Research article

Association of DRD2 gene polymorphisms with schizophrenia in the young Bangladeshi population: A pilot study

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A B S T R A C T

Purpose: DRD2 gene is considered one of the most important candidate genes for the schizophrenia (SCZ) development due to its role in dopamine signaling and no genetic association study has been conducted yet on the Bangladeshi SCZ patients. The objective of the present study was to investigate the association of DRD2 genetic polymorphisms (rs4648317, rs4936270, and rs7131056) with SCZ in the Bangladeshi population.
Patients and methods: This case-control study consisted of 101 SCZ patients and 101 controls. Genotyping was performed by the polymerase chain reaction and restriction fragment length polymorphism (PCR–RFLP) method.
Results: The average ages were 22.15 and 22.09 years in patients and controls, respectively (p > 0.05). CT genotype of rs4936270 showed a significantly higher risk for the development of SCZ compared to CC genotype (OR = 2.0, p = 0.023), whereas no association was found for TT genotype. For the dominant model and T allele, rs4936270 showed a higher risk for the development of SCZ (OR = 2.01, p = 0.020; OR = 1.76, p = 0.021, respectively), while the recessive model had no association with SCZ. A statistically significant (OR = 2.70, p = 0.036) higher risk was found for the AA genotype, but no association was found for GA genotype of rs4648317 SNP compared to GG genotype. In case of dominant and recessive models, rs4648317 showed no association with SCZ. 'A' allele of rs4648317 SNP was found to be significantly associated with the elevated risk of SCZ (OR = 1.50, p = 0.044). No association with SCZ of rs7131056 SNP was found for AC, CC genotypes, dominant, recessive, and allele models. Furthermore, from the haplotyping analysis, we found that CAA and TAA haplotypes of rs4936270, rs7131056 and rs4648317 SNPs are associated with SCZ (χ2 = 8.26, p = 0.004; χ2 = 5.31, p = 0.021, respectively). After Bonferroni correction, the association of SCZ did not withstand with any genotype, allele and haplotype (p < 0.017) except CAA haplotype.
Conclusion: Our results suggest that DRD2 gene polymorphisms may be associated with the susceptibility of SCZ in the young Bangladeshi population.

1. Introduction

Central nervous system (CNS) disorders are considered as the third most common health problem in developed countries, representing 10%–15% of deaths preceded by cardiovascular abnormalities (25%) and cancers (20%) [1, 2]. Among all of these, schizophrenia (SCZ) is one of the most prevalent neuropsychiatric disorders all over the world. SCZ is a typical type of mental disease with an incidence of 1% and a high socio-economic impact in Bangladesh [3, 4]. SCZ and associated problems are quite heritable but cannot be defined by way of presently acknowledged genetic threat elements. SCZ has a heritability anticipated at 70%–90% [1, 5, 6, 7]. Significant linkages with particular chromosomal regions had been diagnosed [8], but no mutations or ailment-predisposing polymorphisms have been identified. The mode of

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inheritance of SCZ is likely to be polygenic/multifactorial [9], and association studies are ideally fitted to analyze the candidate genes implicated on this disorder. Although the biological basis of SCZ is unknown, several neurobiological hypotheses were postulated as answerable for SCZ pathogenesis: polygenic/multifactorial genomic defects, perinatal and intrauterine environment-genome interactions, neurodevelopmental defects, dopaminergic, serotonergic, cholinergic, GABAergic, neuropeptidergic and glutamatergic/NMDA dysfunctions, neuroimmune dysfunction, and epigenetic dysregulation. The dopamine speculation of SCZ has been one of the maximum enduring thoughts in psychiatry. Initially, the emphasis became on the function of hyperdopaminergia within the etiology of SCZ. However, it changed into eventually conceptualized to specify subcortical hyperdopaminergia with prefrontal hypodopaminergia [10].

Genes involved in dopaminergic pathways are consequently being studied as candidate genes for SCZ [11]. Five different subtypes of G-protein coupled dopamine receptors mediate the movements of dopamine, 3 of which belong to the dopamine D2 circle: the dopamine D2 receptor (DRD2), the dopamine D3 receptor (DRD3) and the dopamine D4 receptor (DRD4) [12]. The DRD2 gene, found at 11q23.1 location of the chromosome [13, 14], has been implicated as a possible candidate gene for SCZ. A meta-analysis also supported the involvement of the DRD2 in the pathogenesis of SCZ [11]. Several studies, therefore, advocate the valuable role of the dopamine receptor within the risk of SCZ [15, 16], Numerous research groups have tried to detect the association of DRD2 polymorphisms to validate the effect of DRD2 on the disease etiology. However, association studies, to this point, have now not been capable of yielding constant consequences throughout one of a kind ethnic group [17, 18, 19, 20, 21, 22, 23].

A case-control study between DRD2-polymorphism and SCZ was conducted on the Korean population where four single nucleotide polymorphisms (SNPs) (rs4648317, rs7131056, rs4936270, and rs1076562) of DRD2 had analyzed, which suggested that the genotype and allelic frequency of DRD2 rs4936270 was associated with the development of SCZ [24]. Cho et al. also reported an association of haplotype of rs4648317, rs7131056, rs4936270 polymorphisms with SCZ [24]. Several case-control and meta-analyses reported association [25, 26, 27] of different SNPs of DRD2, and one study reported no significant correlation with risperidone-induced amenorrhea with the DRD2 polymorphisms in Chinese female SCZ patients [28]. However, these studies did not include the rs4648317, rs7131056, rs4936270 polymorphisms. The SNP rs4648317 has been found to influence dopaminergic neurotransmission by modulating DRD2 expression and function [29], whereas the function of rs4936270 is not clear yet. The C allele of rs7131056 enhances transcription activity [30], whereas A allele has higher transcriptional silencer activity [31].

Though several studies were conducted on SCZ in different ethnic groups, no such type of genetic association study has been carried out on the Bangladeshi population. We got an idea from the 1000 genome database of BEB (Bengali from Bangladesh) population that there might be a possibility of linkage disequilibrium among the studied SNPs (rs4936270, rs7131056 and rs4648317). Therefore, this case-control based pilot study was designed to investigate whether the DRD2 polymorphisms are associated with SCZ based on genotype and haplotype data in our population.

2. Methods and materials

2.1. Study setting, sample and data collection

This research work has been conducted over the patients suffering from SCZ in Bangladesh. Our target age group was 18–25 years that is the equivalent age of undergraduate and postgraduate students. Blood samples of SCZ patients were collected from the National Institute of Mental Health (NIMH), Dhaka, Bangladesh. The study recruited 101 young SCZ patients and 101 young, healthy controls. At least two

independent psychiatrists confirmed the diagnosis of SCZ based on the Statistical Manual of Mental Disorders (DSM-V), International Classification of Diseases 11th revision (ICD-11), and Affective Disorders and Schizophrenia-Lifetime (SADS-L). No patient had a history of Alzheimer's disease, dementia, Parkinson's disease, autism spectrum disorder, and other comorbid psychiatric disorders. All patients and respective guardians were informed about the study, and written consent was taken before the recruitment. The socio-demographic parameters, family history of schizophrenia, and other diseases were collected from the patients and/or the guardians through interviewing by trained nurses in an expert's presence. The controls were recruited by matching age and sex with the patients from the different parts of Dhaka city, Bangladesh. The Schedule for Affective Disorders and Schizophrenia-Lifetime (SADS-L) was used to exclude individuals with psychiatric conditions from the control group. The ethical committee of Noakhali Science and Technology University approved the research protocol and consent form. The ID of ethical approval was 05/2018, and the academic council of the Noakhali Science and Technology University approved the ethical committee [NSTU/REG/AC/2017/4627(09)].

The study was carried out according to the declaration of Helsinki and its further amendments [32].

2.2. DNA extraction and genotyping of rs4648317, rs4936270, and rs7131056

In this study, we selected 3 SNPs from the intron 1 of DRD2 gene based on the following criteria 1) the frequency of minor allele should be higher than 5% in BEB (Bengali from Bangladesh) population collected from 1000 genome database 2) Heterozygosity should be more than 10% in BEB population, 3) We conceived an idea from the online resource (https://ldlink.nci.nih.gov/) by using the 1000 genome database of BEB population that there might be a linkage disequilibrium of rs4936270 rs7131056, rs4648317 SNPs and 4) SNP having monomorphic genotype in BEB population was avoided. About 3 ml of the blood sample was drawn into a tube containing ethylenediaminetetraacetic acid disodium from all the patients and controls and stored at $-80\,^{\circ}$C until the isolation of DNA. Genomic DNA was isolated from 101 SCZ patients and 101 controls by a chemical method routinely used in our laboratory [33]. The purity (OD 260/OD 280) of all the genomic DNA samples was between 1.7 and 1.9, and the average concentration was 50–70 μg/ml. Primers required for these SNPs were designed with the help of primer blast (Table 1), and the annealing temperatures of the respective SNPs were validated. After completing PCR amplification rs4648317, rs4936270, and rs7131056 alleles at appropriate conditions, three PCR products of 430, 186, and 484 bp were obtained, respectively, and these were visualized in 1% (w/v) agarose gel. PCR-RFLP method was carried out to detect the rs4648317, rs4936270, and rs7131056 SNPs in the SCZ patients and controls with the respective restriction enzymes according to the conditions mentioned in Table 1. The digested PCR products were resolved on agarose gel (2%) electrophoresis (Figure 1, Figure 2 and Figure 3). All mutant homozygous and 20% of heterozygotes were analyzed twice to confirm the genotypes [34, 35, 36, 37].

2.3. Statistical analysis

Distributions of demographic variables were compared between cases and controls using χ^2-tests and two-sided unpaired t-tests. For assessing the deviation from Hardy-Weinberg equilibrium (HWE) in the reported genotype frequencies among the cases and controls, the appropriate goodness-of-fit χ^2-test was carried out. Genotype and allelic frequencies were reported as a percentage. χ^2-test was also used to estimate the odds ratio (OR) and 95% confidence intervals (CIs) using SPSS version 17.0 (SPSS, Inc., Chicago, IL). Haploview (v4.2) was used to construct the linkage disequilibrium (LD) block and calculate haplotype frequencies and association of these three SNPs. $p < 0.05$ (two-tailed) was considered statistically significant. Bonferroni correction was made to avoid the false

Table 1. Primer sequences, restriction enzyme, digestion condition and length of the expected fragments on digestion to diagnose DRD2 (rs4648317, rs4936270, and rs7131056) allele.

Allele	Primer sequences	Restriction enzyme (RE)	Digestion condition	Expected fragment
rs4648317	FP: 5′-ATTGGGCCTTCACTACCTCC-3′ RP: 5′-TGACCTAGGGAGACACTCGA-3′	BmrI	Incubation at 37 °C, overnight	NH: GG: 41,389 HE: GA: 41,389,430 MH: AA: 430
rs4936270	FP: 5′-CGAACAAACACACACGGGTT-3′ RP: 5′-GCTTGAGGTCACATGGTTGG-3′	HpyCH4V	Incubation at 37 °C, overnight	NH: CC: 83,103 HE: CT: 83,103,186 MH: TT: 186
rs7131056	FP: 5′-TGCTGGTCCACCACAACTTA-3′ RP: 5′-GGTGGTGAGAACTGAACCTG-3′	BstNI	Incubation at 37 °C, overnight	NH: AA: 484 HE: AC: 19,465,484 MH: CC: 19,465

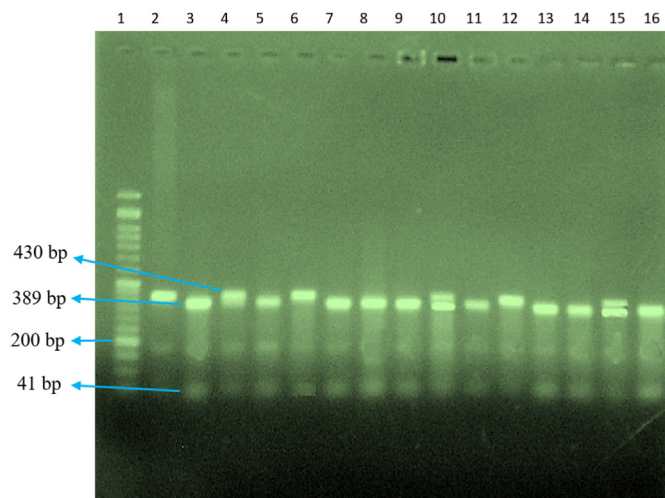**NH:** Normal Homozygote; **HE:** Heterozygote; **MH:** Mutant Homozygote.

Figure 1. PCR-RFLP assay for rs4648317. Lane 1 indicates 50 bp DNA Ladder; Lanes 3,5,7–9,11,13,14,16 indicate GG genotype; Lanes 4,10,15 indicate GA genotype and Lanes 2,6,12 indicate AA genotype.

positive by considering $p < 0.017$ statistically significant for 3 SNPs. Statistical power for each SNP was calculated online by OSSE tool (http://osse.bii.a-star.edu.sg/).

3. Results

3.1. Characteristics of cases and controls

The distribution of socio-demographic characteristics among study subjects is listed in Table 2. This case-control study consisted of 101 SCZ patients and 101 controls, and among the patients, 85.15% were male, and 14.85% were female. Whereas 81.19% were male, and 18.81% were female in case of controls. The average ages were 22.15, 22.09 years in patients and controls, respectively, and this result was not statistically significant compared to control subjects ($p > 0.05$). No statistically significant differences were found in the case of smoking status, education, the socioeconomic status between the cases and controls ($p > 0.05$).

3.2. Distribution of genotype data

Table 3 summarizes genotypes and allele frequencies for the three SNPs of the DRD2 gene, according to the Hardy-Weinberg equation. For rs4648317, 23.76 % of patients and 35.64 % of controls carried GG genotype, whereas 58.42% of patients and 54.46% of controls carried GA genotype. The occurrence of AA genotypes was 17.82% and 9.90% in SCZ patients and controls, respectively. The distribution of genotype data

in patients followed the Hardy-Weinberg equilibrium ($\chi^2 = 3.003$, $p = 0.083$), and that of the controls also obeyed the Hardy-Weinberg equilibrium ($\chi^2 = 2.80$, $p = 0.094$). The distribution of minor allele (A) was 47.03% and 37.13%, respectively, for patients and controls.

For rs4936270, 56.44% of the patients and 72.28% of the controls carried CC genotype, whereas 35.64% of cases and 22.77% of controls carried CT genotype. The frequency of TT genotypes was 7.92% and 4.95% for patient and control groups, respectively. Both the patients and control groups obeyed the Hardy-Weinberg equilibrium ($\chi^2 = 0.46$, $p = 0.50$; $\chi^2 = 2.81$, $p = 0.093$), whereas the observed minor allele (T) frequency was 25.74% and 16.34% for patients and controls, respectively.

About 40.60% of the SCZ patients and 32.67% of controls carried rs7131056AA genotype, whereas 44.55% of cases and 43.57% of controls carried rs7131056AC genotype. On the other hand, 14.85% of cases and 23.76% of controls carried rs7131056CC genotype. The variant rs7131056 showed no deviation from Hardy-Weinberg equilibrium in the SCZ group and control group ($\chi^2 = 2.10$, $p = 0.646$; $\chi^2 = 1.50$, $p = 0.221$, respectively). The percentage of the minor allele (C) was 37.13% in the SCZ patients and 45.54% in controls.

3.3. Association between SCZ risk with rs4648317, rs4936270, and rs7131056 polymorphisms

Correlation between the three SNPs (rs4648317, rs4936270, and rs7131056) of DRD2 gene for the susceptibility of developing SCZ is represented in Table 3. In the case of rs4648317, subjects with GA genotype had 1.60 times more risk for the development of SCZ compared to those carrying GG genotype, and the result is not statistically significant ($p > 0.05$). On the other hand, subjects carrying AA genotype had 2.70 times elevated risk for the development of SCZ compared to GG genotype, and the result was statistically significant (OR = 2.70, 95% CI = 1.07–6.84, $p = 0.036$). In the dominant model, i.e., GA + AA genotype carriers showed 1.78 times more risk for the development of SCZ ($p > 0.05$), and the result is not statistically significant. The AA genotype carriers (AA vs. GG + GA, recessive model) had a lower risk for the development of SCZ compared with patients carrying GG + GA genotype ($p > 0.05$). 'A' allele (A vs. G) also significantly increased the risk of SCZ (OR = 1.50, 95%CI = 1.01–2.24, $p = 0.044$).

In the case of rs4936270 SNP, subjects with CT genotype had almost 2 times higher risk for development of SCZ compared to CC carriers, and the result is statistically significant (OR = 2.00, 95% CI = 1.07–3.75, $p = 0.023$). In contrast, TT genotype carriers possessed 2.05 times higher risk for the development of SCZ, but this result is not statistically significant ($p > 0.05$). The dominant model (CT + TT vs. CC) showed a significantly higher risk for the development of SCZ (OR = 2.01, 95% CI = 1.11–3.61, $p = 0.020$). For the recessive model (TT vs. CC + CT), the TT genotype carriers had a higher risk for the development of SCZ compared to CC + CT genotype, but the result is not statistically significant ($p > 0.05$). T allele carriers showed 1.76 times more risk in comparison to C allele carriers for the development of SCZ, and the result is also found statistically significant (OR = 1.76, 95% CI = 1.09–2.89, $p = 0.021$).
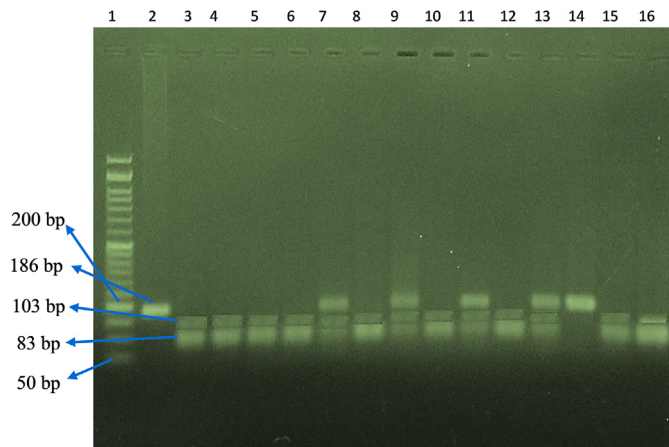
Figure 2. PCR-RFLP assay for rs4936270. Lane 1 indicates 50 bp DNA Ladder; Lanes 3–6 8,10,12,15,16 indicate CC genotype; Lanes 7,9,11,13 indicate CT genotype and Lanes 2,14 indicate TT genotype.

For rs7131056 SNP, all the genetic models showed a lower risk for the susceptibility of SCZ development. AC genotype had 0.82 times lower risk for the development of schizophrenia in comparison to AA genotype, and this result is not statistically significant (p > 0.05). CC genotype had 0.50 times lower risk for the development of schizophrenia compared to AA genotype, and this result is statistically not significant (p > 0.05). The dominant model (AC + CC vs. AA) had 0.71 times lower risk for the development of schizophrenia compared to AA genotype. In all cases, the results are not statistically significant as p > 0.05. Again, the recessive model (CC vs. AA + AC) carriers had 0.60 times lower risk for the development of schizophrenia (p > 0.05). C allele also depicted 0.71 times lower risk for the development of SCZ compared to A allele, and the result is not statistically significant (p > 0.05). After performing the Bonferroni correction, the significant associations did not remain for any genotype and allele (p < 0.017) for all the three SNPs.

We performed the haplotyping analysis by using Haploview to evaluate the LD block and association of SCZ with different haplotypes. An LD block was made among the rs4936270, rs7131056 and rs4648317 SNPs (rs4936270 and rs7131056, D' = 0.743, r^2 = 0.09; rs4936270 and rs4648317, D' = 0.407, r^2 = 0.019; rs7131056 and rs4648317, D' = 0.761, r^2 = 0.401) in the control group (Figure 4). The distribution of CAG, CCA, TAG, CCG, CAA, TAA and TCA haplotypes was 0.323, 0.280, 0.147, 0.117, 0.072, 0.045, and 0.016, respectively. Among these frequencies, the CAA and TAA haplotypes were significantly associated with SCZ (χ^2 = 8.258, p = 0.004; χ^2 = 5.313, p = 0.021, respectively) as listed in Table 4. After Bonferroni correction, only the CAA haplotype remained
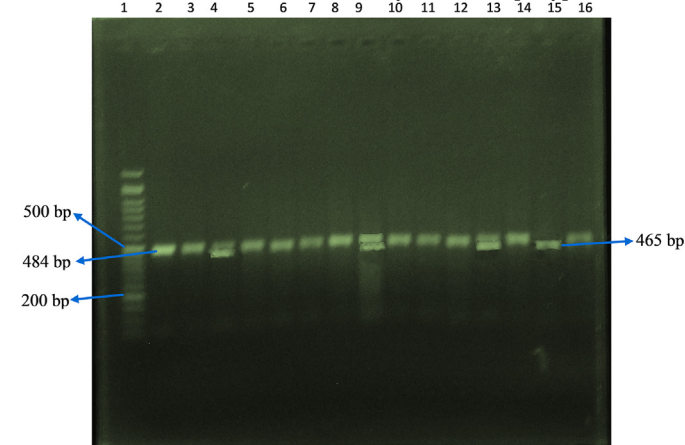

Figure 3. PCR-RFLP assay for rs7131056. Lane 1 indicates 50 bp DNA Ladder; Lanes 2,3,5–8,10-12,14,16 indicate AA genotype; Lanes 4,9,13 indicate AC genotype and Lanes 15 indicate CC genotype.

significantly associated with schizophrenia (p < 0.017).

3.4. Statistical power

Statistical power was calculated for all the SNPs by using the minor allele frequency of the cases and controls at 5% level of significance with the OSSE online sample size estimator. The power obtained for the rs4648317, rs4936270, and rs7131056 variants are 30%, 41.4% and 25.3% respectively.

4. Discussion

Schizophrenia (SCZ) has been considered a complex and subversive central nervous system disorder affecting almost 1% of the world's total population and ranked as one of the most afflicting diseases in humans [38]. However, the unfolding of the underlying genetic factors involved in the pathogenesis of SCZ remains a major concern for researchers. The function of dopamine is widely implicated in the development of schizophrenia and other related neuropsychiatric disorders due to its genetic predisposition [18, 39]. Moreover, a wide variety of biomolecular and pharmacological characteristics of dopaminergic activity justify that it is the key neurotransmitter for the development of SCZ. The D2 dopamine receptor (DRD2) coding genes are considered the most inseparable genes, increasing the risk of SCZ disease [40, 41]. Several studies have been carried out to validate the association of the DRD2 genes with the risk SCZ. To date, several association studies between polymorphisms of DRD2 gene and schizophrenia in different ethnic populations have yielded controversial data [18, 42].

The PCR-RFLP based DNA analysis is an excellent, reasonably priced and commonly used technique for genetic association research and is highly sensitive and reliable [43]. Considering this benefit, we used this technique to detect the association of DRD2 gene polymorphisms with SCZ in the Bangladeshi population. The functional importance of the DRD2 receptor for the pathophysiology of SCZ has been discussed in a few previous research [10, 11]. DRD2 in the prefrontal cortex is a notion to be linked to the improvement of psychotic symptoms [44, 45]. An ancient neurochemical principle of the pathophysiology of psychosis is the dopamine speculation [45]. One of the main persistent and potential reasons for SCZ pathophysiology is dysregulation or dopamine upregulation [40, 41, 44, 45, 46, 47]. The dopamine speculation assumes that psychotic symptoms are manifested via dysregulation of dopaminergic activity within the brain [45, 46].

Several studies suggested the association of DRD2 polymorphisms with SCZ [8, 11, 25, 26, 27, 41, 48, 49, 50, 51]. In this research, we investigated whether the DRD2 gene SNPs mentioned above are associated with SCZ in the Bangladeshi population. We found an association of AA genotype and A allele of rs4648317 with SCZ (OR = 2.70, 95%Cl = 1.07–6.84, p = 0.036; OR = 1.50, 95%Cl = 1.01–2.24, p = 0.044, respectively) in our case-control study. Other genotypic models between the cases and controls were not significant in increasing the risk of SCZ. A signification association was also found for CT genotype, dominant model and T allele of rs4936270 with SCZ (OR = 2.0, 95%Cl = 1.07–3.75, p = 0.023; OR = 2.01, 95%Cl = 1.11–3.6, p = 0.020; OR = 1.76, 95%Cl = 1.09–2.89, p = 0.021, respectively). No association of rs7131056 was found with SCZ in our population. Our present findings are consistent with an earlier study [24].

Among the different models, we found a significant association for the additive model in the case of rs4648317 and rs4936270 SNPs, whereas the significant association was found in the dominant model only for rs4936270. A significant association of SCZ with variant alleles was also found in the case of rs4648317 and rs4936270 SNPs. After Bonfenorri correction, the significant association did not exist with the additive, dominant and allele models. No association of SCZ was also found with the recessive model before and after Bonferroni correction.

Some studies found an association with some SNPs of DRD2 gene with SCZ [9, 25, 26, 27, 52, 53, 54], whereas Zhang et al. found no

Table 2. Distribution of demographic variables among the SCZ patients and controls.

Variables	SCZ (n = 101) (%)	Controls (n = 101) (%)	p-value
Male	86 (85.15)	82 (81.19)	0.453
Female	15 (14.85)	19 (18.81)	
Age range (years)	19–25	20–25	
Mean age, n (±SD)	22.15 (±2.51)	22.09 (±2.27)	0.872
Smoking status			
Ever smoker	30	38	0.234
Never smoker	71	63	
Socioeconomic status			
Low	45	40	0.123
Intermediate	44	38	
High	12	23	
Education			
Below undergraduate	35	28	0.532
Undergraduate	60	65	
Postgraduate	6	8	
Medications use			
Typical antipsychotics drug	6	-	-
Atypical antipsychotics drug	90	-	-
Mixed	5	-	-

Not Significant while p > 0.05.

association of DRD2 polymorphism with SCZ [55]. An association of rs1079727, rs2283265, rs1124492 SNPs with SCZ was found in the Han Chinese population living in Taiwan [56]. A meta-analysis reported an association of DRD2 rs1801028 polymorphism in the Asian population [25], whereas González-Castro et al. reported a protective effect of *Taq*I and C957T polymorphisms in the global population and a risk association of C957T polymorphism in the Chinese population [27]. Glatt et al. reported an association of Cys allele of DRD2 Ser311Cys polymorphism (pooled OR = 1.3, p = 0.007) [49]. In a meta-analysis, Liu et al. also found a positive association of DRD2 C957T polymorphism with SCZ [57]. The -141C Ins/Del polymorphism of DRD2 gene also showed a significant association with schizophrenia in British participants [58].

We conceived an idea from the 1000 genome database that there might be a linkage disequilibrium of DRD2 rs4648317, rs4936270 and rs7131056 SNPs in the Bangladeshi population. To evaluate the significance of this, we constructed an LD block and found that these three SNPs are in linkage disequilibrium. CAA and TAA haplotypes consisting of rs4936270, rs7131056, and rs4648317 in SCZ patients and controls were associated significantly with a risk of SCZ and the significant association withstand only for CAA haplotype after Bonferroni correction. The association of haplotype with SCZ was also reported in a study conducted by Cho et al. [24].

The present case-control study unravels the association between the DRD2 gene rs4648317, rs4936270, rs7131056 polymorphisms, and the risk of schizophrenia development. From our investigation findings, it is

Table 3. Genotypic distributions and the association of rs4648317, rs4936270 and rs7131056 alleles with SCZ.

SNPs			SCZ	%	Controls	%	OR (95% CI)			p
rs4648317	Genotype	GG	24	23.76	36	35.64	Additive model 1 (GA vs.GG)	1.60	0.85–3.03	0.141
							Additive model 2 (AA vs.GG)	2.70	1.07–6.84	**0.036**
		GA	59	58.42	55	54.46	Dominant model (GA + AA vs. GG)	1.78	0.96–3.28	0.066
		AA	18	17.82	10	9.90	Recessive model (AA vs. GG + GA)	0.51	0.22–1.16	0.107
	Allele	**G**	107	52.97	127	62.87		1		
		A	95	47.03	75	37.13		1.50	1.01–2.24	**0.044**
rs4936270	Genotype	CC	57	56.44	73	72.28	Additive model 1 (CT vs.CC)	2.00	1.07–3.75	**0.023**
							Additive model 2 (TT vs.CC)	2.05	0.63–6.60	0.229
		CT	36	35.64	23	22.77	Dominant model (CT + TT vs. CC)	2.01	1.11–3.61	**0.020**
		TT	8	7.92	5	4.95	Recessive model (TT vs. CC + CT)	1.65	0.52–5.23	0.394
	Allele	**C**	150	74.26	169	83.66		1		
		T	52	25.74	33	16.34		1.76	1.09–2.89	**0.021**
rs7131056	Genotype	AA	41	40.60	33	32.67	Additive model 1 (AC vs. AA)	0.82	0.44–1.50	0.538
							Additive model 2 (CC vs. AA)	0.50	0.23–1.11	0.089
		AC	45	44.55	44	43.57	Dominant model (AC + CC vs. AA)	0.71	0.40–1.26	0.243
		CC	15	14.85	24	23.76	Recessive model (CC vs. AA + AC)	0.60	0.27–1.14	0.111
	Allele	**A**	127	62.87	110	54.46		1		
		C	75	37.13	92	45.54		0.71	0.47–1.05	0.086

p < 0.05 was considered as statistically significant, and after Bonferroni correction for 3 SNPs, no association withstands (p < 0.017). Bold values indicate statistically significant (p < 0.05).

Table 4. Haplotype analysis of DRD2 polymorphisms in SCZ patients and controls.

Haplotype	Frequency	χ^2	p-value
CAG	0.323	3.293	0.0696
CCA	0.280	0.24	0.6242
TAG	0.147	1.788	0.1812
CCG	0.117	3.145	0.0761
CAA	0.072	8.258	**0.0041***
TAA	0.045	5.313	**0.0212**
TCA	0.016	0.195	0.6585

$p < 0.05$ was deemed statistically significant, and * indicate the value that is significant after Bonferroni correction ($p < 0.017$). Bold values indicate statistically significant ($p < 0.05$).
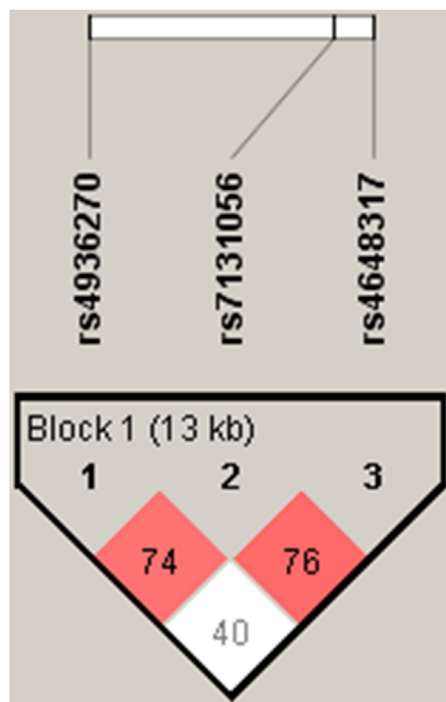

Figure 4. LD block consisting of rs4936270, rs7131056 and rs4648317 SNPs in the control group.

evident that rs4936270 and rs4648317 are associated with SCZ susceptibility, while rs7131056 showed no association. Furthermore, the haplotypic model reported the association of these SNPs with the SCZ. However, as we have identified the genetic basis of the Bangladeshi SCZ patients, we hope it will be helpful for further association studies to investigate the contribution of the other variants of DRD2 in predisposition to SCZ.

Despite the strengths of our present case-control study, it is important to address some notable limitations. The main limitation of this work is the number of the study population that may affect statistical significance. It is very challenging to make a decision of a genetic association study with these small numbers of cases and controls due to the diversity of population genetics. It was not possible to increase the number of samples due to the budget constraints that we have to face as researchers of low and middle-income countries usually. This finding may be considered as the preliminary findings, and we will expand our study to include larger samples and more SNPs of DRD2 and other related genes in the near future. Furthermore, only known SNPs were selected from a public database without novel SNPs. We have overcome these limitations partially by finding the higher frequency of minor alleles for all the tested SNPs and recruiting age and sex-matched cases and controls, although this study's statistical power is not satisfactory.

5. Conclusion

Our findings suggest that rs4648317, rs4936270, and rs7131056 SNPs of the DRD2 gene polymorphism, especially the CAA haplotype, are associated with the susceptibility of schizophrenia in the young Bangladeshi population. In this small pilot study, we have found a potential association of schizophrenia with two alleles of the D2 receptor in a well-characterized Bangladeshi population with schizophrenia. We hope to be able to enlarge the sample and replicate the finding.

Declarations

Author contribution statement

M. Hussain: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

S. Siddiqui and S. Mondal: Performed the experiments; Contributed reagents, materials, analysis tools or data.

M. Millat and S. Marzan: Contributed reagents, materials, analysis tools or data.

M. Uddin and M. Aziz: Analyzed and interpreted the data; Wrote the paper.

M. Alam: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

M. Islam: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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