The European Journal of Psychiatry 36 (2022) 106—113

THE EUROPEAN JOURNAL OF PSYCHIATRY

www.elsevier.es/ejpsy

ORIGINAL ARTICLE

Association of toll-like receptor 2 gene polymorphism (rs3804099) with susceptibility to Schizophrenia risk in the Dogra population of Jammu region, North India

Isar Sharma[a], Indu Priya[a], Sakshi Sharma[a], Suruchi Gupta[a], Manu Arora[b], Ritu Mahajan[a], Nisha Kapoor[a,*]

[a] *School of Biotechnology, University of Jammu Jammu, India*
[b] *Psychiatric Diseases Hospital, Government Medical College, Jammu, India*

Received 17 September 2021; accepted 24 February 2022
Available online 6 April 2022

KEYWORDS
Schizophrenia (SCZ);
TLR;
Polymorphism;
Inflammation;
Pathophysiology

Abstract
Background and objective: Schizophrenia (SCZ) is a severe mental biological disorder with a multifactorial manner of transmission and inheritance associated with environmental, developmental, and genetic set-off. It is a heritable disorder that involves genes and metabolic mechanisms in a combined effect, each conferring a small increase in the overall disease burden. Its etiology is not fully understood, although recent studies showed a relationship between SCZ and inflammation. Evidence from various studies indicates that dysregulation of *TLR* genes may have a role in the physiopathology of schizophrenia. In the present study, 4 polymorphisms, each in *TLR1, TLR2, TLR4,* and *TLR6*, were studied to explore their role in susceptibility to SCZ in the Dogra population of the Jammu region.
Methods: Five hundred (500) individuals including 200 SCZ and 300 healthy controls were included in the study. DNA was isolated and Sanger's sequencing was performed after PCR amplification.
Results: Statistically significant association of *TLR2* (rs3804099) was observed in the study population, the C allele of rs3804099 is associated with the increased risk for SCZ (OR=2.667; [1.4196 −5.0093 at 95%CI] *P* = 0.0023). No statistically significant associations with SCZ were observed in the target population at *TLR1, TLR4,* and *TLR6*.
Conclusion: Study concludes that TLR2 (rs3804099) may be associated with schizophrenia in the targeted population. Advance studies can be carried out focusing on finding potential SNPs for establishing a candidate gene approach.

* Corresponding author.
E-mail address: immunogenomicsbtju@gmail.com (N. Kapoor).

https://doi.org/10.1016/j.ejpsy.2022.02.001

Introduction

Schizophrenia (SCZ) is a withering neuropsychiatric disorder that impacts about 1% of the worldwide population. It is characterized by certain symptoms like delusions, hallucination, disorganized speech and thought, impaired cognition, social withdrawal, and inability to feel pleasure.[1,2] In addition, schizophrenics may have different mental health problems such as anxiety disorders, major depressive illness, substance use disorder.[3] Symptoms usually come in stages, begin in adolescence and last a long time. The causes of SCZ include each genetic and environmental factor considerably.[4] Conceivable environmental elements consist of habitation, cannabis use, certain infections, parental age, and bad nutrition throughout being pregnant.[4,5] Genetic factors consist of a variety of frequent and rare genetic variants.[6] Based on estimation, the heritability of SCZ can be as excessive as 80%, suggesting that genetic factors can also play an important role in the etiology of the disease.[7] The keystone of treatment is antipsychotic medication in addition to counseling, job training, and social rehabilitation.[4] It is undecided whether typical or atypical antipsychotics are better.[8,9] A vast and illustrious number of attempts have been made to explain the link between altered brain function and SCZ.[1] One of the most frequent is the dopamine hypothesis, which attributes psychosis to the mind's inaccurate interpretation of dopaminergic neurons' misfiring.[1,10,11] Of all the acquainted threat elements for SCZ, genetics is the biggest one. There is no particular mode of inheritance known. Elucidation of etiological factors remains the assignment to SCZ researchers as the most effective method for the identification of genetic risk elements for SCZ is unclear. All this has led to the adoption of several approaches, such as polymorphism, in an attempt to clarify the genetic etiology. The present study is based on exploring the role of innate immunity genes in SCZ with a focus on checking the association of *TLR* genes polymorphism with SCZ. Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system. They are single, membrane-spanning, non-catalytic receptors and are usually expressed on many immune cells to recognize structurally conserved molecules derived from microbes. They recognize microbes that have reached the physical barrier such as the skin or intestinal tract and respiratory tract mucosa and activate immune cell responses.[12] TLRs lead to the activation of the innate immunity and adaptive immune system through the production of cytokines and the expression of co-stimulator molecules which, in turn, also affect neurodevelopment and may have a role in neuropsychiatric disorders.[13—16] Innate immunity has a major role in the progression of neuropsychiatric disorders[17] and the role of immuno-inflammatory abnormalities has been seen in neuroprogression.[18] Although the immune system is mostly linked with infectious diseases, the expression and functioning of certain innate immunity genes like *TLR*s affect neuronal development.[13] Their dysregulation is linked with innate immunity genes. A disturbed cytokine profile is generally seen in patients with schizophrenia and there is an increased level of proinflammatory cytokines (like TNFα, INFγ, IL6, etc).[19-21] The cytokine expression is also regulated by TLRs.[13] Any disruption in *TLR* genes can disturb the cytokine expression resulting in an altered profile. It is evident from various studies that there is inflammatory stress in patients with schizophrenia, which can be due to variations in the *TLR*s.[22,23] It is important to study the role of TLRs in neurodevelopment disorders like SCZ as they are known to regulate cytokines and co-stimulatory molecules. As disruption of the immune system and disturbance in the cytokine profile of patients is evident from various studies, any variation in the genes of *TLR*s (like SNPs) can be checked. TLR encoding genes play an important role in host immunity, and variants in these genes can lead to structural and functional changes in these receptors, causing an altered immune response, inflammatory stress, and influencing neuropsychiatric disease progression.[13,17,18] Different studies in different populations have tried to explain the role of TLRs and the association of *TLR* genes polymorphism with SCZ risk. An advanced and better understanding of the stimuli and mechanisms responsible for these responses could lead to improved SCZ treatment and a better understanding of the innate immunity gene markers for SCZ. Hence, it is important to study the polymorphism in *TLR* genes in different populations for its association with SCZ risk and establish its role as a candidate gene associated with SCZ. Genetic studies can describe disease diagnosis, prognosis, treatment, or clinical stratification and help achieve personalized treatment plans for each patient. This study involves the analysis of SNPs and such SNPs associated with SCZ disease can be used as markers for disease susceptibility. The present study provides great promise for SCZ management because a lot of improvement is required for disease assessment and management. SNP data can be used as a powerful tool for combating SCZ. SNPs are important as medical diagnostic indicators in the progression of diseases and biomedical research, where comparison can be made in a region of the genome between cohorts at the genome-wide level. SNPs are among the forms of variations in the *TLR* genes and these *TLR* polymorphisms are associated with various disorders. This makes studying *TLR* Polymorphism in schizophrenia important.

Material and Methods

Study population

Jammu province is among the most diverse areas of the Union Territory of Jammu and Kashmir in India. It has Dogras, Kashmiris, Gujjars/Bakarwals, and Paharis as the major ethnic groups. Ethnically, the majority of the population of Jammu is Dogra. Dogra is an ethnically, linguistically, and culturally defined group that mostly lives in the Jammu, Udhampur, Reasi, Samba, and Kathua districts of the Union Territory. The participants belonged to this very group.

Ethics statement

All experiments were performed in accordance with the approved guidelines and regulations of the Institutional Ethics Committee (IEC), issued by the Government Medical College, Jammu (IEC/2017/425) and University of Jammu, per the guidelines of the Indian Council of Medical Research (ICMR). Informed consent was obtained from all participants.

Table 1. SNPs selected, chromosomal position, location on whole genome and their allele.

Gene	SNP ID	Chromosome	Position	Allele
TLR1	rs4833095	4	38798089	C/T
TLR2	rs3804099	4	154624656	C/T
TLR4	rs4986790	9	117713024	A/G
TLR6	rs5743810	4	38828729	C/T

Selection criteria

The cases were recruited from Government Psychiatric Diseases Hospital, Jammu, and they were diagnosed by an experienced psychiatrist in accordance with the Diagnostic and Statistical Manual of mental disorders, 4th edition (DSM-IV) (American Psychiatric Association, 1994). The present study consisted of 200 SCZ and 300 healthy controls. Of the 200 SCZ patients, 56.6% were male and 43.4% female, and, among 300 controls, 54.8% were male and 45.2% female. The mean ages were 37.8 $\pm$ 11.6 years for cases and 34.7 $\pm$ 11.3 years for controls. The relevant information for the genetic study was gathered by interviewing the patients with schizophrenia and their family members. Details regarding family history with the disorder and the onset of the disease were also obtained. Exclusion criteria for the cases were: psychiatric disorders caused by physical illness, medications, or other treatments; mentally retarded; family history with epilepsy; head injury; substance abuse history, and drug abuse. SCZ patients having psychiatric disorders caused by physical illness, medications, or other treatments; mentally retarded; family history with epilepsy; head injury; substance abuse history and drug abuse were excluded from the study. Independent non-psychiatric healthy controls were collected from the local region of Jammu and they were screened for drug abuse, any mental illness history, and family history of mental disorders. The study design was explained to both cases and controls and their respective guardians.

Blood sample collection and DNA isolation

3 ml of peripheral blood was collected from all cases and controls. EDTA-containing vacutainer tubes were used to store the blood samples at -20 °C. The utmost care was taken in all steps requiring the handling of blood.

Genomic DNA isolation from human whole blood samples was done by non-enzymatic salting-out method[24] and by DNA extraction protocol from fresh and frozen blood.[25] The quality and size of genomic DNA were checked on 0.8% agarose gel by electrophoresis, and quantification of DNA was done by measuring the absorbance at 260 nm using Nanodrop (Thermofisher Scientific). The concentration of the DNA samples was in the range of 60–700 ng/μl.

SNP selection

The four SNPs, each one in the transmembrane receptor gene *TLR1, TLR2, TLR4,* and *TLR6* were selected for the study Table 1. SNP selection for *TLR1*(rs4833095), *TLR2* (rs3804099), *TLR4*(rs4986790), and *TLR6(*rs5743810) was done by taking the following elements into consideration:

1. International HapMap Project (http://www.hapmap.org) and other public databases within the Genome Variation Server.
2. GWAS central and then confirmed by dbSNP
3. Various other databases were used to cross-check the selected SNPs
4. dbSNP (http://www.ncbi.nlm.nih.gov/SNP/)
5. LocusLink(http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi)
6. TSC (http://snp.cshl.org/)
7. SNPper (http://snpper.chip.org/bio/)
8. Ensemble genome browser and UCSC genome browser were used to study the gene sequences.

Genotyping

SNP genotyping of *TLR1*(rs4833095), *TLR2*(rs3804099), *TLR4*(rs4986790), and *TLR6(*rs5743810) was done by Sanger's sequencing method. Primers were designed by the Primer-BLAST tool, and the primer sequence detail of all the variants is provided in Table 2. PCR was performed using denaturation at 94 °C for 3 min; followed by 35 cycles at 94 °C for 50 s, (at optimum temperature for different gene primer sets) for 30 s and 72 °C for 50 s; and a final extension at 72 °C for 5 min. PCR products were analyzed using 1% agarose gel electrophoresis and visualized after staining with ethidium bromide. The amplified products were purified and then sent for sequencing by ABI 3500 genetic analyzer (Applied Biosystems, Carlsbad, CA, USA).

Statistical analysis

Genotype distribution and allele frequencies were calculated by the gene-counting method. Hardy–Weinberg

Table 2 Forward and reverse primer sets used for amplification of gene fragments of interest.

Gene	Primer Sequence	Amplicon Size
TLR1/ rs4833095	CCGAACATCGCTGA-CAAC TTGCCACCCTAC TGTGAACC	612 bp
TLR2/ rs3804099	TCCTGGTTCAAGCCCC TTTC GTGAGCAAAGT CTCTCCGGT	881 bp
TLR4/ rs4986790	AGAGGGCCTGTGC AATTTGA CTGCCTCTG GTCCTTGATCC	967 bp
TLR6/ rs5743810	TCTTGGTTCAAGC CCCTTTC GTGAGCAA AGTCTCTGCGGT	647 bp

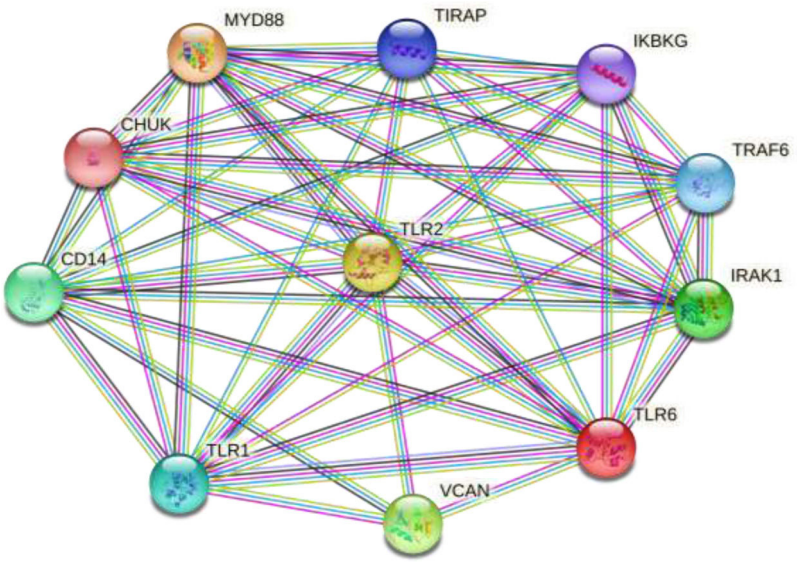

Fig. 1 Interaction of TLR 1, 2 and 6 genes with one another and other genes (https://string-db.org/cgi/network). Thickness of nodes is directly proportional to relationship with the genes.

equilibrium assumption was tested among controls. Genotype and allele frequencies between cases and controls were compared using the chi-square test. The magnitude of association was expressed as an odds ratio (OR) with 95% CI. $P < 0.05$ was considered significant for all analyses. The statistical analyses were done by using SPSS 21.0 version. $P < 0.05$ was considered significant for all analyses.

Results

Four SNPs *TLR1*(rs4833095), *TLR2*(rs3804099), *TLR4* (rs4986790), and *TLR6(*rs5743810) were successfully genotyped and analyzed in a total of 500 subjects. Also, the gene interaction was investigated by String Software (https://string-db.org/). *TLR1, TLR2, TLR6* genes were found interacting with each other, which is shown in Fig. 1.

Hardy Weinberg equilibrium

The allelic distribution of two SNPs, *TLR1*(rs4833095) and *TLR2*(rs3804099) were under the Hardy—Weinberg equilibrium ($p > 0.05$) when tested for chi-square goodness-of-the-fit test. Two SNPs, *TLR4*(rs4986790) and *TLR6 (*rs5743810), showed monomorphic distribution in the study population.

Genotypic analysis of *TLR1*(rs4833095), *TLR2* (rs3804099), *TLR4*(rs4986790), and *TLR6 (*rs5743810)

The frequencies of genotype or allele distribution of all the four SNPs were studied in SCZ patients and healthy controls. Table 3 shows the genotypic distribution and Table 4 shows the allelic frequencies of *TLR1*rs4833095(C/T), *TLR2*rs3804099 (C/T), *TLR4*rs4986790, *TLR6*rs5743810 polymorphisms along

Table 3. Genotyping distribution were compared between the cases and control subjects.

Gene/SNP	Risk Allele	Subjects	Genotype	HWE
TLR1/rs4833095	C		CC CT TT	
		Controls = 300	24 198 78	0.23
		Cases = 200	32 108 60	
TLR2/rs3804099	C		CC CT TT	
		Controls = 300	18 132 150	0.52
		Cases = 200	32 68 100	
TLR4/rs4986790	G		AA AG GG	
		Controls = 300	295 5 0	—
		Cases = 200	109 10 0	
TLR6/rs5743810	T		CC CT TT	
		Controls = 300	264 36 0	—
		Cases = 200	196 4 0	

Table 4. Allelic frequencies were compared between the cases and control subjects.

Gene/SNP	Risk Allele	Controls (n = 300)	Cases (n = 200)	HWE	Allelic OR	P Value
TLR1/ rs4833095	C	C = 0. 41 T = 0.59	C = 0.43 T = 0.57	0.23	1.0856 (0.62−1.90)	0.77
TLR2/ rs3804099	C	C = 0.28 T =0.72	C = 0.33 T = 0.67	0.52	2.667 (1.4196−5.0093)	0.0023
TLR4/ rs4986790	G	A = 0.983 G = 0.017	A = 0.99 G = 0.01	—	2.0204 (0.18−22.61)	0.57
TLR6/ rs5743810	T	C = 0.94 T = 0.06	C = 0.99 T = 0.01	—	6.3191 (0.75−53.48)	0.09

with association analysis. The difference between SCZ and healthy individuals for the *TLR1* rs4833095 risk allele was found statistically insignificant (p = 0.77, OR=1.0856, 95% CI 0.62−1.90). The frequency of *TLR2* rs3804099 allele C is (0.33) in SCZ cases and (0.28) in controls, (OR=2.667; [1.4196 −5.0093 at 95%CI] P = 0.0023) and the difference was found to be statistically significant. The genotypic frequencies were also found not significantly different between cases and controls for *TLR4* (rs4986790) and the population under study showed the monomorphic distribution of allele A (frequency 98.3% & 99% in controls and cases, respectively). However, in a few samples, the heterozygous condition was seen. For *TLR6* rs5743810, the difference between SCZ and healthy individuals was not statistically significant (p = 0.09, OR=6.3191, 95%CI 0.75−53.48). Genotype CC was prevalent in the study population for rs5743810.

Discussion

The current study explored the allelic and genotypic frequencies of polymorphisms in four genes, one each in the exonic region of *TLR1, TLR2, TLR4*, and *TLR6* by a case−control set up in the Dogra population of the Jammu region. Further, the role of the variants was observed using the SNIPA online tool. It was observed that the genetic variant rs4833095 has a direct effect on the transcript (as it is a mis-sense variant), as shown in Fig. 2, whereas the variant rs3804099 has a direct regulatory effect, as shown in Fig. 3. Also, the genetic variant rs5743810 has a direct effect on the transcript, as shown in Fig. 4, so, polymorphism in any of the regions could affect the neighboring SNPs and disturb the overall physiology of the genes. This is the first study addressing the association of the polymorphism in *TLR* genes

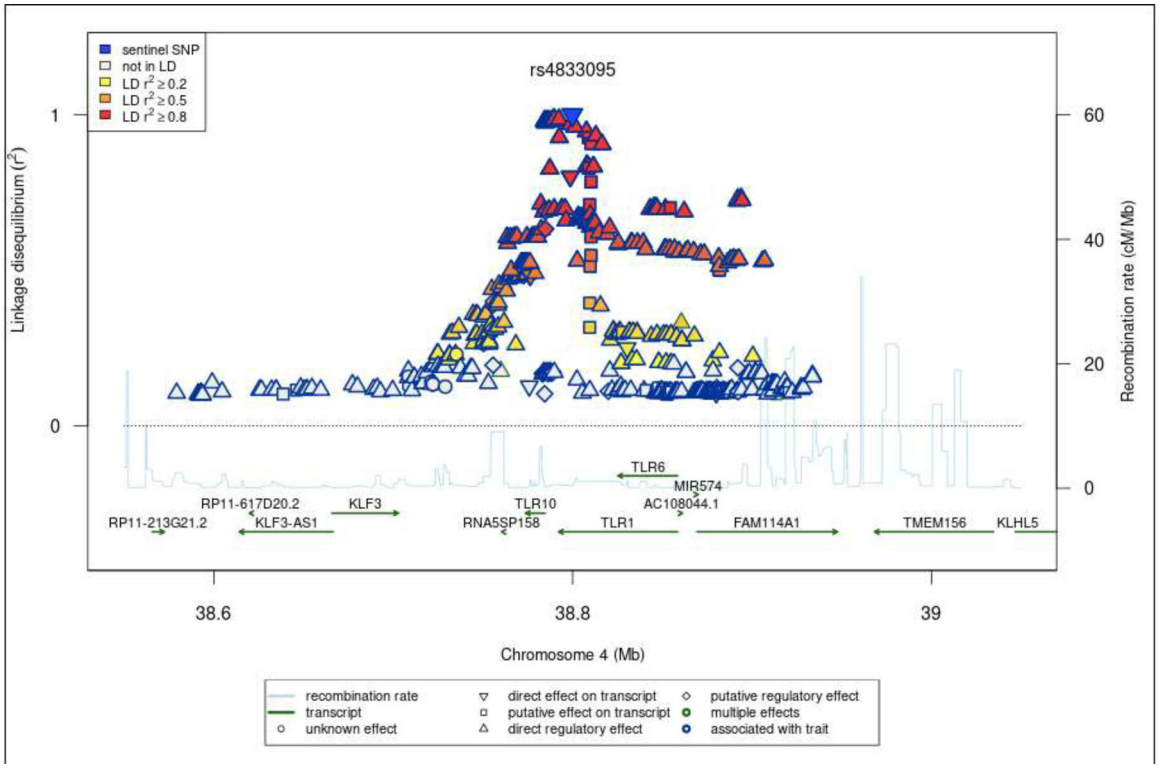

Fig. 2 Linkage disequilibrium plot shows the amount of correlation between a sentinel variant rs4833095(blue colored) and its surrounding variants (red colored). The TLR1 variant rs4833095 has transcript effect on the gene as it is mis-sense variant. The Y-axis signifies the correlation coefficient (r2); the X-axis signifies the chromosomal position of each SNP. The plot symbol of each variant designates its functional observations (http://snipa.helmholtz-muenchen.de).

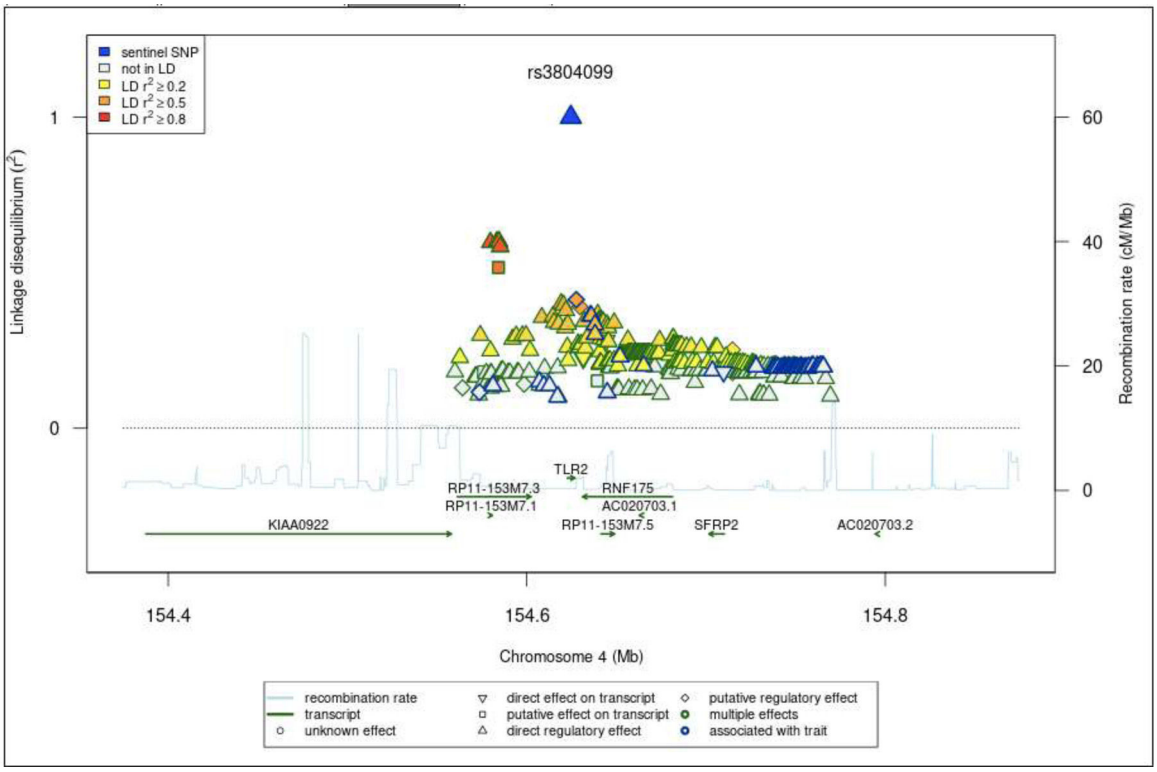

Fig. 3 Linkage disequilibrium plot which shows the amount of correlation between a sentinel variant (blue colored) and its surrounding variants. The TLR2 sentinel variant (rs3804099) is upward triangle thus signifies that variant has direct regulatory effect (http://snipa.helmholtz-muenchen.de).

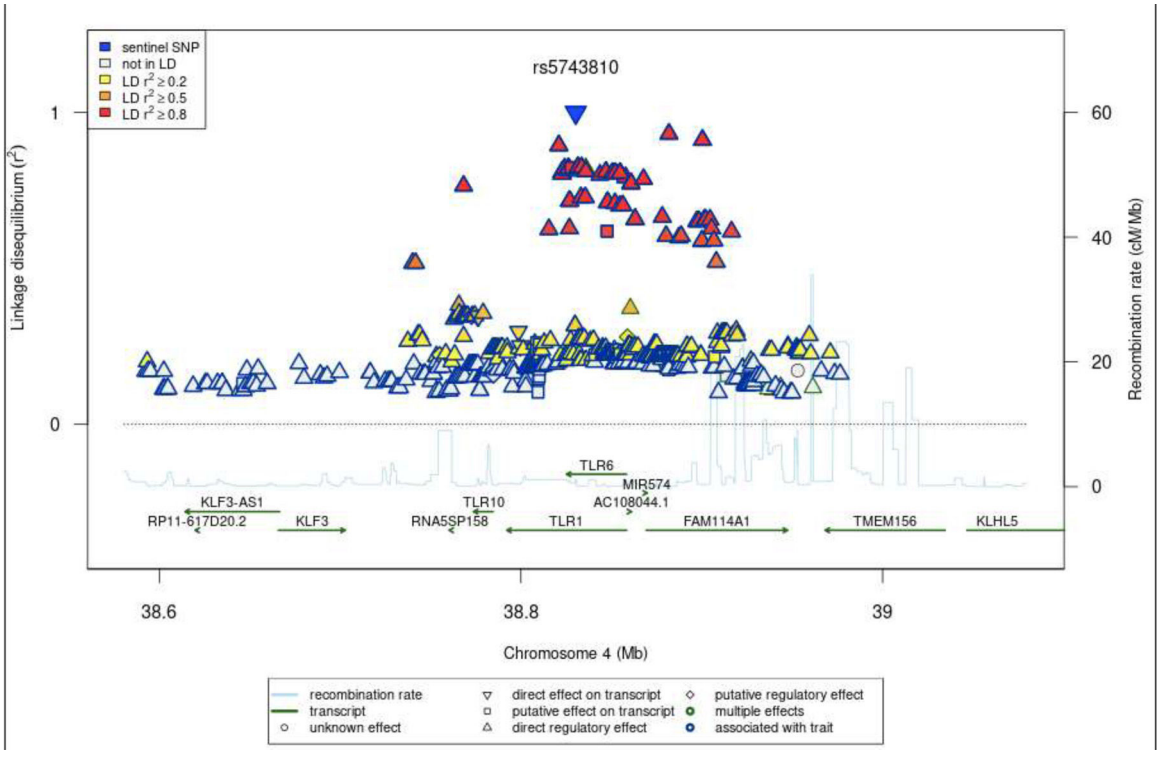

Fig. 4 Linkage disequilibrium plot which shows the amount of correlation between a sentinel variant (blue colored) and its surrounding variants. The TLR6 (rs5743810) sentinel variant is downward triangle thus signifies that variant has direct Transcript effect.

with schizophrenia (SCZ) in this population. All the *TLR* genes selected for the study code for membrane-bound TLRs are present on the cell surface.

In various studies, the association of single nucleotide polymorphism in different *TLR* genes with a set of diseases including neuropsychiatric disorders has been reported. A French study observed the combined effect of SNPs of *TLR2* (rs4696480 and rs3804099) and *TLR4* (rs1927914 and rs11536891) for their association with bipolar disorder and found that *TLR2* rs3804099 TT genotype can be associated with the risk of bipolar disorder, which is also a neuropsychiatric disorder.[26] A 2014 study found that the *TLR2* rs3804099 TT is significantly more prevalent in early-onset bipolar disorder.[27]

A Korean study investigated the coding region of single nucleotide polymorphisms (SNPs) of the *TLR2* gene for their association with the SCZ as well as with clinical symptoms in SCZ patients. The results provide the possibility of association of C allele of rs3804099 and rs3804100 with a poor concentration in SCZ patients.[28] In this study, we have found significant differences in genotypes between cases and controls; hence, the genetic association could be established between *TLR2* rs3804099 C allele and SCZ in the Dogra population of the Jammu region (OR=2.667; [1.4196-5.0093 at 95%CI] $P = 0.0023$).

A study by Keri et al. investigated a molecular pathway traditionally linked with neurodevelopmental hypothesis (neuregulin 1 - ErbB) and pathogen-associated pattern-recognition receptors associated with the immune hypothesis. The expressions of *TLR4*/*TLR5* and *ErbB* were measured and results revealed increased *TLR4*/*TLR5* and decreased *ErbB4* expression in SCZ relative to the control subjects.[29] It was concluded from the results that the stimulation of *TLR4* and *ErbB* induces opposite pro-inflammatory cytokine responses in SCZ.

In a 2016 study, the association of *TLR4* polymorphism including rs4986790 with SCZ was checked. It was also suggested that there is an altered innate immune response in patients with chronic SCZ, wherein the TLR4 proinflammatory pathway could be affected. The study suggested activation of the toll-like receptor-4 proinflammatory pathway in patients, although rs4986790 was not found to be significantly associated with SCZ.[23] Researchers found evidence of alterations in the expression of initial elements of the TLR4 signaling pathway (TLR4, Myeloid differentiation primary response gene 88 [MyD88], and nuclear factor-κ B [NF-κB]) in the Pre-Frontal Cortex (PFC) of patients with SCZ.[23] In our study, we could also not find a significant association between *TLR4* rs4986790 and SCZ. Genotype AA was found prevalent in both cases as well as controls (97% in controls and 98% in cases).

In the *TLR6* gene, the SNP rs5743810 showed no polymorphism, genotype CC was seen prevalent in our population, and it was concordant with the genotypic frequencies found in the GIH population in the HapMap project. Although some other populations have shown polymorphism at this position but in the population of the study, the presence of allele C is found significantly unassociated with the SCZ.

Also, in the case of *TLR1* rs4833095 polymorphism, we could not find a significant association with the SCZ. This nonsynonymous polymorphism, located in the extracellular domain, results in an asparagine-to-serine amino acid change.[30] This polymorphism has been extensively studied worldwide in the case of infectious diseases and, in some studies, polymorphism at this SNP location has shown to provide resistance against various diseases like tuberculosis (TB). However, there is no report of association of this SNP with SCZ. The CT genotype of *TLR1* SNP rs4833095 is associated with resistance to TB across ethnic groups.[30] Two independent studies, one by Dittrich et al., from Hyderabad, and another by Sinha et al. (2014) from Agra, India, have reported that CT genotype protects against TB.[31,32]

Variants *TLR1* rs4833095, *TLR4* rs4986790, and *TLR6* rs5743810 are not significantly associated with SCZ in the Dogra population of the Jammu region in this study. *TLR6* rs5743810 and *TLR4* rs4986790 were found to be largely monomorphic in this population (unlike in the case of other populations) with very few heterozygous genotypes. The present study showed interesting findings that some of the SNPs which are found to be associated with SCZ and other neuropsychiatric disorders in different populations are not associated with the disease in this region. Further association studies involving larger study cohorts are needed to substantiate our results.

Conclusion

Our results suggest that polymorphism at *TLR2* rs3804099 was significantly associated with the disease risk. If the association is validated on large sample size and replicated in other populations, the SNP can be used for the establishment of a biomarker for the disorder diagnosis. However, our outcome may give important data to help clarify the etiology of schizophrenia. Eventually, the information can be utilized to accurately ascertain an individual's risk of developing SCZ and assist in preventing this disease that negatively affects many individuals and families across the world. Further exploration of such polymorphism studies could lead to an important understanding of the genetic pathophysiology of SCZ. Hopefully, further research in this area, especially SNPs which are found associated with SCZ can help in assessing the SCZ risk determination.

Ethical consideration

All procedures involving human participants performed in this study were under the ethical standards of the Institutional Ethics committee which was constituted as per the guidelines formulated by the Indian Council of Medical Research (ICMR).

Informed consent

Informed consent was obtained from all individual participants included in this study.

Funding

The research used no funding or financial support at any stage.

Data availability statement

Authors are supposed not to share data. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors are grateful to all the participants who donated their blood samples for the study and all the Doctors who diagnosed patients with the disorder. Additionally, the authors are thankful to RUSA, UGC-SAP, DST PURSE, JKSTIC-DST, CSIR-New Delhi, the Central facility, and the DBT-funded Bioinformatics center at the School of Biotechnology, University of Jammu.

References

1. Van Os J., Kapur S. Schizophrenia. Lancet Lond Engl 374, 635−645.
2. Patel KR, Cherian J, Gohil K, Atkinson D. Schizophrenia: overview and treatment options. Pharm. Ther. 2014;39(9):638.
3. Buckley PF, Miller BJ, Lehrer DS, Castle DJ. Psychiatric comorbidities and schizophrenia. Schizophr. Bull. 2009;35(2):383−402.
4. Owen MJ, Sawa A, Mortensen PB. Schizophrenia. Lancet. 2016;388:86−97.
5. Chadwick B, Miller ML, Hurd YL. Cannabis use during adolescent development: susceptibility to psychiatric illness. Front. Psychiatry. 2013;4:129.
6. Kavanagh DH, Tansey KE, O'Donovan MC, Owen MJ. Schizophrenia genetics: emerging themes for a complex disorder. Mol. Psychiatry, 20; 2015. p. 201572−6.
7. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch. Gen. Psychiatry. 2003;60(12):1187−92.
8. Kane JM, Correll CU. Past and present progress in the pharmacologic treatment of schizophrenia. J. Clin. Psychiatry. 2010;71(9):13909.
9. Meltzer HY. Update on typical and atypical antipsychotic drugs. Annu. Rev. Med. 2013;64:393−406.
10. Howes OD, Nour MM. Dopamine and the aberrant salience hypothesis of schizophrenia. World Psychiatry. 2016;15(1):3.
11. Howes OD, McCutcheon R, Owen MJ, Murray RM. The role of genes, stress, and dopamine in the development of schizophrenia. Biol. Psychiatry. 2017;81(1):9−20.
12. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat. Immunol. 2010;11(5):373−84.
13. McKernan DP, Dennison U, Gaszner G, Cryan JF, Dinan TG. Enhanced peripheral toll-like receptor responses in psychosis: further evidence of a pro-inflammatory phenotype. Transl. Psychiatry. 2011;1(8):e36.
14. Okun E, Griffioen K, Barak B, Roberts NJ, Castro K, Pita MA, et al. Toll-like receptor 3 inhibits memory retention and constrains adult hippocampal neurogenesis. Proc. Natl. Acad. Sci. 2010;107(35):15625−30.
15. Okun E, Griffioen KJ, Mattson MP. Toll-like receptor signaling in neural plasticity and disease. Trends Neurosci. 2011;34(5):269−81.
16. Rolls A, Shechter R, London A, Ziv Y, Ronen A, Levy R, et al. Toll-like receptors modulate adult hippocampal neurogenesis. Nat. Cell Biol. 2007;9(9):1081−8.
17. Gibney SM, Drexhage HA. Evidence for a dysregulated immune system in the etiology of psychiatric disorders. J. Neuroimmune Pharmacol. 2013;8(4):900−20.
18. Brietzke E, Yamagata AS, Cerqueira R, Rizzo LB, McIntyre RS, Mansur RB. The role of immuno-inflammatory abnormalities in neuroprogression. Neuroprogression in Psychiatry. Oxford University Press; 2019. 2019 Mar 5.
19. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. Biol. Psychiatry. 2008;63(8):801−8.
20. Kim YK, Jung HG, Myint AM, Kim H, Park SH. Imbalance between pro-inflammatory and anti-inflammatory cytokines in bipolar disorder. J. Affect. Disord. 2007;104(1-3):91−5.
21. O'Brien SM, Scully P, Scott LV, Dinan TG. Cytokine profiles in bipolar affective disorder: focus on acutely ill patients. J. Affect. Disord. 2006;90(2-3):263−7.
22. Kéri S, Szabó C, Kelemen O. Antipsychotics influence Toll-like receptor (TLR) expression and its relationship with cognitive functions in schizophrenia. Brain Behav. Immun. 2017;62:256−64.
23. García-Bueno B, Gassó P, MacDowell KS, Callado LF, Mas S, Bernardo M, et al. Evidence of activation of the toll-like receptor-4 proinflammatory pathway in patients with schizophrenia. J. Psychiatry Neurosci. JPN. 2016;41(3):E46.
24. Suguna SA, Nandal DH, Kamble SU, Bharatha AM, Kunkulol RA. Genomic DNA isolation from human whole blood samples by non-enzymatic salting out method. Int. J. Pharm. Pharm. Sci. 2014;6(6):198−9.
25. Guha P, Das A, Dutta S, Chaudhuri TK. A rapid and efficient DNA extraction protocol from fresh and frozen human blood samples. J. Clin. Lab. Anal. 2018;32(1):e22181.
26. Oliveira J, Etain B, Lajnef M, Hamdani N, Bennabi M, Bengoufa D, et al. Combined effect of TLR2 gene polymorphism and early life stress on the age at onset of bipolar disorders. PLoS One. 2015;10(3):e0119702.
27. Oliveira J, Hamdani N, Busson M, Etain B, Bennabi M, Amokrane K, et al. Association between toll-like receptor 2 gene diversity and early-onset bipolar disorder. J. Affect. Disord. 2014;165:135−41.
28. Kang WS, Park JK, Lee SM, Kim SK, Park HJ, Kim JW. Association between genetic polymorphisms of toll-like receptor 2 (TLR2) and schizophrenia in the Korean population. Gene. 2013;526(2):182−6.
29. Kéri S, Szabó C, Kelemen O. Uniting the neurodevelopmental and immunological hypotheses: neuregulin 1 receptor ErbB and toll-like receptor activation in first-episode schizophrenia. Sci. Rep. 2017;7(1):1−8.
30. Ma X, Liu Y, Gowen BB, Graviss EA, Clark AG, Musser JM. Full-exon resequencing reveals toll-like receptor variants contribute to human susceptibility to tuberculosis disease. PLoS One. 2007;2(12):e1318.
31. Dittrich N, Berrocal-Almanza LC, Thada S, Goyal S, Slevogt H, Sumanlatha G, et al. Toll-like receptor 1 variations influence susceptibility and immune response to Mycobacterium tuberculosis. Tuberculosis. 2015;95(3):328−35.
32. Sinha E, Biswas SK, Mittal M, Bajaj B, Singh V, Arela N, et al. Toll-like receptor 1 743 A>G, 1805 T>G & toll-like receptor 6 745 C>T gene polymorphism and tuberculosis: a case control study of north Indian population from Agra (India). Hum. Immunol. 2014;75(8):880−6.