Original Article

Genetic Variants of Interleukin-10 Gene Promoter are Associated with Schizophrenia in Saudi Patients: A Case-Control Study

Saeed Mohammad Al-Asmary, Saeed Kadasah[1], Misbahul Arfin[2], Mohammad Tariq[2], Abdulrahman Al-Asmari[2]

Departments of Neuropsychiatry and [1]Psychiatry, [2]Department of Molecular Biology and Genetics, Research Center, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

Abstract

Background: Interleukin-10 (IL-10) gene is considered as a potential candidate gene in schizophrenia association studies. The polymorphisms on IL-10 gene have been reported to be linked with susceptibility to the development of schizophrenia within consistent results. **Aims:** The aim of this case-control study was to examine whether the -1082A/G, -819T/C, and -592A/C polymorphisms in IL-10 gene are implicated in schizophrenia development in the Saudi population. **Materials and Methods:** Molecular genotyping of IL-10 gene polymorphisms was performed to analyze the genotypes and alleles distribution of three single-nucleotide polymorphisms (SNPs) in patients (n = 181) and healthy individuals as control group (n = 211). **Results:** The frequencies of GA genotype at -1082, and CC genotype at positions -592 and -819 were significantly higher in schizophrenia patients compared to healthy subjects suggesting that GA, CC, and CC genotypes are susceptible to schizophrenia. The ACC haplotype known to be associated with intermediate production of IL-10 are more prevalent in our schizophrenia patients. On the other hand, genotypes -1082 GG, -819 CT, and -592 CA of IL-10 were more prevalent in healthy controls suggesting protective effects of GA, CT, and CA genotypes against schizophrenia. There was no significant association of IL-10 polymorphisms with sex or positive or negative symptoms of schizophrenia. **Conclusion:** This study indicates that the IL-10 gene polymorphisms play a significant role in the etiology of schizophrenia in Saudi Arabians patients.

Keywords: Interleukin-10, Polymorphism, Saudis, Schizophrenia

Address for correspondence: Dr. Abudlrahman Al-Asmari, Senior Consultant and Director of Research Center, Prince Sultan Military Medical City, P.O. Box 7897, Riyadh 11159, Saudi Arabia. E-mail: abdulrahman.alasmari@gmail.com

Introduction

Schizophrenia (MIM 181500) is one of the most disabling psychiatric disorders affecting around 24 million people worldwide, with a prevalence ranging from 0.7 to 1.1% and with a lifetime morbidity risk of 0.5-2.7%.[1,2] Schizophrenia affects men and women equally; however, most researchers agree that the onset of schizophrenia in women appears later than in men.[3] Due to its early age of onset and the lifelong disability, schizophrenia is considered as one of the most catastrophic mental illnesses.[4,5] One of the most vital aspect for developing treatment and prevention strategies for schizophrenia is identifying the causes of the disorder. Research over the decades has led us to suggest that the etiology of schizophrenia involves the interplay of complex polygenic influences and environmental risk factors operating on brain maturational processes.

Access this article online

Quick Response Code:

Website: www.najms.org

DOI: 10.4103/1947-2714.145466

Schizophrenia has long been considered as a disorder involving immune system. Possible role of the immune response system in the pathogenesis of schizophrenia is indicated by several authors and meta-analyzed.[6-10] An immune response shifting from Type 1 to Type 2 is one of the strong proposition supporting immunoinflammatory origin of schizophrenia.[11,12] Variations in the concentration of cytokines in patients

with schizophrenia have also been reported.[7] It has been shown that the concentration of cytokines in blood serum in patients suffering from schizophrenia varies depending on whether the patient is in active or resting phase of the disease.[13]

Interleukin (IL)-10, a Th2 cytokine is one of the many cytokines that seems to play a vital role in immune response that is generated in brain. Increased level of cytokine IL-10 in the cerebrospinal fluid of schizophrenics has been reported.[14,15] Production of cytokine IL-10 is controlled by IL-10 gene located on human chromosome 1 (1q31-q32) in a region reported to be related to schizophrenia in genetic association studies.[16] Given the strong hereditary background of schizophrenia, it is assumed that genetic factors may also underlie immune system deregulation and aberrant cytokine production observed in schizophrenia. IL-10 is secreted by a variety of cells including monocytes/macrophages, T-cells, β-cells, and mast cells. It is responsible for various functions. It shifts the Th1/Th2 balance by downregulating the Th1 responses and by suppression of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) secretion.[17] A number of single-nucleotide polymorphisms (SNPs) were reported in the proximal and distal regions of the IL-10 gene.[18,19] Three promoter polymorphisms (rs18000896-1082A/G, rs1800871-819T/C, and rs1800872-592A/C) are reportedly involved in IL-10 transcription rate, thereby directly affecting its production level.[19,20] The -1082G, -819C, and -592C (GCC) alleles have been associated with elevated levels of IL-10 production,[21] while ACC and ATA haplotypes exhibit intermediate and low IL-10 gene transcription, respectively.[22] These polymorphisms on IL-10 gene are reported to be linked with susceptibility to the development of schizophrenia.[23-26] However, data is limited and inconsistent,therefore do not allow drawing unequivocal conclusions. Inthis study, we aimed to investigate the association between IL-10 gene polymorphismsin promoter region at -1082, -819, and -592 loci and susceptibility to schizophrenia in Saudi cohort.

Materials and Methods

Subjects

The study population was composed of a total of 392 unrelated Saudi subjects including 181 schizophrenia patients recruited from the outpatient psychiatric clinic of Prince Sultan Military Medical City (PSMMC) Riyadh, Saudi Arabia and 211 age- and sex-matched healthy volunteers. The diagnosis of schizophrenia was based on the criteria mentioned in American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, DSM-IV-TR version. This criterion uses the self-reported experience of the patient and reported abnormalities in behavior and a comprehensive clinical assessment by a neuropsychiatrist. To ensure the diagnostic reliability, a systemic search into the case notes of the patients was made. Out of 200 initially selected schizophrenia patients,19 patients failedto meet explicit stated criteria, hence were excluded and only 181 patients were included in this study. Among the confirmed 181 cases of schizophrenia, there were 53 females and 128 males with mean age of 39 ± 12.5 years and mean disease duration of 9 ± 4.5 years. Age of onset of disease ranged from 19 to 64 years. The female to male ratio of schizophrenia patients in our study was 1:2.5. The control group consisted of 61 females and 150 males with mean age of 36 ± 10 years.

If diagnoses of schizophrenia was confirmed by above mentioned criteria, patients were further assessed for positive and negative symptoms using Positive or Negative Syndrome Scale (PANSS) involving further clinical interview, cognitive testing, motor assessment, and careful review of medical and historical records as described by Kay *et al*.[27] All the subjects in control group were screened using a questionnaire about the health status and excluded if they had any history of neurological, psychiatric, or medical disorders or had a past or present involvement in substance abuse. Control subjects less than 26-years-old and having first- or second-degree relatives with schizophrenia or any psychiatric disorder were excluded. A number of baseline parameters to rule out any psychotic illness were adopted as described by Johnstone *et al*.[28] This study was approved by the ethical committee of the hospital and informed consent was obtained from each subject.

Polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from the blood of schizophrenia patients and controls using the QIAamp^{R}DNA mini kit (Qiagen, USA). IL-10 gene was amplified using amplification refractory mutation systems (ARMS)-PCR methodology[29] to detect any polymorphism involved at various loci viz:-592,-819, and -1082.The sets of primers used to amplify various types of polymorphism were as reported earlier.[30]

PCR amplification was carried out in Ready-To-Go PCR Beads (Amersham Biosciences, USA). Reaction consisted of 10 temperature cycles of denaturation for 15 s at 94°C, annealing for 50 s at 65°C, and extension for 40 s at 72°C. Then 25 cycles of denaturation for 20 s at 94°C, annealing for 50 s at 59°C, and extension for 50s at 72°C. Final extension was performed at 72°C for 7 min. A positive control was included in the PCR assay by amplification of the human growth hormones (HGH) gene. Electrophoresis of the PCR product was performed

in 1.5% agarose gel, stained with ethidium bromide, and photographed.

Statistical analysis

The differences in genotype and allele frequencies between patients and controls were analyzed with the Fisher's exact test. $P \leq 0.05$ was considered significant. The strength of the association of disease with respect to a particular genotype/allele was expressed with the odds ratio interpreted as relative risk (RR). The strength of the association of disease with respect to a particular genotype/allele was expressed with odds ratio interpreted as RR following the method of Woolf as described by Schallreuter *et al*.[31] RR indicates how many times more frequent a disease is in the positive subjects compared with allele/genotype-negative subjects. It is calculated for a genotype/allele that is increased or decreased in psoriasis patients compared to the frequency in normal Saudi subjects. RR was calculated using the following formula:

$$RR = \frac{a \times d}{b \times c}$$

a = Number of patients expressing the allele or genotype

b = Number of patients without allele or genotype expression

c = Number of controls expressing the allele or genotype

d = Number of controls without allele or genotype expression

The etiologic fraction (EF) indicates the hypothetical genetic component of the disease. EF values of >0.00-0.99 are significant. It is calculated for positive associations (RR > 1) using the following formula proposed by Savejgaard *et al*:[32]

$$EF = \frac{(RR - 1)f}{RR}, \text{ where } f = \frac{a}{a + b}$$

Preventive fraction (PF) indicates the hypothetical protective effect of one allele/genotype for a disease. It is calculated for negative associations (RR < 1) using the following formula.[32] Values of <1.0 indicate the protective effect of an allele/genotype against the manifestation of disease.

$$PF = \frac{(1 - RR)f}{RR\,(1 - f) + f}, \text{ where } f = \frac{a}{a + b}$$

Results

The demographic features of the participants are summarized in [Table 1]. The results of SNP for IL-10 G (-1082) A, IL-10 C (-592) A, IL-10 C (-819) T, and corresponding genotypesusing ARMS-PCR method are summarized in [Tables 2-4].

The frequency of -1082GG genotype was found to be significantly lower ($P = 0.027$) in schizophrenia patients (2.21%) as compared to controls subjects (7.53%). On the contrary, the frequency of heterozygous genotype GA was significantly higher ($P = 0.0002$) in patients (91.71%) as compared to control subjects (75.27%), whereas frequency of homozygous AA genotype was lower in patients (6.08%) as compared with controls (12.20 %). These results indicate that genotype -1082GA is susceptible to schizophrenia (RR = 3.636, EF = 0.393) while genotypes GG and AA are resistant to schizophrenia (RR = 0.278, PF = 0.363 and RR = 0.311, PF = 0.362 respectively, [Table 2].

The frequency of -819 CC genotype was significantly higher ($P = 0.05$) in the schizophrenia patients (51.93%)

Table 1: Demographic features of patients/controls

Number of patients 181
Gender F:M=53:128 (1:2.5)
Age of patients mean 39±12.5 (range 26-74 years)
Age of onset mean 29±12.5 (range 19-64 years)
Duration of disease mean 9±4.5
Type of schizophrenia:
With negative symptoms: 94 (52.22%)
With positive symptoms:86 (47.78%)
Number of controls 211
Gender F:M=61:150 (1:2.5)
Age mean 36±10 (range 26-60 years)

Table 2: Genotype and allele frequencies of interleukin-10 (-1082G/A) variants in schizophrenia patients and matched controls

Genotype/allele	Schizophrenia (*N* = 181)		Control (*N* = 186)		*P*-value	RR	EF*/PF
	N	Frequency (%)	*N*	Frequency (%)			
GG	4	2.21	14	7.53	0.02†	0.278	0.363
GA	166	91.71	140	75.27	<0.001†	3.636	0.393*
AA	11	6.08	32	12.20	<0.001†	0.311	0.362
G-allele	174	48.07	168	45.16	0.45	1.124	0.056*
A-allele	188	51.93	204	54.84	0.45	0.889	0.056

†Statistically significant, *N* = Number of subjects, RR = Relative risk, EF = Etiological fraction, PF = Preventive fraction

compared to controls (41.71 %), while CT showed a reverse pattern with lower frequency (38.12 %) in schizophrenia patients as compared to 48.34% in controls (P = 0.05). The frequency of homozygous TT genotype was similar in both schizophrenia and control samples (9.95%). The CT genotype is more common among the healthy controls indicating that the 819 CT genotype may have a protective effect on the susceptibility to schizophrenia (RR = 0.66,PF = 0.17), whereas CC genotype appears to be susceptible to schizophrenia (RR = 1.51, EF = 0.17) [Table 3].

The frequency of -592 CC genotype was significantly higher in schizophrenia patients as compared to controls (51.93 vs 41.71%, P = 0.05), while CA genotype was found to be significantly lower (P = 0.05) in schizophrenia patients (38.12%) compared to control subjects (48.34%). The frequency of homozygous AA genotype was similarin both schizophrenia and control samples (9.95%). The higher frequency of CA genotype among the healthy controls indicated that the individuals with -592CA genotype are protected against schizophrenia (RR = 0.66, PF = 0.17), while CC genotype at position -592 of IL-10 are susceptible to schizophrenia (P = 0.05, RR = 1.51, and EF = 0.17) [Table 4].

Upon stratification of subjects into gender and with positive or negative symptoms, no significant difference was found in distribution of alleles and genotypes of all the three polymorphisms either with sex of patients or schizophrenia with positive or negative symptoms [Tables 5 and 6].

Discussion

In the present study, intermediate IL-10 producer genotype -1082 G/A showed positive association with schizophrenia as 91.71% of the patients have this genotype as compared to 75.27% of control subjects,

Table 3: Genotype and allele frequencies of interleukin-10 (-819C/T) variants in schizophrenia patients and matched controls

Genotype/allele	Schizophrenia (N = 181)		Control (N = 211)		P-value	RR	EF*/PF
	N	Frequency (%)	N	Frequency (%)			
CC	94	51.93	88	41.71	0.05†	1.51	0.174*
CT	69	38.12	102	48.34	0.05†	0.66	0.173
TT	18	9.95	21	9.95	1.00	1.00	–
C-allele	257	70.99	278	65.88	0.14	1.27	0.101*
T-allele	105	29.01	144	34.12	014	0.79	0.101

†Statistically significant, N = Number of subjects, RR = Relative risk, EF = Etiological fraction, PF = Preventive fraction

Table 4: Genotype and allele frequencies of interleukin-10 (-592C/A) variants in schizophrenia patients and matched controls

Genotype/allele	Schizophrenia (N = 181)		Control (N = 211)		P-value	RR	EF*/PF
	N	Frequency (%)	N	Frequency (%)			
CC	94	51.93	88	41.71	0.05†	1.51	0.174*
CA	69	38.12	102	48.34	0.05†	0.66	0.173
AA	18	9.95	21	9.95	1.00	1.00	–
C-allele	257	70.99	278	65.88	0.14	1.27	0.101*
A-allele	105	29.01	144	34.12	014	0.79	0.101

†Statistically significant, N = Number of subjects, RR = Relative risk, EF = Etiological fraction, PF = Preventive fraction

Table 5: Comparison of frequencies of interleukin-10 (-1082G/A) variants with negative or positive symptoms in schizophrenia patients and controls

Genotype/allele	With negative symptoms (N = 94)		With positive symptoms (N = 86)		Control (N = 186)	
	N	Frequency (%)	N	Frequency (%)	N	Frequency (%)
GG	3	3.19	1	1.16*	14	7.53
GA	84	89.36*	81	94.19*	140	75.27
AA	7	7.45*	4	4.65*	32	12.20
G-allele	90	47.87	83	48.26	168	45.16
A-allele	98	52.13	89	51.74	204	54.84

N = Number of subjects,*P-value < 0.05 as compared to the frequency in controls

Table 6: Comparison of frequencies of interleukin-10 (-1082G/A) variants in female and male schizophrenia patients

Genotype/allele	Female (*N* = 53)		Male (*N* = 128)		*P*-value	RR
	N	Frequency (%)	*N*	Frequency (%)		
GG	1	1.89	3	2.34	0.99	0.80
GA	49	92.45	117	91.41	1.00	1.15
AA	3	5.66	8	6.25	1.00	0.90
G-allele	51	48.11	123	48.05	0.99	1.00
A-allele	55	51.89	133	51.95	0.99	1.00

N = Number of subjects, RR = Relative risk

suggesting that individuals having 1082 G/A genotype are more prone to schizophrenia. Similarly, another study showed that intermediate IL-10 producer genotype -1082 GA is susceptible to schizophrenia in Taiwanese.[25] Almoguera *et al*.,[33] reported a significant association of IL-10-1082A allele with Spanish schizophrenia females. Contrary to these, significantly increased frequency of G allele was reported in the schizophrenia group in Polish,[24] Chinese,[23,34] and Italians.[35] By contrast, the association between the schizophrenia and the presence of-1082G has not been found in Korean and Turkish populations.[16,36]

The frequency of IL-10-819CC was significantly higher, whereas the frequency of IL-10-819CT, was significantly lower in schizophrenia patients compared to healthy controls. The genotype -819 CC has been reported to be a high producer of IL10, whereas CT and TT as intermediate and low producer, respectively.[37] These findings further indicated that genotypes associated with intermediate-high production of IL-10 are more prevalent in schizophrenia patients. The genotypes of IL-10- (592), being in linkage disequilibrium with the genotypes of IL-10- (819) followed the pattern similar to those for frequencies of -819 in schizophrenia patients and healthy controls. The frequency of IL-10-592CC was also significantly higher in schizophrenia patients compared to healthy controls. However, contradictory reports are available from different ethnicities on the association between schizophrenia and IL-10-592C/A polymorphism. It has been reported as the statistically significant polymorphism in Chinese.[23] and Turkish patients with schizophrenia.[16] On the other hand,-592 C/A polymorphism had no association with both allele and genotype distributions in Italian.[35] and Korean schizophrenia patients.[36]

The genotypes of IL-10; -1082GA, -819CC, and -592CC (ACC haplotype); known to be associated with intermediate production of IL-10 are more prevalent in our schizophrenia patients indicating the possible association with susceptibility of schizophrenia which is supported by the finding that the schizophrenia patients show statistically significant increased expression of IL-10 as compared to controls.[7,9,13-15] Considering IL-10 gene promoter haplotypes, GCC in Italians.[35] and Chinese,(34) whereas GTA in Turkish.[16] and Chinese population,[23] both associated with high IL-10 production, have also been linked to schizophrenia. It seems that IL-10 might correlate to schizophrenia based on the hypotheses of Th2-like immunity shift of susceptible allele carrier.

There was no significant association of IL-10 polymorphisms with positive or negative symptoms of schizophrenia in our study. However, recently Sun *et al*,[38] reported that the interaction of the promoter of IL-10 rs1800872 and dopamine beta-hydroxylase (DBH) rs72393728 polymorphism is significantly associated with the PANSS general score, and suggested that the interaction of their variants may play a role in psychopathology of the general symptoms on PANSS in schizophrenic patients in a northern Chinese Han population.

Earlier published reports and present study indicate that IL-10 promoter polymorphisms may enhance the vulnerability of schizophrenia and many diseases,[26,39-49] while may also exert a protective effect against others.[50-53] It has been confirmed by the twin and family studies that ~75% of the variations in IL-10 production is genetically determined.[54] Furthermore, there are intrapopulation differences in the genetic control of IL-10 production by various alleles of the promoter region. Hence, the share of the promoter polymorphism may be different depending on the population.[36,55]

The exact mechanism by which IL-10 affects the susceptibility/pathogenesis of schizophrenia is far from clear. It participates in the regulation of the immune response at several levels.[56] IL-10 regulates the inflammatory response, by inhibiting proinflammatory Th1 cytokines production,[57,58] and it is constitutively expressed during fetal brain development in humans.[59] IL-10 cytokine downregulates the expression of major histocompatibility complex (MHC) class I and II molecules.[60-63] It also has potent stimulatory effects on B lymphocytes, resulting in increased production of immunoglobulin and DNA replication.[60] Some immune-stimulating effects of IL-10 have been documented, where it induces activated B cells to secrete large

amounts of IgG, IgA, and IgM and in combination with IL-4 results in the secretion of four immunoglobulin isotypes. Thus, increased levels of IL-10 may also play a role in the amplification of humoral responses in some diseases as suggested by Rousset *et al*.[60]

There is evidence of the role of IL-10 in the neurodevelopmental abnormalities found in schizophrenia.[64] It has been suggested that the genetically determined differences in IL-10 production could lead to behavioral abnormalities in the adulthood after prenatal immune challenge or innate immune imbalances. It has also been pointed out that not only an excess of proinflammatory cytokines, but also an imbalance between both classes of cytokines during development might alter normal brain functions in adult life.[64]

The immune system have been linked to schizophrenia, through candidate-gene,[24,25,35] pathway-based,[38] and genome-wide association.[65] approaches that have produced evidence of the immune system involvement in schizophrenia. The present study addresses the significant association of IL-10 promoter polymorphisms with Saudi schizophrenia patients as a case-control study.

Conclusion

In conclusion, our results of IL-10 polymorphism in schizophrenia suggested that GA genotype at -1082 position together with CC genotypes at positions -592 and -819 of IL-10 genemight be susceptible to the development of schizophrenia. The genotypes 592 CA, 819 CT, 1082 GG, and 1082 AA of IL-10 gene, maybe protective against schizophrenia in Saudi test population. To our knowledge, this is the first study that examined IL-10 polymorphism in schizophrenia patients from Arabian ethnicity. However, further studies are warranted to investigate IL-10 polymorphisms in larger population samples as well as in schizophrenia affected families.

Acknowledgment

The authors thank S. Sadaf Rizvi and Mohammad Al-Asmari for their help with laboratory work.

References

1. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. PLoS Med 2005;2:e141.
2. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: A concise overview of incidence, prevalence, and mortality. Epidemiol Rev 2008;30:67-76.
3. Abel KM, Drake R, Goldstein JM. Sex differences in schizophrenia. Int Rev Psychiatry 2010;22:417-28.
4. Paek MJ, Kang UG. How many genes are involved in schizophrenia? A simple simulation. Prog Neuropsychopharmacol Biol Psychiatry 2012;38:302-9.
5. Ayalew M, Le-Niculescu H, Levey DF, Jain N, Changala B, Patel SD, *et al*. Convergent functional genomics of schizophrenia: From comprehensive understanding to genetic risk prediction. Mol Psychiatry 2012;17:887-905.
6. Plata-Salamán C, Turrin N. Cytokine action in the brain. Mol Psychiatr 1999;4:302.
7. Kunz M, Ceresér KM, Goi PD, Fries GR, Teixeira AL, Fernandes BS, *et al*. Serum levels of IL-6, IL-10 and TNF-α in patients with bipolar disorder and schizophrenia: Differences in pro- and anti-inflammatory balance. Rev Bras Psiquiatr 2011;33:268-74.
8. Singh B, Bera NK, Nayak CR, Chaudhuri TK. Decreased serum levels of interleukin-2 and interleukin-6 in Indian Bengalee schizophrenic patients. Cytokine 2009;47:1-5.
9. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: Clinical status and antipsychotic effects. Biol Psychiatry 2011;70: 663-71.
10. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory cytokine alterations in schizophrenia: A systematic quantitative review. Biol Psychiatry 2008;63:801-8.
11. Schwarz MJ, Muller N, Riedel M, Ackenheil M. The Th2-hypothesis of schizophrenia: A strategy to identify a subgroup of schizophrenia caused by immune mechanisms. Med Hypotheses 2001;56:483-6.
12. Freudenreich O, Brockman MA, Henderson DC, Evins AE, Fan X, Walsh JP, *et al*. Analysis of peripheral immune activation in schizophrenia using quantitative reverse-transcription polymerase chain reaction (RT-PCR). Psychiatry Res 2010;176:99-102.
13. Maes M, Bocchio Chiavetto L, Bignotti S, Battisa TG, Pioli R, Boin F, *et al*. Effects of atypical antipsychotics on the inflammatory response system in schizophrenic patients resistant to treatment with typical neuroleptics. Eur Neuropsychopharmacol 2000;10:119-24.
14. Cazzulo CL, Scarone S, Grassi B, Vismara C, Trabattoni D, Clerici M. Cytokines production in chronic schizophrenia patients with or without paranoid behavior. Prog Neuropsychopharmacol Biol Psychiatry 1998;22:947-57.
15. Kamińska T, Wysocka A, Marmurowska-Michalowska H, Dubas-Slemp H, Kandefer-Szerszeń M. Investigation of serum cytokine levels and cytokine production in whole blood cultures of paranoid schizophrenic patients. Arch Immunol Ther Exp (Warsz) 2001;49:439-45.
16. Ozbey U, Tug E, Namli M. Interleukin-10 gene promoter polymorphism in patients with schizophrenia in a region of East Turkey. World J Biol Psychiatry 2009;10:461-8.
17. Thio CL. Host genetic factors and antiviral immune responses to hepatitis C virus. Clin Liver Dis 2008;12:713-26.
18. D'Alfonso S, Rampi M, Rolando V, Giordano M, Momigliano-Richiardi P. New polymorphisms in the IL-10 promoter region. Genes Immun 2000;1:231-3.
19. Mormann M, Rieth H, Hua TD, Assohou C, Roupelieva M, Hu SL, *et al*. Mosaics of gene variations in the interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used. Genes Immun 2004;5:246-55.
20. Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci USA 1998;95: 9465-70.

21. Koss K, Satsangi J, Fanning GC, Welsh KI, Jewell DP. Cytokine (TNF alpha, LT alpha and IL-10) polymorphisms in inflammatory bowel diseases and normal controls: Differential effects on production and allele frequencies. Genes Immun 2000;1:185-90.
22. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet 1997;24:1-8.
23. Yu L, Yang MS, Zhao J. Shi YY, Zhao XZ, Yang JD, *et al*. An association between polymorphisms of the interleukin-10 gene promoter and schizophrenia in the Chinese population. Schizophr Res 2004;71:179-83.
24. Paul-Samojedny M, Kowalczyk M, Suchanek R, Owczarek A, Fila-Danilow A, Szczygiel A, *et al*. Functional polymorphism in the interleukin-6 and interleukin-10 genes in patients with paranoid schizophrenia — A case control study. J Mol Neurosci 2010;42:112-9.
25. Lung FW, Yang MC, Shu BC. The interleukin 10 promoter haplotype ACA and the long-form variant of the DRD4 uVNTR polymorphism are associated with vulnerability to schizophrenia. Psychiatry Res 2011;188:294-6.
26. Gao L, Li Z, Chang S, Wang J. Association of interleukin-10 polymorphisms with schizophrenia: A meta-analysis. PLoS One 2014;9:e90407.
27. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261-76.
28. Johnstone EC, Ebmeier KP, Miller P, Owens DG, Lawrie SM. Predicting schizophrenia: Findings from the Edinburgh high-risk study. Br J Psychiatry 2005;186:18-25.
29. Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-α, TNF-β and TGF-β1 gene polymorphisms. Transpl Immunol 1999;7:127-8.
30. Abanmi A, Al Harthi F, Al Baqami R, Al Assaf S, Zouman A, Arfin M, *et al*. Association of HLA loci alleles and antigens in Saudi patients with vitiligo. Arch Dermatol Res 2006;298: 347-52.
31. Schallreuter KU, Levenig C, Kuhnl P, Loliger C, Hohl-Tehari M, Berger J. Histocompatability antigens in vitiligo: Hamburg study on 102 patients from Northern Germany. Dermatology 1993;187:186-92.
32. Savejgaard A, Platz P, Ryder LP. HLA and disease 1982 — A survey. Immunol Rev 1983;70:193-218.
33. Almoguera B, Riveiro-Alvarez R, Lopez-Castroman J, Dorado P, Lopez-Rodriguez R, Fernandez-Navarro P, *et al*; Spanish Consortium of Pharmacogenetics Research in Schizophrenia. ATA homozigosity in the IL-10 gene promoter is a risk factor for schizophrenia in Spanish females: A case control study. BMC Med Genet 2011;12:81.
34. He G, Zhang J, Li XW, Chen WY, Pan YX, Yang FP, *et al*. Interleukin-10-1082 promoter polymorphism is associated with schizophrenia in a Han Chinese sib-pair study. Neurosci Lett 2006;394:1-4.
35. Bocchio Chiavetto L, Boin F, Zanardini R, Popoli M, Michelato A, Bignotti S, *et al*. Association between promoter polymorphic haplotypes of interleukin-10 gene and schizophrenia. Biol Psychiatry 2002;51:480-4.
36. Jun TY, Lee KU, Pae CU, Chae JH, Bahk WM, Kim KS, *et al*. Polymorphisms of interleukin-4 promoter and receptor gene for schizophrenia in the Korean population. Psychiatry Clin Neurosci 2003;53:283-8.
37. Addas-Carvalho M, Salles TS, Saad ST. The association of cytokine gene polymorphism with febrile non-hemolytic transfusion reaction in multitransfused patients. Transfus Med 2006;19:184-91.
38. Sun J, Jia P, Fanous AH, van den Oord E, Chen X, Riley BP, *et al*. Schizophrenia gene networks and pathways and their applications for novel candidate gene selection. PLoS One 2010;5:e11351.
39. Inoue N, Watanabe M, Wada M, Morita M, Hidaka Y, Iwatani Y. IL-10-592A/C polymorphism is associated with severity of Hashimoto's disease. Cytokine 2013;64:370-4.
40. Qin SY, Jiang HX, Lu DH, Zhou Y. Association of interleukin-10 polymorphisms with risk of irritable bowel syndrome: A meta-analysis. World J Gastroenterol 2013;19:9472-80.
41. Peng H, Wang W, Zhou M, Li R, Pan HF, Ye DQ. Role of interleukin-10 and interleukin-10 receptor in systemic lupus erythematosus. Clin Rheumatol 2013;32:1255-66.
42. Kutluturk F, Yarman S, Sarvan FO, Kekik C. Association of cytokine gene polymorphisms (IL6, IL10, TNF-α, TGF-β and IFN-γ) and Graves' disease in Turkish population. Endocr Metab Immune Disord Drug Targets 2013;13:163-7.
43. Liu P, Song J, Su H, Li L, Lu N, Yang R, *et al*. IL-10 gene polymorphisms and susceptibility to systemic lupus erythematosus: A meta-analysis. PLoS One 2013;8:e69547.
44. Davis LA, Whitfield E, Cannon GW, Wolff RK, Johnson DS, Reimold AM, *et al*. Association of rheumatoid arthritis susceptibility gene with lipid profiles in patients with rheumatoid arthritis. Rheumatology (Oxford) 2014;53:1014-21.
45. Shih CM, Lee YL, Chiou HL, Hsu WF, Chen WE, Chou MC, *et al*. The involvement of genetic polymorphism of IL-10 promoter in non-small cell lung cancer. Lung Cancer 2005;50:291-7.
46. Monneret G, Finck ME, Venet F, Debard AL, Bohe J, Bienvenu J, *et al*. The anti-inflammatory response dominates after septic shock: Association of low moncyte HLA-DR expression and high interleukin-10 concentration. Immunol Lett 2004;95: 193-8.
47. Fernandez L, Martinez A, Mendoza JL, Urcelay E, Fernandez-Arquero M, Garcia-Paredes J, *et al*. Interleukin-10 polymorphisms in Spanish patients with IBD. Inflamm Bowel Dis 2005;11:739-43.
48. Gong MN, Thompson BT, Williams PL, Zhou W, Wang MZ, Pothier L, *et al*. Interleukin-10 polymorphism in position-1082 and acute respiratory distress syndrome. Eur Respir J 2006;27:674-81.
49. Munshi A, Rajeshwar K, Kaul S, Al-Hazzani A, Alshatwi AA, Sai Babu M, *et al*. Interleukin-10-1082 promoter polymorphism and ischemic stroke risk in a South Indian population. Cytokine 2010;52:221-4.
50. Lio D, Candore G, Crivello A, Scola L, Colonna-Romano G, Cavallone L, *et al*. Opposite effects of interleukin 10 common gene polymorphisms in cardiovascular diseases and in successful ageing: Genetic background of male centenarians is protective against coronary heart disease. J Med Genet 2004;41:790-4.
51. Langsenlehner U, Krippl P, Renner W, Yazdani-Biuki B, Eder T, Koppel H, *et al*. Interleukin-10 promoter polymorphism is associated with decreased breast cancer risk. Breast Cancer Res 2005;90:113-5.
52. Shin HD, Park BL, Kim YH, Cheong HS, Lee IH, Park SK. Common interleukin 10 polymorphism associated with decreased risk of tuberculosis. Exp Mol Med 2005;37: 128-32.

53. Malhotra D, Darvishi K, Sood S, Sharma S, Grover C, Relhan V, *et al*. IL-10 promoter single nucleotide polymorphisms are significantly associated with resistance to leprosy. Hum Genet 2005;118:295-300.
54. Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI, *et al*. Genetic influence on cytokine production and fatal meningococcal disease. Lancet 1997;349:170-3.
55. Kimball P, Elswick RK, Shiffman M. Ethnicity and cytokine production gauge response of patients with hepatitis C to interferon- therapy. J Med Virol 2001;65:510-6
56. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin-10. Annu Rev Immunol 1993;11: 165-90.
57. Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, *et al*. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 1991;146:3444-51.
58. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. J Immunol 1991;147:3815-22.
59. Mousa A, Seiger A, Kjaeldgaard A, Bakhiet M. Human first trimester forebrain cells express genes for inflammatory and anti-inflammatory cytokines. Cytokine 1999;11:55-60.
60. Rousset F, Garcia E, Defrance T, Vezzio N, Peronne C, Hsu DH, *et al*. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 1992;89:1890-3.
61. Yue FY, Dummer R, Geertsen R, Hofbauer G, Laine E, Manolio S, *et al*. Interleukin-10 is a growth factor for human melanoma cells and down-regulates HLA class-I, HLA class-II and ICAM-1 molecules. Int J Cancer 1997;71:630-7.
62. de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, *et al*. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via down regulation of class II major histocompatibility complex expression. J Exp Med 1991;174:915-24.
63. Tsuruma T, Yagihashi A, Torigoe T, Sato N, Kijuchi K, Watanabe N, *et al*. Interleukin-10 reduces natural killer sensitivity and down regulates MHC class I expression on H-ras-Transformed cells. Cell Immunol 1998;184:121-8.
64. Meyer U, Murray PJ, Urwyler A, Yee BK, Schedlowski M, Feldon J. Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. Mol Psychiatry 2008;13:208-21.
65. Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, *et al*; Genetic Risk and Outcome in Psychosis (GROUP). Common variants conferring risk of schizophrenia. Nature 2009;460:744-7.

How to cite this article: Al-Asmary SM, Kadasah S, Arfin M, Tariq M, Al-Asmari A. Genetic variants of interleukin-10 gene promoter are associated with schizophrenia in Saudi patients: A case-control study. North Am J Med Sci 2014;6:558-65.

Source of Support: Nil, **Conflict of Interest:** None declared.