ORIGINAL RESEARCH

Inducible Nitric Oxide Synthase iNOS-954-G>C and Ex16+14-C>T Gene Polymorphisms and Susceptibility to Vitiligo in the Saudi Population

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Objective: Vitiligo is an acquired pigmentary skin disorder with regional disappearance of melanocytes. Multigenic inheritance has been proposed in the pathogenesis of vitiligo. The present study aimed to investigate the possible association of inducible nitric oxide synthase polymorphisms iNOS-954-G/C (rs1800482 G>C) and iNOS-Ex16+14-C/T (rs2297518 C>T) with vitiligo in the Saudi population, if any.

Methods: We included 120 vitiligo cases and an equal number of age matched healthy controls. Polymerase chain reaction with restriction fragment length polymorphism method was used for the analysis of genetic polymorphisms.

Results: The heterozygous (GC), (GC + CC) combined genotype and variant allele; C allele of rs1800482 G>C were associated significantly ($p < 0.005$, after Bonferroni correction) with increased risk of vitiligo (OR = 3.46, 95% CI = 1.99–6.01, $p = 0.001$), (OR = 3.30, 95% CI= 1.93–5.65, $p = 0.001$) and (OR = 1.94, 95% CI = 1.31–2.87, $p = 0.001$) respectively. When GC genotype of rs1800482 G>C was co-inherited with common genotype (CC) and heterozygous genotype (CT) of rs2297518 C>T, the risk of vitiligo was significantly increased ((OR = 4.51, 95% CI = 2.18–9.33, $p = 0.001$) and (OR = 3.60, 95% CI = 1.61–8.01, $p = 0.001$)) respectively. None of the rs1800482 G>C and rs2297518 C>T genotypes and alleles have been associated with non-segmental vitiligo in terms of gender, age of onset, and types of vitiligo.

Conclusion: The heterozygous (GC), (GC+CC) combined genotype and variants allele; C allele of rs1800482 G>C, may cause overproduction of NO, which has been linked to melanocyte loss by increasing oxidative stress and decreasing melanocyte adhesion to the extracellular matrix components, and thus could be an associative risk factor for vitiligo.

Keywords: vitiligo, non-segmental, genetic polymorphism, rs1800482 G>C, rs2297518 C>T, nitric oxide, nitric oxide synthase

Introduction

Vitiligo is an acquired multifactorial progressive depigmentary disorder characterized by the appearance of defined white macules resulting from the progressive loss of functional melanocytes from the skin and mucous. It affects 0.1–2% of the world population without sex predilection.[1,2] Although the precise etiology of vitiligo is still illusive the role of autoimmunity, genetics, oxidative stress, auto cytotoxic, neurogenic, viral, apoptotic, cell adhesion disorder, etc. have all been reported.[3–5] The most widely accepted theory of its development is multifactorial according to which genetic changes predispose risk in the presence of favorable environmental conditions i.e. gene-environment interaction may be involved in melanocyte destruction.[1]

The high occurrence rate of vitiligo within the families suggested that genetic risk factors may be one of the predominant causes which is associated with this skin abnormality.[6,7] Genome wide association studies confirmed that more than 40 robust genetic susceptible loci have been associated with vitiligo. Beside this, many more susceptible loci with unknown functions have also been identified in the pathogenesis of vitiligo.[1,8,9] The inducible nitric oxide

synthesize (iNOS) gene of 48Kb is located on the chromosome 17q11.2-q12, which encodes a protein of 131 kDa.[10] Several SNPs in *iNOS* have been revealed, including the G>C in *iNOS*-954 (rs1800482) in the promoter region, which has been linked to increased *iNOS* expression, and the C>T substitution in *iNOS* exon 16 (rs2297518), which induces Ser608Leu amino acid substitution resulting in higher nitric oxide (NO) production[11,12] Oxidative stress mediated toxicity in melanocyte destruction is another widely accepted hypothesis for the development of vitiligo.

The excessive generation of NO may cause increased oxidative stress and imbalance of the antioxidant system in melanocytes resulting in an accumulation of huge toxic intermediate of melanin, breaking the melanocyte redox homeostasis and reducing melanogenesis, all of which contribute to the onset of vitiligo.[13,14] Polymorphism in oxidative stress related genes like inducible nitric oxide synthase (iNOS),[15] glutathioneS-transferase,[16] NF-E2-related factor2,[17] catechol-O-methyltransferase, catalase,[18] and angiotensinogen converting enzymes[19] have previously been associated with a higher risk of vitiligo. The iNOS promoter polymorphisms have shown strong association with vitiligo in Chinese and Egyptian populations.[15,20]

Many factors, including lipopolysaccharides (LPO), cytokines, interleukins, and others, can influence the expression of *iNOS* in normal human melanocytes, affecting NO production.[13,21] Previous studies from our laboratory have shown the association of inflammatory genes SNPs with vitiligo.[22,23] So far, no studies have reported a genetic association of these genes with vitiligo in a Saudi population. Considering the importance of iNOS in NO production and its subsequent role in the pathogenesis of vitiligo, the present study intends to examine the potential association of functional polymorphisms in the iNOS rs1800482 G>C and rs2297518 C>T with vitiligo in the Saudi population.

Materials and Methods

In the present study, a total of a hundred and twenty (n = 120) vitiligo cases and an equal number of controls were recruited from the Dermatology Clinic of the Prince Sultan Military Medical City (PSMMC), Riyadh, Saudi Arabia. Patients who routinely visited the outpatient clinic without any known health risk were recruited as controls. All the subjects were age and sex matched and biologically unrelated Saudis. The ethical clearance was obtained from the institutional ethical clearance committee, PSMMC, Riyadh to conduct this research work. We collected required data of vitiligo in accordance with principles of the Declaration of Helsinki. The informed written consent was obtained from all the participants prior to the recruitment. The patient and control samples were collected after following strict exclusion and inclusion criteria. Only those vitiligo patients were included in this study who were not subject to any therapy in the last six months and had no history of autoimmune disorders. We recruited gender and age matched controls and excluded those with clinical evidence or a family history of a first- or second-degree relative with vitiligo or any autoimmune disorders to minimize genetic heterogeneity. All vitiligo cases recruited in the present study are non-segmental. The patient group consisted of n = 61 males and n = 59 females with age at diagnosis ranging from 6 to 79 years (mean ± SD = 27.04 ± 12.32). Venous blood (3 mL) was drawn in an EDTA coated vial from each subject, brought to the laboratory in ice boxes and stored at −80 °C before extraction of genomic DNA.

Genotyping Analysis

Genomic DNA for genotyping was isolated from whole blood by using commercially available QIAamp DNA mini kit (Qiagen, Venlo, Limburg, the Netherlands) as per the manufacturer's protocol. The quality of DNA was checked on agarose gel and quantitation was done by using Nano Drop-2000 at 260/280 nm (ThermoFisherScientific Inc, Waltham, MA, USA). The gradient PCR (Ependorf, Hamburg, Germany) was used to amplify the fragments that contained the selected *iNOS* polymorphic sites. The specific set of primers were used for the rs1800482 G>C amplification was 5'-CATATGTATGGGAATACTGTATTTCAG-3,5-TCTGAACTAGTCACTTGAGG-3'. DNA amplification was done in a 25 μL reaction mixture consisting of 50 ng of genomic DNA, 10 pM of each primer, 200 μM of dNTPs mixture, 1.5 mM MgCl$_2$, 2.5 μL of 10x PCR buffer (10x 500 mM KCl, 100 mM TrisHCl, pH 9.0) and 1 U *Taq* DNA polymerase (New Englad Biolab Ipswich, MA, USA). Genomic DNA was amplified for 35 cycles. Initial denaturation was applied for 3 minutes at 95 °C. Each cycle consisted of: 95 °C for 30 sec, 59 °C for 30 sec, and 72 °C for 45 sec.

For rs2297518 C>T amplification, the following set of primers was used 5'-CATATGTAAACCAACTTCCGTG-3',5'-GGCAGGGCTAGGAGTAGGAC-3'. The thermal cycler program was similar except the annealing temperature was kept at 60 °C. Both the PCR products obtained were separated by electrophoresis on 2% agarose gel in TBE buffer, visualized by ethidium bromide fluorescence. The PCR product of rs2297518 C>T was purified by gel

band purification kit as per the manufacturer's protocol (GE Healthcare, Buckinghamshire, HP7 9NA, UK). Genotyping has been performed by previously standardized PCR-RFLP method by Shen et al.[12] The PCR product of rs1800482 G>C was digested with BsaI enzyme and iNOS- rs2297518 C>T by MluCI (New England Biolab, Ipswich, MA, USA) for three hours at 37 °C. The restriction fragment was separated by 2.5% and 3% agarose gel electrophoresis to identify the genotypes of rs1800482 G>C and rs2297518 C>T respectively.

Statistical Analysis

The statistical analysis was carried out by using SPSS software, windows version 21.0 (Chicago, IL, USA). The age of patients and onset age (years) of disease were presented as mean ± SD, gender, types, stage, family history of vitiligo and autoimmune immune diseases are presented in frequency and percentage values. We computed odds ratio (OR) and 95% confidence interval (CI) for *iNOS* polymorphisms by univariable logistic regression taking the group as dependent and *iNOS* polymorphic variants as independent covariates. Bonferroni correction was applied to reduce the type-1 error due to multiple comparison testing. The corrected significant p value is <0.005.

Results

In our study, mean age of cases (27.04 ± 12.32 years) are matching with the mean age of controls (26.84 ± 4.89) years. The age of onset of vitiligo was (22.51 ± 15.40) years. The mean value of duration of disease was 6.2 (1–19) years. The proportion of male and female patients with vitiligo was almost the same. The family history of vitiligo and other autoimmune diseases were observed in 15% and 6.67% cases, respectively, the frequency of generalized vitiligo and focal were 33.34% (Table 1). The distribution of the genotypic frequencies of *iNOS*-954 were in Hardy-Weinberg

Table 1 Demographical Characteristics of Vitiligo Patients and Controls

Characteristics	Vitiligo Patients (N = 120)	Controls (N = 120)
Age of patients (years, mean ± SD)	27.04 ± 12.32	28.68 ±12.12
Gender Male Female	 61 (50.83%) 59 (49.16%)	 64 (50%) 56 (50%)
Age of onset (years)	22.51 ± 15.40	NA
Duration of disease (mean age in years)	6.2 (1–19)	
Family history of vitiligo Present Absent	 18/120 (15%) 102/120 (85%)	
History of autoimmune disease Present Absent	 8/120 (6.67%) 112/120 (93.33%)	
Type of vitiligo	N (%)	
Generalized	40 (33.33%)	
Focal	40 (33.33%)	
Acrofacial	23 (19.16%)	
Lip tip	15 (12.5%)	
Universalis	2 (1.66%)	

Note: Figure in parenthesis shows the percentage values.

equilibrium (HWE) in controls (p = 0.901) which implies similar genetic background to the Mendelian population. However, iNOS-954 G/C genotype ($\chi 2$ = 14.84, p < 0.001) in cases was not compatible with HWE (Table 2). In cases, the frequency of rs1800482 G>C common (GG), heterozygous (GC) and homozygous variants (CC) genotypes were 29.16%, 64.16% and 6.68%, while in controls 56.67%, 37.5% and 5.83% respectively. Calculated risk estimation revealed the GC genotype of rs1800482 was found to have a threefold risk of vitiligo in Saudis. However, the frequency of CC genotype was higher in cases but did not reach the level of significance. When, GC genotype was combined with CC genotype of rs1800482 G>C imposes significant risk (OR = 3.30, 95% CI = 1.93–5.65, p = 0.001) of vitiligo in cases as compared to controls. The variants allele; C allele, was found to be significantly (OR = 1.94, 95% CI = 1.34–2.87, p = 0.001) higher in cases as compared to controls (Table 2). Similarly, when rs2297518 C>T genetic polymorphism were evaluated between patients and controls using the CC genotype as a reference group, we observed that CT and TT genotypes were greater in cases but did not meet the level of significance (p > 0.005). Further, no significant difference was observed between cases and controls for the variant allele; T allele of rs2297518 C>T (Table 2). When GC genotype of rs1800482 G>C was co-inherited with the CT genotype of rs2297518 C>T, the risk of vitiligo was significantly increased (OR = 5.07, 95% CI = 2.46–10.43, p =< 0.001) in cases as compared to controls respectively. Similarly, when CC genotype of rs1800482 G>C was co-inherited with the CT genotype of rs2297518 C>T, the risk of vitiligo was also increased significantly (OR = 9.77, 95% CI = 1.02–93.6, p = 0.036) as shown in Table 3. None of the rs1800482 G>C and rs2297518 C>T genotypes have been associated with non-segmental vitiligo in terms of gender, age of onset, and types of vitiligo (Tables 4–6).

Table 2 Genotypic Frequencies of rs1800482 G>C and rs2297518 C>T in Cases and Controls and Their Associations with Risk of Vitiligo

Genotypes/Allele	Controls, N = 120 (%)	Cases, N = 120 (%)	Odds Ratio (OR)	95% CI	p-value	P_{HWE}
rs1800482 G>C						0.754**
GG	68 (56.67)	35 (29.16)	Ref	-	-	0.612***
GC	45 (37.50)	77 (64.16)	3.46	1.99–6.01	0.001*	
CC	7 (5.83)	8 (6.68)	2.28	0.765–6.83	0.139	
GC+CC	52 (43.33)	85 (70.83)	3.30	1.93–5.65	0.001*	
G	181 (75.41)	147 (61.25)	Ref	-		
C	59 (24.58)	93 (38.75)	1.94	1.31–2.87	0.001*	
rs2297518 C>T						0.804**
CC	76 (63.33)	70 (58.33)	Ref	-	-	0.775***
CT	41 (34.17)	46 (38.33)	1.19	0.707–2.03	0.501	
TT	3 (2.50)	4 (3.33)	1.60	0.261–9.90	0.609	
CT+TT	44 (36.67)	50 (41.67)	1.21	0.724–2.04	0.458	
C	193 (80.42)	186 (77.5)	Ref	-		
T	47 (19.58)	54 (22.5)	1.19	0.767–1.85	0.501	

Notes: The Binary logistic regression analysis was performed for variants analysis of rs1800482 G>C and rs2297518 C>T, GG & CC = common genotypes, GC & CT = heterozygous genotypes, CC & TT = homozygous variants genotypes. After Bonferroni correction, p*< 0.005 was declared statistically significant. ^{P}HWE <0.05 are not consistent, **^{P}HWE for controls, ***^{P}HWE for patients, figure in parenthesis indicate percent values.

606

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Pharmacogenomics and Personalized Medicine 2022:15

Table 3 Combined Genotypes Analysis of rs1800482 G>C and rs2297518 C>T in Cases and Controls

Combined Genotypes of rs1800482 and rs2297518	Controls, N = 120 (%)	Cases, N = 120 (%)	OR	95% CI	p- value
GG/CC	44 (36.67)	18 (15.0)	Ref	-	-
GG/CT	22 (18.33)	15 (12.5)	1.66	0.708–3.91	0.274
GG/TT	2 (1.67)	1 (0.83)	1.22	0.104–14.33	1.00
GC/CC	26 (21.67)	48 (40)	4.51	2.18–9.33	0.001*
GC/CT	19 (15.83)	28 (23.33)	3.60	1.61–8.01	0.001*
GC/TT	0	2 (1.67)	ND	-	-
CC/CC	6 (5.0)	4 (3.33)	1.62	0.410–6.47	0.713
CC/CT	1 (0.83)	4 (3.33)	9.77	1.02–93.6	0.036
CC/TT	0	0	ND	-	-

Notes: The Chi square test was performed for polymorphic analysis of combined genotypes, GG & CC = common genotypes, GC & CT = heterozygous genotypes, CC & TT = homozygous variants genotypes, *$p < 0.005$ was considered statistically significant after Bonferroni correction, figure in parenthesis indicate percent values.

Table 4 Comparison of Genotypic Frequencies of rs1800482 G>C and rs2297518 C>T in Male and Female Cases of Vitiligo

Genotypes/Allele	Male N = 61 (%)	Female N = 59 (%)	OR	95% CI	p- value
rs1800482 G>C					
GG	16 (26.22)	18 (30.50)	Ref		
GC	42 (68.85)	37 (62.71)	1.27	0.57–2.85	0.378
CC	03 (4.91)	04 (6.78)	0.843	0.16–4.35	0.881
GC+CC	45 (73.78)	41 (69.49)	1.23	0.56–2.73	0.375
G	74 (60.66)	73 (61.86)	Ref		
C	48 (39.34)	45 (38.13)	1.05	0.62–1.77	0.476
rs2297518 C>T					
CC	35 (57.38)	36 (61.02)	Ref		
CT	25 (40.98)	20 (33.90)	1.28	0.60–2.72	0.320
TT	01 (1.63)	03 (5.08)	0.34	0.034–3.42	0.338
CT+TT	26 (42.62)	23 (38.98)	1.16	0.56–2.41	0.413
C	95 (77.87)	92 (77.97)	Ref		
T	27 (22.13)	26 (22.03)	1.05	0.54–1.185	0.554

Notes: The Binary logistic regression analysis was performed for polymorphic analysis of rs1800482 G>C and rs2297518 C>T, GG & CC = common genotypes, GC & CT = heterozygous genotypes, CC & TT = homozygous variant genotypes, $p < 0.005$ was considered statistically significant (after Bonferroni correction), figure in parenthesis indicate percent values.

Table 5 Comparison of Genotypic Frequencies of rs1800482 G>C and rs2297518 C>T Between Two Groups of Onset Age in Vitiligo Patients

Genotypes/Allele	≤ 30 Years N = 57 (%)	>30 Years N = 63 (%)	OR	95% CI	p- value
rs1800482 G>C					
GG	20 (35.09)	23 (36.50)	Ref		
GC	32 (56.14)	33 (52.38)	1.11	0.515–2.41	0.468
CC	05 (8.77)	07 (11.12)	0.821	0.225–2.99	0.514
GC+CC	37 (64.91)	40 (63.49)	1.06	0.503–2.24	0.511
G	72 (63.15)	79 (62.69)	Ref		
C	42 (36.84)	47 (37.30)	0.980	0.580–1.77	0.524
rs2297518 C>T					
CC	38 (66.67)	35 (55.56)	Ref		
CT	15 (26.32)	23 (36.50)	0.600	0.270–1.38	0.144
TT	04 (7.01)	05 (7.94)	0.736	0.83–2.96	0.468
CT+TT	19 (33.34)	28 (44.45)	0.625	0.297–1.31	0.145
C	91 (79.82)	93 (73.81)	Ref		
T	23 (20.18)	33 (26.19)	0.712	0.388–1.30	0.171

Notes: The Binary logistic regression analysis was performed for polymorphic analysis of rs1800482 G>C and rs2297518 C>T, GG & CC = common genotypes, GC & CT = heterozygous genotypes, CC & TT = homozygous variant genotypes, $p < 0.005$ was considered statistically significant (after Bonferroni correction), figure in parenthesis indicate percent values.

Discussion

The expression of the iNOS gene is regulated at the level of transcription. The presence of variation in the regulatory promoter region affects the gene product in the cell, while variation in the coding region bring structural changes in proteins and its activity. This change in gene product level and protein molecule structure could be the source of genetic predisposition in many human diseases.[24,25] The promoter polymorphisms rs1800482 G>C have been shown to modify iNOS transcription and mRNA levels[26,27] whereas the rs2297518 C>T exon 16 polymorphism, which causes an amino acid substitution from serine to leucine, may be of functional interest in vitiligo.[28]

In our study, when GG genotype of rs1800482 was considered as a reference group, the frequency of GC genotype was found significantly higher in cases as compared to controls. In addition, the combined (GC+CC) genotypes and variant allele; C allele of rs1800482 were also significantly associated with increased risk of vitiligo (Table 2). Our findings demonstrate that the risk factors aggregation may amplify the risk of vitiligo in the Saudi population. Similar to our study, Zayed et al,[20] reported that GC genotype of rs1800482 may increase the risk of vitiligo by 3.35 times while combined (GC+CC) genotype of *iNOS*-954 may increase it by 3.02 times, when compared to controls in Egyptians. They also reported variants allele; C allele was significantly higher and a 2.21 fold risk of vitiligo. In another study, Zhang et al,[15] did not find any significant association of GC genotype of rs1800482 with cases in the Chinese population while they have reported combined (GC+CC) genotype may increase by 1.36 times the risk of vitiligo. In their study, C allele was also reported to be significantly associated with the cases. The difference in genotypic frequency of rs1800482 in our study with Chinese and similarity with Egyptians populations, indicate that the genotypic distribution of the rs1800482 G>C polymorphism varies by ethnicity.

When we considered the CC genotype of rs2297518 C>T as a reference group, we did not find any significant difference for individuals with CT and TT or combined (CC+TT) genotypes between cases and controls (Table 2). Our observations are in accordance with the previous genotypic frequencies of rs2297518 reported among Chinese, Spanish

Table 6 Comparison of Genotypic Frequencies of rs1800482 G>C and rs2297518 C>T Among Different Types of Vitiligo
Type of Vitiligo

	Generalized N = 40	Focal N = 40	Acrofacial N = 23	Lip Tip N = 15	Universalis N = 2	p- value
rs1800482G>C						
GG	09 (22.50)	07 (17.50)	06 (26.08)	03 (20.00)	0.0 (0.00)	0.885
GC	28 (70.00)	27 (67.50)	15 (65.21)	10 (66.67)	01 (50.00)	
CC	03 (7.50)	06 (15.00)	02 (08.71)	02 (13.33)	01 (50.00)	
GC+CC	31 (77.50)	33 (82.50)	17 (73.91)	12 (80.00)	02 (100.0)	0.920
G	46 (57.50)	41 (51.25)	27 (58.70)	16 (53.33)	01 (25.00)	0.646
C	34 (42.50)	39 (48.75)	19 (41.30)	14 (46.67)	03 (75.00)	
rs2297518 C>T						
CC	8 (20.00)	10 (25.00)	05 (21.73)	02 (13.33)	01 (50.00)	0.862
CT	30 (75.00)	26 (65.00)	16 (69.57)	12 (80.00)	01 (50.00)	
TT	02 (5.00)	04 (10.00)	02 (8.69)	01 (6.67)	0.0 (0.00)	
CT+TT	32 (80.00)	23 (57.50)	18 (78.26)	13 (86.67)	01 (50.00)	0.624
C	46 (57.50)	46 (57.50)	26 (56.52)	16 (53.33)	02 (50.00)	0.841
T	34 (42.50)	34 (42.50)	20 (43.48)	14 (46.67)	02 (50.00)	

Notes: Fisher's exact test was performed for polymorphic analysis of rs1800482 G>C and rs2297518 C>T, GG & CC = common genotypes, GC & CT = heterozygous genotypes, CC & TT = homozygous variant genotypes, $p < 0.005$ was considered statistically significant (after Bonferroni correction), figure in parenthesis indicate percent values.

and Japanese populations.[12,15,27] In contrast to our findings, Zayed et al,[20] found significant associations of CT and combined (CT+TT) genotypes of rs2297518 with vitiligo in Egyptian patients. Further, they have also reported a significantly high frequency of allelic variant; T allele in cases as compared to controls.

In the present study, when rs1800482 GC genotype was co-inherited with CC and CT genotypes of rs2297518, the risk of vitiligo was further increased significantly by 4.51 and 3.60 times in cases as compared to controls, respectively. When the CC genotype of rs1800482 was co-inherited with the CT genotype of rs2297518, the risk increased 9.77 times however, the data became non-significant after Bonferroni correction ($p < 0.005$), as shown in Table 3. Similar findings were reported by Zayed et al[20] while Zhang et al[15] identified a substantial difference only when the GC genotype of rs1800482 was co-inherited with the CC genotype of rs2297518. We found no significant association between non-segmental vitiligo and any genotype of rs1800482 G>C and rs2297518 C>T in terms of gender, onset age, and types of vitiligo. Zhang et al[15] noticed a similar outcome in the Chinese population. In the Egyptian population, however, rs1800482 G>C were found to be significantly associated with female patients when compared to its counterpart in controls.[20] Our data showed that there are no haplotypes or LD, and one of the markers did not follow the Hardy-Weinberg formula. The reported spacing between markers is approximately 31.9 kb.

The role of NO in the inhibition of cell proliferation, differentiation and death, and thus possible contribution in the pathogenesis of autoimmune disorders has been reported previously. In addition, as a regulatory mediator, NO has been linked to the onset of autoimmune disease.[29] A study was carried out in the south Indian population to evaluate the association of the *iNOS* promoter polymorphism with rheumatoid arthritis (RA). They found that iNOS-277A/G and −1026 G/T promoter's polymorphism may increase the risk of RA.[30] Also, Sato et al, 2016 suggested the importance of the iNOS genotype in the clinical application of FeNO for asthmatic patients.[31]

The association of rs1800482 G>C promoter gene polymorphisms with vitiligo in our study may have a greater significance because variations in the regulatory promoter region of a gene can directly affect the level of gene product,

subsequently it may play a causative role in the development of diseases. Several hypotheses have been proposed to understand the pathophysiology of vitiligo, however the actual mechanism underlying the onset of vitiligo remains unknown. The rs1800482 G>C promoter polymorphism is linked with the increased iNOS expression and also the C/T substitution in exon 16 causing an amino acid substitution (Ser608Leu), resulting in increased production of NO.[32,33] Furthermore, C allele of *iNOS* may have a higher affinity to bind with DNA binding protein as compared to G allele therefore, the transcriptional activity and the mRNA expression will be higher for C allele than G allele which ultimately increases the iNOS activity and NO production.[15] The increased iNOS activity triggers overproduction of NO and O^{-2} which leads to accumulation of hydrogen peroxide (H_2O_2) and exposure of melanocytes to H_2O_2 results in their destruction and depigmentation. Tetrahydrobiopterin, a cofactor, is essential for the production of NO by all nitric oxide synthase enzymes. Defective recycling of 6-tetrahydrobiopterin increases H_2O_2 production and decreases catalase levels.[34] In vitiligo, an increase in iNOS activity caused by the overexpression of tetrahydrobiopterin or LPS/cytokines resulted in a large amount of NO production. Nitric oxide has been linked to melanocyte loss by increasing oxidative stress and decreasing melanocyte adhesion to the extracellular matrix component, which could be a risk factor for vitiligo.[35,36] The identification of genetic susceptibility variants in vitiligo will lead to a better understanding of the pathophysiology of the disease and also enable us to screen the high risk patients at the early stages of the disease, development of new diagnostic markers, and treatment strategies.

Conclusion

Our findings supported the genetic basis of vitiligo. The present study clearly demonstrates the association of rs1800482 G>C polymorphism with vitiligo and it could be used as a genetic marker for screening vitiligo patients among Saudis. The functional polymorphisms could be an activator of gene expression therefore, our findings may be useful in exploring the molecular mechanism underlying the onset of vitiligo. Further study with a larger sample size, estimation of iNOS activity, oxidative stress parameters, and gene environment interaction will strengthen the current findings.

Ethical Approval

Institutional ethical approval has been taken to conduct this research study.

Disclosure

The authors declare that they have no conflict of interests in relation to this work.

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