Molecular Vision 2016; 22:311-318 <http://www.molvis.org/molvis/v22/311>
Received 4 November 2015 | Accepted 1 April 2016 | Published 3 April 2016

Association of a *NOS3* gene polymorphism with Behçet's disease but not with Vogt-Koyanagi-Harada syndrome in Han Chinese

Yan Zhou,[1,2] Hongsong Yu,[1] Shengping Hou,[1] Jing Fang,[1] Jieying Qin,[1] Gangxiang Yuan,[1] Aize Kijlstra,[3] Peizeng Yang[1]

(The first two authors contributed equally to this work)

[1]The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing, P. R. China; [2]Department of Ophthalmology, Yongchuan Hospital, Chongqing Medical University, Chongqing, China; [3]University Eye Clinic Maastricht, Maastricht, The Netherlands

Purpose: Previous studies have identified that *nitric oxide synthase (NOS)* genes are associated with several immune-mediated diseases. This study aimed to investigate whether *NOS2* and *NOS3* gene polymorphisms are associated with Behçet's disease (BD) and Vogt-Koyanagi-Harada (VKH) syndrome in a Han Chinese population.
Methods: An association analysis of *NOS2*/rs4795067, *NOS3*/rs1799983 and *NOS3*/rs1800779 was performed in 733 patients with BD, 800 patients with VKH syndrome, and 1,359 controls using PCR restriction fragment length polymorphism (PCR-RFLP) assay. Statistical analysis was performed with the chi-square test followed by the Bonferroni correction.
Results: The result showed a decreased frequency of the *NOS3*/rs1799983 GG genotype and an increased frequency of *NOS3*/rs1799983 GT genotype in the patients with BD (Bonferroni correction test [Pc]=0.02, odds ratio [OR]=0.74; Pc=2.1×10^{-3}, OR=1.57, respectively). No significant association was found between rs1799983 and VKH syndrome. *NOS2*/rs4795067 and *NOS3*/rs1800779 were not associated with either BD or VKH syndrome.
Conclusions: Our findings suggest that a *NOS3*/rs1799983polymorphism is associated with susceptibility to BD in Han Chinese.

Uveitis is characterized by intraocular inflammatory disease and can be caused by infectious or non-infectious mechanisms. This immune-mediated disease has occasional systemic involvement and is an important cause of blindness [1,2]. Two important uveitis entities with systemic involvement are Vogt-Koyanagi-Harada (VKH) syndrome and Behçet's disease (BD). VKH syndrome is characterized by bilateral ocular involvement, sunset glow fundus, choroiditis, headache, tinnitus, dysacusis, neck rigidity, pleocytosis, alopecia, leukotrichia, and vitiligo [3]. VKH syndrome mainly occurs in individuals with dark skin pigmentation, such as Asians, Native Americans, and Hispanics and is rare in Caucasians. Several studies have suggested that the *HLA-DR4* (Gene ID: 3126), *HLA-DRB1/DQA1* (Gene ID: 3123, OMIM: 142857), *CTLA-4* (Gene ID: 1493, OMIM:123890), *IL*-17F (Gene ID: 112744, OMIM: 606496), *miRNA-182* (Gene ID: 406958, OMIM: 611607), and *FAS* (Gene ID: 355, OMIM: 134637) genes are associated with VKH syndrome [4-9]. BD is characterized by recurrent uveitis, eye lesions, skin lesions, positive pathergy test, retinal vasculitis, arthritis, and oral and genital mucous ulcers. BD mainly occurs in countries along the ancient Silk Road with a frequency of 80–370 cases per 100,000 population in Turkey and 10/100,000 in Japan and is not common in Caucasians (0.6/100,000 in the United Kingdom) [10]. The exact reason for this situation is not yet clear, but it might be caused by environmental factors. Additionally, a local genetic predisposition may play an important role. Previous studies have shown that *HLA* genes, such as *HLA-B51 (*Gene ID: 3106, OMIM:142830), non-HLA genes, such as *IL23R* (Gene ID: 149233, OMIM: 607562)-*IL12RB2* (Gene ID: 3595, OMIM: 601642), *IL-10* (Gene ID: 3586, OMIM: 124092), *STAT4* (Gene ID: 6775, OMIM: 600558), *miRNA-146a* (Gene ID: 406938, OMIM: 610566), *DHCR7* (Gene ID: 1717, OMIM: 602858), *PDGFRL* (Gene ID: 5157, OMIM: 604584), *miRNA-182*, and *FAS* genes predispose individuals to the occurrence of BD [8,9,11-16].

Since uveitis often leads to visual impairment, it is essential to control the intraocular inflammation as soon as possible. Research directed at unraveling the various pathways of inflammation operative in the eye may lead to new therapies. The analysis of immunogenetic associations with uveitis may help to identify the role of various inflammatory or immune response–related factors in this disease. Gene

Correspondence to: Peizeng Yang, The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing, P. R. China, 400016; Phone: 0086-23-89012851; FAX: 0086-23-89012851; email: peizengycmu@126.com.

polymorphisms that have recently received a great deal of attention in the pathogenesis of autoimmune disease include the nitric oxide synthase (NOS) family. NOS catalyzes the production of NO from L-arginine. The NOS family has three well-known isoforms: neuronal NOS (nNOS, NOS1, Gene ID: 4842, OMIM: 163731), inducible NOS (iNOS, NOS2, Gene ID: 4843, OMIM: 163730), and endothelial (eNOS, NOS3, Gene ID: 4846, OMIM: 163729). NOS1 and NOS3 can rapidly produce small amounts of NO. Its function is mostly physiologic and is short-lived. However, NOS2 produces large amounts of potentially toxic NO that can persist for longer periods [17]. NO generated by NOS2 is important in immune processes and is increased after chronic inflammatory and immunologic stimuli. Its cytotoxic and cytostatic effects can attack abnormal and healthy cells [18]. Various studies have addressed the role of *NOS1*, *NOS2*, and *NOS3* polymorphisms in a large number of autoimmune diseases, such as BD [19], rheumatoid arthritis [20], systemic lupus erythematosus [21], type 1 diabetes mellitus [22], multiple sclerosis [23], psoriasis [24], vitiligo [17], and non-Hodgkin's lymphoma [25].

The role of *NOS* gene polymorphisms has not yet been reported in patients with uveitis and was therefore the subject of the study presented here. We chose two relatively common uveitis entities observed in China to obtain a sufficient sample size, BD and VKH syndrome. In this study, the potential association of *NOS2*/rs4795067 (intronic variant), *NOS3*/rs1799983 (coding variant), and *NOS3*/rs1800779 (intronic variant) polymorphisms with VKH syndrome and BD was investigated in a Han Chinese population. Of the combinations tested, only rs1799983 was shown to be significantly involved in the genetic susceptibility of BD in Han Chinese.

METHODS

Case–control cohorts: A case–control study was performed including 733 patients with BD, 800 patients with VKH syndrome, and 1,359 healthy controls. All patients and controls were Han Chinese and were recruited at the Zhongshan Ophthalmic Center of Sun Yat-sen University (Guangzhou, China) and the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) from April 2005 to November 2014. The diagnosis of BD and VKH syndrome was based on the criteria of the International Study Group for BD [26] and VKH syndrome [27], respectively. All participants provided written informed consent, and the study was approved by the Clinical Research Ethics Committee of the Zhongshan Ophthalmic Center of Sun Yat-sen University and the First Affiliated Hospital of Chongqing Medical University (Permit Number: 2009–201008) and adhered to the tenets of the Declaration of Helsinki as well as the ARVO statement on human subjects.

Genotyping: Peripheral blood from the patients and controls was obtained from the elbow vein, collected in vacuum blood tubes with EDTA, and cryopreserved at −20 °C. DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. Single nucleotide polymorphism (SNP) genotyping was performed using the PCR restriction fragment length polymorphism (PCR-RFLP) method. The 7 µl PCR mixtures contained 10 ng of DNA, 0.25 µM of each primer, 3 µl Gotaq® Green Master Mix (Promega, Madison, WI), and 2 µl Nuclease-Free Water (Promega). The PCR amplification conditions were as follows: 95 °C for 5 min, 37–43 cycles of 95 °C for 30 s, 58–65 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min, following storage at 4 °C (Table 1). The PCR products (7 µl) were digested with 3U restriction endonuclease (Table 1) and incubated at 37 °C for 16 h. The digested fragments were analyzed with electrophoresis on a 4% agarose gel, which were stained with GoldView™ (SBS Genetech, Beijing, China). DNA bands were analyzed by Vilber Lourmat (Marne la Vallée, France) under ultraviolet (UV) light. Approximately 5% of the samples were randomly selected for direct sequencing to check the accuracy of the PCR-RFLP method used in the study. All SNPs tested in the study showed a genotyping success rate greater than or equal to 95% and accuracy greater than 99% in the case and control groups.

Statistical analysis: SHEsis software was used to test whether the experimental data were in accordance with Hardy–Weinberg equilibrium. The chi-square test was used to compare the genotype and allele frequencies between patients and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated with SPSS version 17.0 (Chicago, IL). To account for multiple testing, p values were corrected with the Bonferroni correction (Pc). A Pc of less than 0.05 was considered statistically significant.

RESULTS

Clinical features of patients with BD and patients with VKH syndrome: The detailed clinical characteristics of the patients with BD, patients with VKH syndrome, and healthy controls are shown in Table 2. The genotype frequencies of rs4795067and rs1800779 were in accordance with the Hardy–Weinberg equilibrium in the controls ($\chi2=2.97$, $p=0.09$; $\chi2=1.92$, $p=0.17$, respectively). However, it appeared that rs1799983 deviated from the Hardy–Weinberg equilibrium ($p<0.05$).

Table 1. Primers, restriction enzymes and PCR conditions used to analyze the nitric oxide synthase (NOS)2 and NOS3 polymorphism.

Gene	SNP ID	Primer (5'-3')	Restriction enzyme	Restriction fragment	Tm (°C)
NOS2 (iNOS)	rs4795067	F: TAATCCCAGCCAGAGAGA CG	KspAI (MBI)	A:117 G:23, 94bp	64
		R: AGCCTGGCACATACTTGAAGGTTAA			
	rs1799983	F: CATGAGGCTCAGCCCCAGAA	SduI (MBI)	T:206bp G:124, 82bp	60
		R: AGTCAATCCCTTTGGTGCTCAC			
NOS3 (eNOS)	rs1800779	F: TCTGCCTCTCCCAGTCTCTCA	BccI (NEB)	A:189bp G:124,65bp	65
		R: AGCACTCTCCAGGCACTTCAG			

Table 2. The clinical features in BD patients, VKH syndrome patients and healthy controls.

Disease	Clinical features	Total	%
Patients with BD		733	100
	Mean age ±SD	29.6±10.2	
	Male	628	85.7
	Female	105	14.3
	Uveitis	733	100
	Oral ulcer	733	100
	Genital ulcer	410	55.9
	Skin lesions	526	71.8
	Arthritis	115	15.7
	Positive pathergy test	167	22.8
	Retinal vasculitis	40	5.5
Patients with VKH syndrome		800	
	Mean age ±SD	38.0±12.3	
	Male	442	55.3
	Female	358	44.7
	Uveitis	800	100
	Sunset-like eyes	451	56.4
	Neck stiffness	88	22
	Headache	329	41.1
	Tinnitus	367	45.9
	Hearing loss	258	32.2
	Vitiligo	140	17.5
	Alopecia	311	38.9
	Poliosis	296	37
Controls		1359	
	Mean age±SD	39.1±10.8	
	Male	762	56.1
	Female	597	43.9

Table 3. Allele and genotype frequencies of rs4795067, rs1799983 and rs1800779 in BD.

SNPs	Genotype / Allele	BD patients n(freq)	Controls n(freq)	P value	Pc value	OR(95%CI)
NOS2/rs4795067	AA	484(0.66)	929(0.68)	0.28	NS	0.90(0.74–1.09)
	AG	231(0.32)	400(0.29)	0.32	NS	1.10(0.91–1.34)
	GG	18(0.03)	30(0.02)	0.72	NS	1.12(0.62–2.02)
	A	1199(0.82)	2258(0.83)	0.29	NS	0.92(0.78–1.08)
	G	267(0.18)	460(0.17)	0.29	NS	1.09(0.93–1.29)
NOS3/rs1799983	GG	509(0.69)	1027(0.76)	2.5×10^{-3}	0.02	0.74(0.60–0.90)
	GT	143(0.20)	182(0.13)	2.3×10^{-4}	2.1×10^{-3}	1.57(1.23–1.99)
	TT	81(0.11)	150(0.11)	0.99	NS	1.00(0.75–1.33)
	G	1161(0.79)	2236(0.82)	0.02	NS	0.82(0.70–0.96)
	T	305(0.21)	482(0.18)	0.02	NS	1.22(1.04–1.43)
NOS3/rs1800779	AA	658(0.90)	1201(0.88)	0.33	NS	1.15(0.86–1.54)
	AG	67(0.09)	150(0.11)	0.18	NS	0.81(0.60–1.10)
	GG	8(0.01)	8(0.01)	0.21	NS	1.86(0.70–4.99)
	A	1383(0.94)	2552(0.94)	0.56	NS	1.08(0.83–1.42)
	G	83(0.06)	166(0.06)	0.56	NS	0.92(0.70–1.21)

Pc, Bonferroni corrected p value; OR, odds ratio; NS, not significant; SNP, single nucleotide polymorphism.

Table 4. Allele and genotype frequencies of rs4795067, rs1799983 and rs1800779 in VKH patients and controls.

SNPs	Genotype / Allele	VKH patients n(freq)	Controls n(freq)	P value	Pc value	OR(95%CI)
NOS2/rs4795067	AA	513(0.64)	929(0.68)	0.04	NS	0.83(0.69–0.10)
	AG	261(0.33)	400(0.29)	0.12	NS	1.16(0.96–1.40)
	GG	26(0.03)	30(0.02)	0.14	NS	1.49(0.87–2.54)
	A	1287(0.80)	2258(0.83)	0.03	NS	0.84(0.71–0.98)
	G	313(0.20)	460(0.17)	0.03	NS	1.19(1.02–1.40)
NOS3/rs1799983	GG	584(0.73)	1027(0.76)	0.19	NS	0.87(0.72–1.07)
	GT	138(0.17)	182(0.13)	0.02	NS	1.35(1.06–1.72)
	TT	78(0.10)	150(0.11)	0.35	NS	0.87(0.65–1.16)
	G	1306(0.82)	2236(0.82)	0.60	NS	0.96(0.82–1.12)
	T	294(0.18)	482(0.18)	0.60	NS	1.04(0.89–1.23)
NOS3/rs1800779	AA	729(0.91)	1201(0.88)	0.05	NS	1.35(1.01–1.81)
	AG	68(0.09)	150(0.11)	0.06	NS	0.75(0.55–1.01)
	GG	3(0.004)	8(0.01)	0.50	NS	0.64(0.17–2.40)
	A	1526(0.95)	2552(0.94)	0.04	NS	1.34(1.01–1.78)
	G	74(0.05)	166(0.06)	0.04	NS	0.75(0.56–0.99)

Pc, Bonferroni corrected p value; OR, odds ratio; NS, not significant; SNP, single nucleotide polymorphism.

The genotype and allele frequency distribution of NOS2 and NOS3 in BD and VKH syndrome: Genotyping for the three SNPs in the patients with BD, patients with VKH, and healthy controls was performed with PCR-RFLP. The results showed that the genotype frequencies of *NOS3*/rs1799983 were significantly different between the patients with BD and the healthy controls (Appendix 1, Figure S1, Table 3). The frequency of the heterozygous GT genotype was significantly higher in patients with BD (Pc=2.1×10^{-3}, OR=1.57; Table 3). No statistical differences for the genotype and allele frequencies of*NOS2*/rs4795067 and *NOS3*/rs1800779 were found in the BD group (Pc>0.05; Table 3). Furthermore, no association between the three SNPs and VKH syndrome was detected (Pc>0.05; Table 4).

Stratified analysis for NOS3/rs1799983 with main clinical manifestations of BD: A stratified analysis was conducted to investigate the association of rs1799983 with the main clinical manifestations of BD. They included arthritis, skin lesions, genital ulcer, positive pathergy reaction, and retinal vasculitis. We could not find a significant association of the genotype frequency of *NOS3*/rs1799983 with any clinical manifestation of BD (Table 5).

Table 5. Main effects of rs1799983 on clinical feature risk of BD,

Clinical features	Genotype / Allele	BD with n(freq)	BD without n(freq)	P value	Pc value	OR(95%CI)
Genital ulcer		n=410	n=323			
	GG	274(0.67)	235(0.73)	0.08	NS	0.75 (0.55–1.04)
	GT	88(0.21)	55(0.17)	0.13	NS	1.33 (0.92–1.94)
	TT	48(0.12)	33(0.10)	0.52	NS	1.17 (0.73–1.86)
	G allele	636(0.78)	525(0.81)	0.08	NS	0.80 (0.62–1.03)
	T allele	184(0.22)	121(0.19)	0.08	NS	1.26 (0.97–1.62)
Skin lesions		n=526	n=207			
	GG	361(0.69)	148(0.71)	0.45	NS	0.87 (0.61–1.24)
	GT	102(0.19)	41(0.20)	0.90	NS	0.97 (0.65–1.50)
	TT	63(0.12)	18(0.09)	0.20	NS	1.43 (0.82–2.48)
	G allele	824(0.78)	337(0.81)	0.19	NS	0.83 (0.62–1.10)
	T allele	228(0.22)	77(0.19)	0.19	NS	1.21 (0.91–1.62)
Arthritis		n=115	n=618			
	GG	86(0.75)	423(0.68)	0.18	NS	1.37 (0.87–2.15)
	GT	19(0.16)	124(0.20)	0.38	NS	0.79 (0.46–1.34)
	TT	10(0.09)	71(0.12)	0.77	NS	0.90 (0.45–1.80)
	G allele	191(0.83)	970(0.79)	0.12	NS	1.34 (0.93–1.95)
	T allele	39(0.17)	266(0.22)	0.12	NS	0.75 (0.51–1.08)
Positive pathergy test		n=167	n=566			
	GG	120(0.72)	389(0.69)	0.44	NS	1.16 (0.79–1.70)
	GT	33(0.20)	110(0.19)	0.93	NS	1.02 (0.66–1.58)
	TT	14(0.08)	67(0.12)	0.21	NS	0.68 (0.37–1.25)
	G allele	273(0.82)	888(0.78)	0.19	NS	1.23 (0.90–1.68)
	T allele	61(0.18)	244(0.22)	0.19	NS	0.81 (0.60–1.11)
Retinal vasculitis		n=40	n=693			
	GG	27(0.67)	482(0.70)	0.78	NS	0.91 (0.46–1.80)
	GT	10(0.25)	133(0.19)	0.37	NS	1.40 (0.67–2.94)
	TT	3(0.08)	78(0.11)	0.46	NS	0.64 (0.19–2.12)
	G allele	64(0.80)	1097(0.79)	0.86	NS	1.05 (0.80–1.85)
	T allele	16(0.20)	289(0.21)	0.86	NS	0.95 (0.54–1.67)

Pc, Bonferroni corrected p value; OR, odds ratio; NS, not significant; SNP, single nucleotide polymorphism.

DISCUSSION

In this study, we examined the association between *NOS2*/rs4795067, *NOS3*/ rs1799983, and *NOS3*/rs1800779 gene polymorphisms with BD and VKH syndrome and found that the frequency of the *NOS3*/rs1799983 GT genotype is significantly increased in patients with BD. Individuals with this genotype show an odds ratio of 1.57 of developing BD. An association was found only with BD and not with VKH syndrome perhaps because the immunopathogenesis of the two uveitis entities differs markedly. BD is currently seen as an autoinflammatory disease caused by an exaggerated response to microbial stimuli, whereas VKH syndrome is an autoimmune disease that is directed against melanocytes [28,29]. Vasculitis is the main feature of BD, and the endothelial nitric oxide synthase (eNOS=NOS3) Glu298Asp polymorphism (rs1799983) may play an important role in the vascular response to inflammatory triggers in the blood vessel wall.

Comparison of NOS3/rs1799983 genotype frequencies in patients with BD uveitis with a specific extraocular symptom with those without the symptom did not reveal significant differences. This result indicates that the observed association is not restricted to a certain subgroup of BD uveitis cases. It would be interesting to study the NOS3 polymorphisms in patients with BD without uveitis to see whether the association is confined to ocular BD cases. NOS3 (eNOS) has unique functions in the eye in that it may play an important role in the breakdown of the retinal–blood barrier following an inflammatory stimulus. It may be possible that certain *NOS3 gene* polymorphisms may affect eNOS expression in ocular blood vessels. Further studies are needed to validate this hypothesis.

We chose the candidate SNPs (*NOS2*/rs4795067, *NOS3*/rs1799983, rs1800779) based on earlier reports concerning the association of NOS2 and NOS3 SNPs with autoimmune disease [17,19-22,24]. Our previous genome-wide association study (GWAS) and replication studies for BD were focused on SNPs with an association p value of less than 1.0×10^{-4}. Although the SNPs in *NOS2* and *NOS3* did not reach the threshold p value smaller than 1.0×10^{-4}, the SNPs in these two genes showed a suggestive association with BD ($p<0.05$). Our findings in BD are in agreement with earlier findings from Korea and Italy [19,30]. Analysis of rs1799983 in 65 Korean patients with BD and 80 controls showed that the frequency of the GT genotype and T allele were significantly higher in the patients with BD than in the controls ($Pc=6.0\times10^{-3}$; $Pc=6.0\times10^{-3}$; separately) [19]. A study concerning rs1799983 among 73 Italian patients with BD and 135 controls also showed that the GT genotype was significantly associated with BD ($Pc=9.0\times10^{-5}$) [30]. This study also showed that the T allele was significantly higher in patients with BD compared with healthy controls ($Pc=6.0\times10^{-4}$). However, several studies were not able to find an association between BD and rs1799983 [31,32]. The discrepancies between the studies might be due to the small sample size resulting in insufficient statistical power.

The *NOS3* gene is located on chromosome 7q35–36 and contains 26 exons [33]. The variant of the *NOS3* gene can result in deficient expression of NOS that may subsequently lead to disease [34]. Thus far, many studies have confirmed that the variants of the *NOS3* gene are closely related to several vascular diseases [35]. Different types of arterial or venous vasculitis as well as superficial thrombophlebitis and deep venous thrombosis have been reported as important features of BD [36] but were only occasionally observed in the patients in the present study. The reason is not clear but might be because we recruited patients from an ophthalmology department or that it might be caused by racial differences.

To ensure the validity of our findings, the following measures were taken. First, all participants were from a Han Chinese population, and we chose to work with a large sample size of patients, which was larger than previous studies in this field. Second, the diagnosis of patients with BD and VKH syndrome was made by the same senior ophthalmologist (Peizeng Yang), and patients with a doubtful diagnosis were eliminated from the study. Third, careful inquiry of the medical history of the controls was performed to exclude individuals with intraocular or extraocular inflammation or those who have an autoimmune disease. An interesting finding in our study was the observation that despite our large sample size we observed Hardy–Weinberg disequilibrium in rs1799983 in the healthy controls. This phenomenon has also been described previously by others [33]. It has been suggested that selection pressure in rs1799983 caused the Hardy–Weinberg disequilibrium in this SNP.

Our study has several limitations. We focused on the *NOS gene* but did not test other genes that are involved in the pathway that regulates the production of NO. Although we found that the SNP rs1799983 may be a susceptibility factor for patients with BD in a Han Chinese population, we have not yet been able to identify a possible mechanism how this gene polymorphism affects BD. In addition, we tested only three SNPs in the *NOS2* and *NOS3* genes and found that SNP rs1799983 in *NOS3* is associated with BD. As more than 100 SNPs are located in *NOS2* and *NOS3*, the association of other SNPs cannot be excluded from this study. Further studies including detailed fine mapping of the region and an analysis of functional effects must be performed to address this issue.

In conclusion, our results showed that *NOS3*/rs1799983, but not *NOS2*/rs4795067 and *NOS3*/rs1800779, contributes to the genetic susceptibility to BD in a Han Chinese population.

APPENDIX 1. AGAROSE GEL ELECTROPHORETIC ANALYSIS OF NITRIC OXIDE SYNTHASE (NOS)3/ RS1799983 POLYMORPHISM AFTER DIGESTION WITH SDUI ENZYME.

The TT genotype shows one band at 206 bp, and the TG genotype shows three bands at 206 bp,124 bp and 82 bp, whereas the GG genotype shows two bands at 124 bp and 82 bp. To access the data, click or select the words "Appendix 1."

ACKNOWLEDGMENTS

This work was supported by Natural Science Foundation Major International (Regional) Joint Research Project (81320108009), Key Project of Natural Science Foundation (81130019), National Natural Science Foundation Project (31370893, 81200678), Basic Research program of Chongqing (cstc2013jcyjC10001), Fundamental and Advanced Research Program of Chongqing (cstc2015jcyjA10022), Science and Technology Project of Chongqing Municipal Education Commission (KJ1500236), Scientific Research Program of Science and Technology Commission of Yuzhong District of Chongqing (20150102), Chongqing Key Laboratory of Ophthalmology (CSTC, 2008CA5003), National Key Clinical Specialties Construction Program of China, Key Project of Health Bureau of Chongqing (2012–1-003), Research fund for Traditional Chinese Medicine of Chongqing Health and Family Planning Commission (ZY201401013), Chongqing Science & Technology Platform and Base Construction Program(cstc2014pt-sy10002)and Fund for PAR-EU Scholars Program.

REFERENCES

1. Nuzzi R, Amerio G. Uveitis and systemic diseases. Minerva Med 1992; 83:239-48. [PMID: 1589128].
2. Rothova A, Suttorp-van Schulten MS, Frits Treffers W, Kijlstra A. Causes and frequency of blindness in patients with intraocular inflammatory disease. Br J Ophthalmol 1996; 80:332-6. [PMID: 8703885].
3. Yang P, Ren Y, Li B, Fang W, Meng Q, Kijlstra A. Clinical characteristics of Vogt-Koyanagi-Harada syndrome in Chinese patients. Ophthalmology 2007; 114:606-14. [PMID: 17123618].
4. Zhao M, Jiang Y, Abrahams IW. Association of HLA antigens with Vogt-Koyanagi-Harada syndrome in a Han Chinese population. Arch Ophthalmol 1991; 109:368-70. [PMID: 2003797].
5. Hou S, Du L, Lei B, Pang CP, Zhang M, Zhuang W, Zhang M, Huang L, Gong B, Wang M, Zhang Q, Hu K, Zhou Q, Qi J, Wang C, Tian Y, Ye Z, Liang L, Yu H, Li H, Zhou Y, Cao Q, Liu Y, Bai L, Liao D, Kijlstra A, Xu J, Yang Z, Yang P. Genome-wide association analysis of Vogt-Koyanagi-Harada syndrome identifies two new susceptibility loci at 1p31.2 and 10q21.3. Nat Genet 2014; 46:1007-11. [PMID: 25108386].
6. Du L, Yang P, Hou S, Lin X, Zhou H, Huang X, Wang L, Kijlstra A. Association of the CTLA-4 gene with Vogt-Koyanagi-Harada syndrome. Clin Immunol 2008; 127:43-8. [PMID: 18282809].
7. Shu Q, Yang P, Hou S, Li F, Chen Y, Du L, Jiang Z. Interleukin-17 gene polymorphism is associated with Vogt-Koyanagi-Harada syndrome but not with Behcet's disease in a Chinese Han population. Hum Immunol 2010; 71:988-91. [PMID: 20620187].
8. Yu H, Liu Y, Bai L, Kijlstra A, Yang P. Predisposition to Behcet's disease and VKH syndrome by genetic variants of miR-182. J Mol Med (Berl) 2014; 92:961-7. [PMID: 24801147].
9. Yu H, Luo L, Wu L, Zheng M, Zhang L, Liu Y, Li H, Cao Q, Kijlstra A, Yang P. FAS Gene Copy Numbers are Associated with Susceptibility to Behcet Disease and VKH Syndrome in Han Chinese. Hum Mutat 2015; 36:1064-9. [PMID: 26136352].
10. Saadoun D, Wechsler B. Behcet's disease. Orphanet J Rare Dis 2012; 7:20-[PMID: 22497990].
11. Remmers EF, Cosan F, Kirino Y, Ombrello MJ, Abaci N, Satorius C, Le JM, Yang B, Korman BD, Cakiris A, Aglar O, Emrence Z, Azakli H, Ustek D, Tugal-Tutkun I, Akman-Demir G, Chen W, Amos CI, Dizon MB, Kose AA, Azizlerli G, Erer B, Brand OJ, Kaklamani VG, Kaklamanis P, Benchetrit E, Stanford M, Fortune F, Ghabra M, Ollier WE, Cho YH, Bang D, O'Shea J, Wallace GR, Gadina M, Kastner DL, Gul A. Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R–IL12RB2 regions associated with Behcet's disease. Nat Genet 2010; 42:698-702. [PMID: 20622878].
12. Hou S, Yang Z, Du L, Jiang Z, Shu Q, Chen Y, Li F, Zhou Q, Ohno S, Chen R, Kijlstra A, Rosenbaum JT, Yang P. Identification of a susceptibility locus in STAT4 for Behcet's disease in Han Chinese in a genome-wide association study. Arthritis Rheum 2012; 64:4104-13. [PMID: 23001997].
13. Zhou Q, Hou S, Liang L, Li X, Tan X, Wei L, Lei B, Kijlstra A, Yang P. MicroRNA-146a and Ets-1 gene polymorphisms in ocular Behcet's disease and Vogt-Koyanagi-Harada syndrome. Ann Rheum Dis 2014; 73:170-6. [PMID: 23268366].
14. Fang J, Hou S, Xiang Q, Qi J, Yu H, Shi Y, Zhou Y, Kijlstra A, Yang P. Polymorphisms in genetics of vitamin D metabolism confer susceptibility to ocular Behcet disease in a Chinese Han population. Am J Ophthalmol 2014; 157:488-494.e6. [PMID: 24184224].
15. Hou S, Xiao X, Zhou Y, Zhu X, Li F, Kijlstra A, Yang P. Genetic variant on PDGFRL associated with Behcet disease

in Chinese Han populations. Hum Mutat 2013; 34:74-8. [PMID: 22926996].

16. Hu J, Hou S, Zhu X, Fang J, Zhou Y, Liu Y, Bai L, Kijlstra A, Yang P. Interleukin-10 gene polymorphisms are associated with Behcet's disease but not with Vogt-Koyanagi-Harada syndrome in the Chinese Han population. Mol Vis 2015; 21:589-603. [PMID: 26015771].
17. Zhang Y, Li C, Li K, Liu L, Jian Z, Gao T. Analysis of inducible nitric oxide synthase gene polymorphisms in vitiligo in Han Chinese people. PLoS One 2011; 6:e27077-[PMID: 22205923].
18. Ben Dhifallah I, Houman H, Khanfir M, Hamzaoui K. Endothelial nitric oxide synthase gene polymorphism is associated with Behcet's disease in Tunisian population. Hum Immunol 2008; 69:661-5. [PMID: 18718857].
19. Kim JU, Chang HK, Lee SS, Kim JW, Kim KT, Lee SW, Chung WT. Endothelial nitric oxide synthase gene polymorphisms in Behcet's disease and rheumatic diseases with vasculitis. Ann Rheum Dis 2003; 62:1083-7. [PMID: 14583572].
20. An JD, Li XY, Yu JB, Zhao Y, Jin ZS. Association between the eNOS gene polymorphisms and rheumatoid arthritis risk in a northern Chinese population. Chin Med J (Engl) 2012; 125:1496-9. [PMID: 22613658].
21. Lee YH, Lee HS, Choi SJ, Ji JD, Song GG. Associations between eNOS polymorphisms and susceptibility to systemic lupus erythematosus: a meta-analysis. Inflamm Res 2012; 61:135-41. [PMID: 22105628].
22. Nosikov VV. Genomics of type I diabetes mellitus and its late complications. Mol Biol (Mosk) 2004; 38:150-64. [PMID: 15042845].
23. AlFadhli S, Mohammed EM, Al Shubaili A. Association analysis of nitric oxide synthases: NOS1, NOS2A and NOS3 genes, with multiple sclerosis. Ann Hum Biol 2013; 40:368-75. [PMID: 23826716].
24. Stuart PE, Nair RP, Ellinghaus E, Ding J, Tejasvi T, Gudjonsson JE, Li Y, Weidinger S, Eberlein B, Gieger C, Wichmann HE, Kunz M, Ike R, Krueger GG, Bowcock AM, Mrowietz U, Lim HW, Voorhees JJ, Abecasis GR, Weichenthal M, Franke A, Rahman P, Gladman DD, Elder JT. Genome-wide association analysis identifies three psoriasis susceptibility loci. Nat Genet 2010; 42:1000-4. [PMID: 20953189].
25. Lan Q, Zheng T, Shen M, Zhang Y, Wang SS, Zahm SH, Holford TR, Leaderer B, Boyle P, Chanock S. Genetic polymorphisms in the oxidative stress pathway and susceptibility to non-Hodgkin lymphoma. Hum Genet 2007; 121:161-8. [PMID: 17149600].
26. Tunc R, Uluhan A, Melikoglu M, Ozyazgan Y, Ozdogan H, Yazici H. A reassessment of the International Study Group criteria for the diagnosis (classification) of Behcet's syndrome. Clin Exp Rheumatol 2001; 19:Suppl 24S45-7. [PMID: 11760398].
27. Read RW, Holland GN, Rao NA, Tabbara KF, Ohno S, Arellanes-Garcia L, Pivetti-Pezzi P, Tessler HH, Usui M. Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. Am J Ophthalmol 2001; 131:647-52. [PMID: 11336942].
28. Houman MH, Bel Feki N. Pathophysiology of Behcet's disease. Rev Med Interne 2014; 35:90-6. [PMID: 24210264].
29. Yang MM, Lai TY, Luk FO, Pang CP. The roles of genetic factors in uveitis and their clinical significance. Retina 2014; 34:1-11. [PMID: 23903794].
30. Salvarani C, Boiardi L, Casali B, Olivieri I, Ciancio G, Cantini F, Salvi F, Malatesta R, Govoni M, Trotta F, Filippini D, Paolazzi G, Nicoli D, Farnetti E, Macchioni P. Endothelial nitric oxide synthase gene polymorphisms in Behcet's disease. J Rheumatol 2002; 29:535-40. [PMID: 11908569].
31. Karasneh JA, Hajeer AH, Silman A, Worthington J, Ollier WE, Gul A. Polymorphisms in the endothelial nitric oxide synthase gene are associated with Behcet's disease. Rheumatology (Oxford) 2005; 44:614-7. [PMID: 15705632].
32. Lee YH, Song GG. Associations between eNOS polymorphisms and susceptibility to Behcet's disease: a meta-analysis. J Eur Acad Dermatol Venereol 2012; 26:1266-71. [PMID: 21957880].
33. Li J, Cun Y, Tang WR, Wang Y, Li SN, Ouyang HR, Wu YR, Yu HJ, Xiao CJ. Association of eNOS gene polymorphisms with essential hypertension in the Han population in southwestern China. Genet Mol Res 2011; 10:2202-12. [PMID: 21968727].
34. Dosenko VI, Zahorii V, Moibenko OO. Proteasomal degradation of ribonucleic acids of different NO-synthase isoforms. Fiziol Zh 2006; 52:3-7. [PMID: 16553292].
35. Wang XL, Wang J. Endothelial nitric oxide synthase gene sequence variations and vascular disease. Mol Genet Metab 2000; 70:241-51. [PMID: 10993711].
36. Yang P, Fang W, Meng Q, Ren Y, Xing L, Kijlstra A. Clinical features of chinese patients with Behcet's disease. Ophthalmology 2008; 115:312-318.e4. [PMID: 17692378].

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 3 April 2016. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.