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 Yang4

(The first two authors contributed equally to this work)

1The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing, P. R. China; 2Department of Ophthalmology, Yongchuan Hospital, Chongqing Medical University, Chongqing, China; 3University 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]=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/rs1799983 polymorphism 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.
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 NOS1 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.

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 rs4795067 and rs1800779 were in accordance with the Hardy–Weinberg equilibrium in the controls (χ²=2.97, p=0.09; χ²=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   | rs4795067 | F: TAATCCCAGCCAGAGAGA CG | KspAI              | A:117                | 64      |
|        |         | R: AGCCCTGGCACATCTTGAAGGTTAA | (MBI)              | G:23, 94bp           |         |
|        | rs1799983 | F: CATGAGGCTCAGCCCCAGAA | SduI               | T:206bp G:124, 82bp  | 60      |
|        |         | R: AGTCAATCCCTTTGTTGCTCAC | (MBI)              |                      |         |
| NOS3   | rs1800779 | F: TCTGCCTCTCCAGTCTCTCA | BccI               | A:189bp              | 65      |
|        |         | R: AGCACTCTCCAGGCCATTCAG | (NEB)              | G:124,65bp           |         |

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

| Disease                     | Clinical features       | Total | %  |
|-----------------------------|-------------------------|-------|----|
| Patients with BD            | Mean age ±SD            | 29.6±10.2 | 100 |
|                             | 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  | Mean age ±SD            | 38.0±12.3 | 100 |
|                             | 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                    | Mean age±SD             | 39.1±10.8 | 100 |
|                             | Male                    | 762   | 56.1 |
|                             | Female                  | 597   | 43.9 |
## Table 3. Allele and genotype frequencies of rs4795067, rs1799983 and rs1800779 in BD.

| SNPs       | Genotype | BD patients | Controls | P value | Pc value | OR(95%CI) |
|------------|----------|-------------|----------|---------|----------|-----------|
|            | Allele   | n(freq)     | n(freq)  |         |          |           |
| 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 | VKH patients | Controls | P value | Pc value | OR(95%CI) |
|------------|----------|--------------|----------|---------|----------|-----------|
|            | Allele   | n(freq)      | n(freq)  |         |          |           |
| 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 ($P_c=2.1×10^{-3}$, 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 ($P_c>0.05$; Table 3). Furthermore, no association between the three SNPs and VKH syndrome was detected ($P_c>0.05$; 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 | $P_c$ value | OR(95%CI) |
|-------------------|-----------------|-----------------|--------------------|---------|-------------|-----------|
| Genital ulcer     |                 |                 |                    |         |             |           |
|                   | 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      |                 |                 |                    |         |             |           |
|                   | 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         |                 |                 |                    |         |             |           |
|                   | 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 |             |                 |                    |         |             |           |
|                   | 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 |                 |                 |                    |         |             |           |
|                   | 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) |

$P_c$, 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=6.0×10^{-3}; Pc=6.0×10^{-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=9.0×10^{-7}) [30]. This study also showed that the T allele was significantly higher in patients with BD compared with healthy controls (Pc=6.0×10^{-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 \(\text{NOS2/rs4795067}\) 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 (NOS3)/RS1799983 POLYMORPHISM AFTER DIGESTION WITH SDUI ENZYME.**

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

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