N (%)	Controls N (%)	P	OR (95%CI)
Genotype				
InsIns	62(42.76)	85(58.22)		
InsDel	65(44.82)	58(39.73)	**0.001**	2.91 (1.65-5.14)
DelDel	18(12.41)	3(2.05)		
Allele				
Ins	189(65.17)	228(78.08)	**0.001**	1.9 (1.32-2.75)
Del	101(34.83)	64(21.92)		
Additive model 1 InsDel vs. InsIns	65(44.82)	58(39.73)	0.128	1.65 (0.87-3.14)
Additive model 2 DelDel vs. InsIns	18(12.41)	3(2.05)	**0.042**	6.93 (1.07-44.71)
Dominant model InsDel+DelDel vs InsIns	83(57.24)	61(41.78)	**0.01**	1.87 (1.17-2.97)
Recessive model DelDel vs. InsIns+InsDel	127(87.59)	143(97.95)	**0.001**	6.76 (1.94-23.47)

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

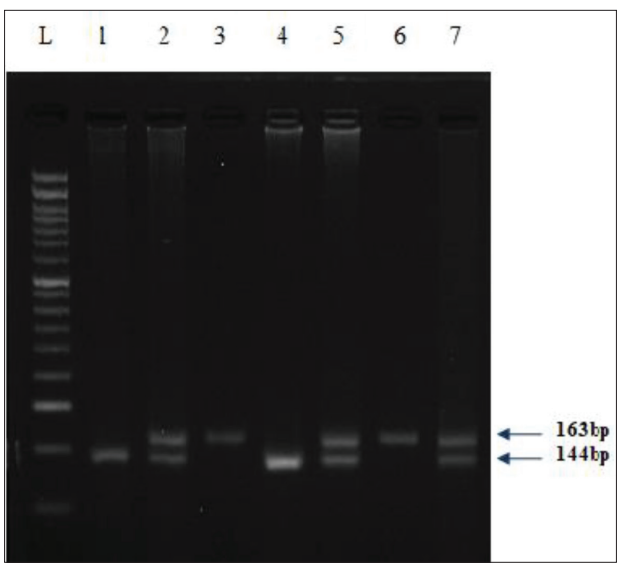

Fig. 3 Electrophoretic profile of PCR products of *DBH* rs72393728 polymorphism. Lane L – Ladder (50bp); lanes 1 and 4 are homozygotes (DelDel); lanes 2, 5, and 7 are heterozygotes (InsDel); lanes 3 and 6 are homozygotes (InsIns).

the Del allele being more common in patients (34.83%) than in controls (21.92%).

Genetic models have been applied for the *DRD2* rs1799732, and results are given in Table 2. When applying the additive model, subjects carrying the DelDel genotype had a 6.93-fold higher risk of developing SCZ compared to those with the InsIns genotype, with a statistically significant result (P=0.042). However, individuals with the InsDel genotype showed a 1.65-fold higher risk of developing SCZ compared to those with the InsIns genotype; this result was not statistically significant (P>0.05). In the dominant model, subjects with InsDel+DelDel genotypes had a 1.87-fold increased risk of developing SCZ, with a statistically significant result (P= 0.01). For the recessive model, the DelDel genotype carriers had a 6.76-fold higher risk of developing SCZ compared to patients with InsDel+InsIns genotypes, with a statistically significant result (P= 0.0007).

Genotype and allele frequencies of *DBH* rs72393728 polymorphism

The genotype frequencies of the DBH rs72393728 polymorphism were consistent with Hardy-Weinberg equilibrium in both patient and control groups (P>0.05), indicating no significant deviation from the expected distribution. The electrophoresis profile corresponding to different genotypes of the *DBH* 19bp Ins/Del is represented in Fig. 3. Homozygous subjects with the insertion genotype (InsIns) show a band at 163 bp, whereas those with the deletion genotype (DelDel) exhibit a band at 144 bp. Heterozygous subjects are detected by the presence of two bands at 163 and 144 bp.

The genotype and allele frequencies of both patient and control groups are presented in Table 3. When patient and control subjects were compared, no significant differences between genotype and allele frequencies were observed (P=0.46 and P=0.73, respectively). After applying the additive model, subjects with the InsDel genotype showed a 0.58-fold lower risk of developing SCZ compared to the InsIns genotype. In contrast, DelDel genotype carriers exhibited a 1.13-fold higher risk of developing SCZ. However, in both additive

Table 3. Genotype, allele frequencies, and genetic models of *DBH* rs72393728 in patient and control groups

***DBH* rs 72393728**	**Patients N (%)**	**Controls N (%)**	**P**	**OR (95%CI)**
Genotype				
InsIns	33 (22.76)	30 (20.55)		
InsDel	66 (45.52)	77 (52.74)	0.46	1.28 (0.94-1.75)
DelDel	46 (31.72)	39 (26.71)		
Allele				
Ins	132 (45.52)	137 (46.92)	0.73	1.06 (0.76-1.47)
Del	158 (54.48)	155 (53.08)		
Additive model 1 InsDel vs. InsIns	66 (45.52)	77 (52.74)	0.14	0.58 (0.27-1.19)
Additive model 2 DelDel vs. InsIns	46 (31.72)	39 (26.71)	0.79	1.13 (0.47-2.73)
Dominant model InsDel+DelDel vs InsIns	110 (75.86)	116 (79.45)	0.65	0.88 (0.50-1.53)
Recessive model DelDel vs. InsIns+InsDel	99 (68.28)	107 (73.29)	0.35	1.27 (0.77-2.12)

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

models, the results were not statistically significant (P>0.05). In the dominant model, individuals carrying InsDel+DelDel genotypes had a 0.88-fold lower risk of developing SCZ compared to those with the InsIns genotype, with a non-statistically significant result (P>0.05). In the recessive model, subjects with the DelDel genotype had a 1.27-fold higher risk of developing SCZ than those carrying InsIns+InsDel genotypes, but the result was not statistically significant (P>0.05).

Table 4 summarizes the combined genotype analysis of *DRD2* rs1799732 and *DBH* rs72393728 polymorphisms in patients and controls. The results showed significant differences between patients and controls for some genotype combinations. Indeed, the frequency of the combined genotype (InsIns (*DRD2*)-InsDel (*DBH*)) was higher in controls (55.4%) compared to patients (44.1%), at P=0.006. The second combination exhibiting a significant difference between patients and controls (P=0.03) was the (DelDel (*DRD2*)-InsIns (*DBH*)) genotype with an elevated frequency in patients (17.5%) compared to controls (11.3%). Similarly, the combined genotype (DelDel (*DRD2*)-DelDel (*DBH*)) was significantly associated with SCZ (P=0.03), with a higher frequency observed in patients (21.3%) than in healthy subjects (14.3%). For the remaining genotype combinations, no significant differences were observed (P>0.05).

The genotype frequencies of the examined polymorphisms were compared among schizophrenia patients based on various clinical variables to identify potential correlations and are shown in Supplementary Table S1. Differences in the distribution of *DRD2* rs1799732 genotypes were observed concerning gender (X^2(df)=9.08(2), P=0.01) among patients. Specifically, the InsIns genotype was predominant in males (48.67%), while in females, the InsDel genotype prevailed (68.75%).

Table 5 compares the allele frequencies of the *DRD2* rs1799732 polymorphism in healthy subjects with those of the five populations in the 1000 Genomes Project (African, American, East Asian, European, and South Asian). The findings indicated low genetic differentiation across most populations, except for the African population, where a significant diversity was

Table 4. Distribution of combined *DRD2* rs1799732 and *DBH* rs72393728 genotypes among patient and control subects

Combined Genotypes	**Patients (N)**	**Patients (frequency)**	**Controls (N)**	**Controls (frequency)**	**X^2(df)**	**P**
InsIns(*DRD2*) InsIns(*DBH*)	95	0.327	115	0.393	2.76(1)	0.1
InsIns(*DRD2*) InsDel(*DBH*)	128	0.441	162	0.554	7.47(1)	**0.006**
InsIns(*DRD2*) DelDe(*DBH*)	108	0.372	124	0.424	1.65(1)	0.2
InsDel(*DRD2*) InsIns(*DBH*)	98	0.337	88	0.301	0.89(1)	0.344
InsDel(*DRD2*) InsDel(*DBH*)	131	0.451	135	0.462	0.07(1)	0.8
InsDel(*DRD2*) DelDe(*DBH*)	111	0.382	97	0.332	1.62(1)	0.20
DelDel(*DRD2*) InsIns(*DBH*)	51	0.175	33	0.113	4.65(1)	**0.03**
DelDel(*DRD2*) InsDel(*DBH*)	84	0.289	80	0.273	0.18(1)	0.67
DelDel(*DRD2*) DelDe(*DBH*)	62	0.213	42	0.143	4.84(1)	**0.03**

N – Number of subjects, X^2 – chi-square statistic, df – degrees of freedom.

Table 5. Genetic variability of *DRD2* rs1799732: comparison between healthy controls and major ethnic groups from the 1000 Genomes Project.

SNP ID	Population	N	MA	MAF	Fst Versus Current
rs1799732	**Current study**	146	Del	0.2192	-
	All populations	5008	Del	0.2416	0.0014
	African	1322	Del	0.5703	0.2580
	American	694	Del	0.157	0.0127
	East Asia	1008	Del	0.1369	0.0231
	European	1006	Del	0.0845	0.0704
	South Asia	978	Del	0.127	0.0297

N – Number of subjects, MA – minor allele, MAF – minor allele frequency, Fst – fixation index.

observed (Fst=0.2580). We were unable to calculate the Fst for the *DBH* rs72393728 polymorphism due to the lack of allele frequency data across multiple populations in public genetic databases, such as the 1000 Genomes Project. In this context, this polymorphism is considered novel, as allele frequency information is not currently available.

DISCUSSION

Schizophrenia is a complex, chronic psychiatric disorder characterized by a loss of reality perception. It is now well-established that genetic factors play a significant role in susceptibility to schizophrenia [5,31]. Several genes are considered potential candidates for schizophrenia (SCZ); however, those most extensively studied for their involvement in the disorder's pathophysiology are those that encode proteins within the dopaminergic pathway [5]. Among these, *DRD2* and *DBH* genes are considered promising candidate risk genes. This case-control study aimed to identify a potential association between two functional polymorphisms, the *DRD2* rs1799732 and the *DBH* rs72393728, and SCZ.

DRD2 polymorphisms have gained significant attention, not only due to their functional implications but also because of the gene's important chromosomal position [11]. The *DRD2* rs1799732 variant, located in the *DRD2* promoter region, is considered a potential risk factor owing to its regulatory activity [13]. Numerous studies have been conducted to substantiate the association between this polymorphism and SCZ. Nevertheless, the outcomes have been controversial, exhibiting variations based on ethnic populations. This underscores the need for further investigations across diverse ethnicities [15,32].

In the present study, the findings highlight a significant association between the *DRD2* rs1799732 polymorphism and susceptibility to SCZ. A greater strength of association of the Del allele was revealed, suggesting that the presence of the Del allele significantly increases the risk of developing SCZ. The genetic models consistently demonstrated that both the additive and recessive models showed an elevated risk of SCZ associated with the DelDel genotype compared to both InsIns and InsDel genotypes. Additionally, the dominant model implied that possessing at least one copy of the Del allele is associated with an elevated risk of SCZ.

Our results agree with British [14] and Brazilian [16] studies. Moreover, Sáiz et al. [6] reported that the Del allele was more common in SCZ patients than in controls in the Spanish population. Additionally, a significant association of the Del allele was observed in both dominant and recessive models [6]. Himei et al. [33] demonstrated that the Del allele is an aggravating factor that could influence the positive symptoms of SCZ. In addition, *in vitro* studies reported a decreased promoter activity being associated with the Del allele and demonstrated that the Del allele reduced gene transcription by an average of 68% [13,16]. Moreover, imaging studies found an elevated DRD2 density in the striatum of schizophrenic patients carrying the Del variant. Thus, by altering the transcriptional regulation, the Del allele may impact the dopaminergic neurotransmission, which plays a critical role in the neurodevelopment processes and synaptic plasticity, thereby increasing vulnerability to SCZ [13,6,16].

In contrast, studies conducted in Japanese[13], Swedish [34], Finnish [15], and Chinese [7] populations have described an opposite association. They reported a significantly lower frequency of the Del allele in patients with SCZ than in controls. However, other reports did not register any association between this polymorphism and SCZ [17-19,35].

To our knowledge, this study represents the first investigation in Algeria to explore the potential association between this polymorphic variant and schizophrenia. Our findings corroborate previous reports of

genetic heterogeneity observed across diverse ethnic populations. Indeed, it has been reported that the frequencies of the Del allele among patients were about 11% in Chinese samples [17-19,34], 12% in Japanese samples [13,33,35], and 17% in European Caucasian samples [6,14,15,18,34]. However, in a Brazilian study, the frequency of the Del allele among patients with schizophrenia was notably higher at 81% [16]. In this study, we observed an intermediate frequency of the Del allele in SCZ patients (32%). Breen et al. [14] proposed that the most plausible explanation for these inconsistent results is the linkage disequilibrium involving different alleles in various ethnic groups. Therefore, the Del allele may act as a risk factor in some populations and a protective factor in others.

The Fst was calculated for *DRD2* rs1799732 polymorphism to measure the proportion of genetic variation between different populations. Our results indicated a general similarity across most populations (American, East Asian, European, and South Asian) with values ranging from 0.0014 to 0.0704. However, the highest Fst value was observed in the African population (Fst=0.2580). This value demonstrated a significant diversity and a distinct genetic profile for this variant in the African population compared to other populations.

Regarding the *DBH* rs72393728 polymorphism, no significant differences were found in the genotype and allele distributions between patient and control groups. No significant associations were observed when genetic models were applied. These results align with studies conducted in Canadian [29] and Southeast Iranian populations [36]. Meanwhile, a Chinese study conducted by Zhou et al. [37] aimed to assess a possible association between the *DBH* rs72393728 polymorphism and SCZ, comparing patients with and without tardive dyskinesia (TD) and controls. The results did not reveal any association. However, they suggested a possible implication of this polymorphism in increasing the vulnerability to psychotic symptoms in schizophrenia. Another Chinese study by Hui et al. [38] confirmed the absence of an association between this variant and schizophrenia. Nevertheless, they observed that the frequency of the Del allele was significantly lower in patients with the first episode of schizophrenia, suggesting that this allele could affect vulnerability to first-episode schizophrenia.

Although neither this study nor any other previous study has found a direct significant association between genotype or allele distributions and SCZ, it has been shown that the *DBH* rs72393728 variant is strongly associated with DBH enzymatic activity [21]. It has also been demonstrated that the minor homozygous allele (Del allele) is associated with reduced promoter activity, leading to decreased DBH levels and enzyme activity in the plasma and CSF. Also, it has been suggested that reduced DBH activity is a biological marker of SCZ [21,38].

Our study highlighted important insights into the potential role of both polymorphisms in SCZ in the examined population when their genotypes were combined. Indeed, the higher frequency of the combined genotype (InsIns (*DRD2*)-InsDel (*DBH*)) in controls suggested a potential protective effect against SCZ, whereas the DelDel genotype of *DRD2* rs1799732, when combined with either InsIns or DelDel genotypes of *DBH* rs72393728, appeared to be associated with an increased risk of SCZ. Thus, these findings suggested that these combinations may influence susceptibility to SCZ. However further research is warranted to confirm the association of these polymorphisms and understand their biological significance.

Our study has several limitations. Firstly, the small sample size increases the risk of false-positive and false-negative results. Secondly, the study's focus on a single geographical region may limit its generalizability to other regions, given the genetic diversity of our population. Thirdly, we examined only two polymorphisms, while schizophrenia is a complex disorder influenced by multiple genetic and environmental factors. Therefore, conducting replication studies with larger sample sizes and diverse populations would be of considerable interest. Expanding the genetic analysis and exploring a panel of variations within the dopaminergic pathway is recommended.

In summary, the findings of this study provide evidence supporting the association of the Del allele of the DRD2 rs1799732 polymorphism with susceptibility to schizophrenia (SCZ), indicating its potential role as a genetic risk factor in our population. However, no significant association was observed with the *DBH* rs72393728. Nevertheless, our results suggested a potential interaction between these two polymorphisms.

These results underscore the genetic complexity of schizophrenia (SCZ), emphasizing the necessity for further investigations to elucidate the precise mechanisms underlying these associations. Identifying these genetic markers will help in the early clinical detection of schizophrenia in similar populations.

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Data availability: Data underlying the reported findings have been provided as a raw dataset which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Boukhenaf%20et%20al_Raw%20dataset.pdf

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SUPPLEMENTARY MATERIAL

Supplementary Table S1: Association between *DRD2* rs1799732 and *DBH* rs72393728 genotypes with Clinical Data

Polymorphism	*DRD2* rs1799732			P	*DBH* rs72393728			P
Genotypes	**InsIns**	**InsDel**	**DelDel**		**InsIns**	**InsDel**	**DelDel**	
Gender								
Male N (%)	55(48.67)	44(38.94)	14(12.39)	**0.001**	36 (31.3)	49 (42.61)	30 (26.09)	0.73
Female N (%)	7(21.88)	22(68.75)	3(9.38)		10 (32.26)	15 (48.39)	6 (19.35)	
Age (years) Mean (SD)	39.29 (10.56)	41.03 (10.19)	40.56 (11.05)	0.64	41.87 (9.33)	39.34 (11.29)	37.77 (10.53)	0.24
Age of onset Mean (SD)	24.7 (6.34)	24.41 (7.07)	24 (8.71)	0.94	25.24 (7.59)	23.2 (6.06)	26.46 (7.22)	0.06
Family history of psychotic disorders				0.91				0.69
Yes N (%)	22 (40.74)	26 (48.15)	6 (11.11)		20 (35.71)	25 (44.64)	11 (19.64)	
No N (%)	35 (43.21)	36 (44.44)	10 (12.35)		24 (30)	36 (45)	20 (25)	
Tobacco								
Smokers N (%)	38 (46.34)	32 (39.02)	12 (14.63)	0.17	29 (34.94)	33 (39.76)	21 (25.3)	0.31
Non smokers N (%)	20 (34.48)	32 (55.17)	6 (10.34)		46 (52.87)	15 (17.24)	26 (29.89)	
Positive symptoms PANSS (+)score Mean (SD)	33.04 (7.48)	31.43 (9.95)	24.14 (9.74)	0.09	32.35 (8.66)	32.52 (10.09)	28.86 (8.79)	0.5
Negative symptoms PANSS (-) score Mean (SD)	24.72 (8.25)	22.76 (10.22)	20.85 (3.53)	0.38	24.5 (7.51)	22.4 (8.89)	23.04 (9.96)	0.78
General psychopathology score Mean (SD)	48.36 (9.95)	42.27 (11.23)	44.14 (8.69)	0.11	43.61 (11.07)	45.38 (11.19)	44.62 (10.5)	0.92

N – Number of subjects, SD – standard deviation, PANSS – Positive and Negative Syndrome Scale, Ins – insertion, Del – deletion

Supplementary Table S2: Genotype and Allele Frequencies of *DRD2* rs1799732 Polymorphism in Schizophrenia Patients and Controls from Various Studies.

Reference	Patients	InsIns N (%)	InsDel N (%)	DelDel N (%)	Ins N (%)	Del N (%)	Controls	InsIns N (%)	InsDel N (%)	DelDel N (%)	Ins N (%)	Del N (%)
[13]	**260**	190(73.08)	66 (25.38)	4 (1.54)	446 (85.77)	74 (14.23)	**312**	193 (61.86)	102 (32.69)	17 (5.45)	488 (78.21)	136 (21.79)
[14]	**439**	320 (72.89)	107 (24.37)	12 (2.72)	747 (85.08)	131 (14.92)	**437**	347 (79.41)	83 (18.99)	7 (1.06)	777 (88.90)	97 (11.10)
[34]	**129**	114 (88.37)	15 (11.63)	0	243 (94.19)	15 (5.81)	**179**	142 (77.33)	34 (18.99)	3 (1.68)	318 (88.83)	40 (11.17)
[35]	**241**	162 (67.22)	71 (29.46)	8 (3.32)	395 (81.95)	87 (18.05)	**201**	142 (70.65)	54 (26.87)	5 (2.49)	338 (84.08	64 (15.92)
[33]	**190**	118 (62.11)	69 (36.32)	3 (1.58)	305 (80.62)	75 (19.74)	**103**	71 (68.93)	27 (26.21)	5 (4.85)	169 (82.04)	37 (17.96)
[15]	**93**	86 (92.47)	7 (7.53)	0	179 (96.24)	7 (3.76)	**94**	88 (93.62)	6 (6.38)	0	182 (96.81)	6 (3.19)
[17]	**211**	165 (78.20)	44 (20.85)	2 (0.95)	374 (88.65)	48 (11.37)	**201**	164 (81.59)	34 (16.92)	3 (1.49)	362 (90.05)	40 (9.95)
[18]	**144**	104 (72.22)	37 (25.69)	3 (2.08)	245 (85.07)	43 (14.93)	**142**	120 (84.51)	21 (14.79)	1 (0.70)	261 (91.90)	23 (8.10)
[6]	**272**	181 (66.54)	76 (24.94)	15 (5.51)	438 (80.51)	106 (19.49)	**404**	301 (74.50)	98 (24.26)	5 (1.24)	700 (86.63)	108 (13.37)
[7]	**120**	96 (80.00)	22 (18.33)	2 (1.67)	214 (89.17)	26 (10.83)	**100**	68 (68.00)	28 (28.00)	4 (4.00)	164 (82.00)	36 (18.00)
[19]	**306**	257 (83.99)	34 (11.11)	15 (4.90)	548 (89.54)	64 (10.46)	**324**	275 (84.88)	44 (13.58)	5 (1.54)	594 (91.67)	54 (8.33)
[16]	**144**	9 (6.25)	36 (25.00)	99 (68.75)	54 (18.75)	234 (81.25)	**72**	9 (12.50)	23 (31.94)	40 (55.56)	41 (28.47)	103 (71.53)

N – Number of subjects, Ins – insertion, Del – deletion