

Associations of Mitochondrial Deoxyribonucleic Acid Polymorphisms With Behçet's Disease in the Korean Population

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ABSTRACT

Objectives: This study aims to examine the possible associations of mitochondrial single nucleotide polymorphisms (SNPs) and Behçet's disease (BD) in a larger patient group.

Patients and methods: Whole blood or buffy coat was collected from 98 BD patients (31 males, 67 females; mean age 48±2.8 years; range 20 to 60 years) from four university hospitals located in the Chung-Cheong district of the Republic of Korea, and 196 age- and sex-matched healthy controls (HCs) (62 males, 134 females; mean age 46.91±12.90 years; range 20 to 68 years) from Konyang University Hospital. Twenty targeted mitochondrial deoxyribonucleic acids (DNAs) were genotyped and compared using the revised Cambridge Reference Sequence. Chi square and Fisher's exact tests were used to analyze association of mitochondrial DNA SNPs with BD susceptibility and its clinical characteristics.

Results: There were no differences for m.248A>G, m.304C>A, m.709G>A, m.3010G>A, m.3970C>T, m.4883C>T, m.5178C>A, m.6392T>C, m.6962G>A, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.14668C>T, m.16129G>A, and m.16304T> between patient and HC groups. However, m.16182A>C and m.16183A>C were more frequently observed in the patient group than the HC group (22 [22.4%] vs. 24 [12.2%], p=0.061 and 32 [32.7%] vs. 42 [21.4%], p=0.092) but without statistical significance. m.4883C>T and m.5178C>A were associated with posterior location of oral ulcers (p=0.025 for each) and m.16183A>C was associated with deep oral ulcers (p=0.001), while m.16189T>C was associated with deep oral ulcers and thrombosis (p=0.042, 0.048, respectively).

Conclusion: m.16182A>C and m.16183A>C may be associated with BD in the Korean population.

Keywords: Behçet's disease; etiology; mitochondrial deoxyribonucleic acid; single nucleotide polymorphism.

Behçet's disease (BD) is a rheumatic disorder characterized by recurrent oral ulcers, genital ulcers, uveitis and erythema nodosum-like skin lesions. It has several features of both autoimmune and autoinflammatory diseases.¹ The predominant pathology of BD is vasculitis in vessels of various sizes and types. However, its cause requires further study. It is multifactorial and multi-genetic, and several nuclear genetic changes have

been suggested as possible pathogenic factors. Mitochondrial genetic alterations have scarcely been considered.

Mitochondrial diseases are emerging as novel and expanding disease entities; they include several inborn neuromuscular diseases, and their associations with other diseases are being investigated. Mitochondrial abnormalities are thought to contribute to cancers, diabetes mellitus,

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and some autoimmune diseases. Although many researches have tried to elucidate pathogenesis and disease mechanisms of the disease, BD is still uncharted territory and mitochondrial genetic aberrations could be one of the hypotheses for a multifactorial disease like BD. A previous report has linked a mitochondrial single nucleotide polymorphism (SNP), m.709G>A, to BD in the Iranian population.² Our group sequenced nearly the entire mitochondrial deoxyribonucleic acid (DNA) sequence in 20 Korean BD patients and 10 sex-, and age-matched healthy controls (HCs) and found that the minor allele frequencies for m.248A>G, m.709G>A, m.3970C>T, m.6392T>C, m.6962G> A, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.16129G>A, and m16304T>C were more frequent in BD patients though the differences were not statistically significant.³ Therefore, in this study, we aimed to examine the possible associations of mitochondrial SNPs and BD in a larger patient group.

PATIENTS AND METHODS

Ninety-eight BD patients (31 males, 67 females; mean age 48±2.8 years; range 20 to 60 years) who met the 1999 Diagnostic Criteria of the International Study Group for Behçet’s disease were enrolled from outpatient rheumatology clinics of Konyang University Hospital, Chungnam National University Hospital, Chungbuk National University Hospital, and Eulji University Hospital. located in Chung-Cheong district of the Republic of Korea between June 2016 and July 2017.⁴ A total of 196 age- and sex-matched HCs (62 males, 134 females; mean age 46.91±12.90 years; range 20 to 68 years) were enrolled from Konyang University Hospital. Patients with other autoimmune or autoinflammatory diseases were excluded. All patients’ peripheral blood was drawn. The case records consisted of patients’ current age, sex, time since diagnosis, symptom duration, family history of BD, clinical manifestations of oral and genital ulcers, erythema nodosum-like skin lesions, pseudofolliculitis, uveitis, optic neuritis, thrombosis, arthralgia and/or arthritis, gastrointestinal involvement and laboratory results including human leukocyte antigen B (HLA-B)*51 positivity. Medication status was also collected. The study protocol was approved by the Konyang

Table 1. Sequences of iPLEX® primers and unextended primers

rCRS position	2 nd -PCR primer sequence	1 st -PCR primer sequence	Amplicon length (bp)	UEP direction	UEP	Nucleotide alterations
709	acgttggatgGTAAGATTACACATGCAAGC	acgttggatgGCATTGCTGCGTGTGATG	113	F	CACATGCAAGCATCCCC	G/A/C
3010	acgttggatgCGAACCTTTAATAGCGGCTG	acgttggatgACAATAGGGTTTACGACCTC	88	R	AATAGCGGCTGCACCAT	G/A
4883	acgttggatgCCTGCTTCTTCTCACATGAC	acgttggatgGAGAAGGCTTACGTTTAGTG	102	F	CTCACATGACAAAACTAGCCCC	C/T/A
6392	acgttggatgTGGTATTGGGTTATGGCAGG	acgttggatgGTGCTCTCTATCTTAGGG	99	R	TTATATTGATAATTGTTGTGATGAA	T/C
6962	acgttggatgACGTGTCGTGTAGTACGATG	acgttggatgTCTAGCCCTAGGATTCATC	116	R	AGTTTGCTAATACAATGCCAGT	G/A/T/C
10310	acgttggatgCTTTTACCCCTACCATGAGC	acgttggatgGACTTAGGGCTAGGATGATG	111	F	GCCCTACAAAACAACATAACCT	G/A/C
10609	acgttggatgGGAATAATACTATCGCTGTTTC	acgttggatgTATTGGCTAAGAGGGAGTGG	90	F	TGTTCAATTAGCTACTCTCA	T/C
12406	acgttggatgCTAATTCCTCCCTACCTTAC	acgttggatgAAAGGTGGATCGACAAATGG	110	F	CCATCCTTACCACCCCTC	G/A
12882	acgttggatgTCCATACACCGTATCGGC	acgttggatgTTGTGGGCTCATGAGTTGG	103	F	ATCGCGGATATCGGTTT	C/T
13928	acgttggatgGGTTTTGGCTCGTAAGAAGG	acgttggatgTCTCCAA CATACTCGGATTC	98	R	ATTGTGCGGTGTGTGATG	G/C/A/T
14668	acgttggatgCCCCATTACTAAACCCACAC	acgttggatgCATTGGTCTGCTGGTTGTAGTC	97	F	TCAACGAAAAACAAGCATA	C/T/G

rCRS: Revised Cambridge Reference Sequence; PCR: Polymerase chain reaction; UEP: Unextended primer.

University Hospital Ethics Committee (Institutional Review Board approval no. KYUH 2015-10-024). A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Genomic DNA was extracted from peripheral blood or buffy coats with the Qiagen GeneAll Exgene™ Blood SV Kit® (GeneAll Biotechnology, Seoul, Korea), and DNA concentration was determined with a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Each sample was diluted to 10 ng/μL.

Eleven mitochondrial DNA nucleotides (m.709, m.3010, m.4883, m.6392, m.6962, m.10310, m.10609, m.12406, m.12882, m.13928, and m.14668) were genotyped using the MassARRAY system (Agena BioScience Inc., San Diego, CA, USA) with the Assay Design Suite tool and iPLEX® assay. All the primers used are listed in Table 1. Polymerase chain reactions (PCRs) were performed in a total volume of 5 μL containing 10 ng genomic DNA, 100 nM of each amplification primer, 500 μM deoxynucleotide triphosphate (dNTP) mix, 1.625 mM magnesium chloride, and 0.5 units HotStar Taq DNA Polymerase (Qiagen, Valencia, CA, USA). The mixture was subjected to the following PCR conditions: a single denaturation cycle at 94°C for 15 minutes, followed by 45 cycles at 94°C for 20 seconds, 56°C for 30 seconds, 72°C for 60 seconds, and a final extension at 72°C for three minutes. Unincorporated nucleotides in the PCR product were deactivated using shrimp alkaline phosphatase. Allele discrimination reactions were conducted by adding the allele-specific extension

primers, DNA polymerase and a cocktail of dNTPs and dideoxynucleotide triphosphates to each well. MassExtend clean resin (Agena BioScience Inc., San Diego, CA, USA) was added to the mixtures to remove extraneous salts that could interfere with matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis. The desalted extension products were spotted onto silicon SpectroChip (Agena BioScience Inc., San Diego, CA, USA). Genotypes were determined by spotting an aliquot of each sample onto a 384 SpectroChip, which was subsequently read by the MALDI-TOF mass spectrometer.

m.3970 was genotyped using TaqMan-based genotyping on an Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The primers used were as follows: forward, 5'-CCCTTCGACCTTGCCGAA-3' and reverse, 5'-GCGTCATATGTTGTTCCCTAGGAAGA-3', wild-type allele probe, 5'-CCTTCGCCCTCTTCT-3', and mutant-type allele probe 5'-CCTTCGCCTTCTTCT-5'.⁵ The 10 μL PCR reaction mix comprised of 10 ng genomic DNA and 5.0 μL of 2× TaqMan Universal PCR master mix without uracil N-glycosylase and 0.25 μL of 40× SNP genotyping assay (Probes and Primers). Genotyping was performed according to the manufacturer's instructions.

m.5178 and the other seven mitochondrial DNA control region nucleotides, m.248, m.304, m.16129, m.16182, m.16183, m.16189, and m.16304 were Sanger sequenced. The PCR/Sanger Sequencing Primer pairs for m.5178 mitochondrially encoded NADH dehydrogenase 2 (MT-ND2) and the mitochondrial DNA control region are listed in Table 2. PCR was performed in

Table 2. Polymerase chain reaction/Sanger sequencing primers used for m.5178 and mitochondrial deoxyribonucleic acid control region

Primer	Sequence(5'-3')	Usage
m.5178F	ATCTCTCCCTCACTAAACGTAAGCCTT	PCR and Sequencing
m.5178R	TTAGTATAAAAAGGGGAGATAGGTAGGAGTAGC	PCR and Sequencing
F15975	CTC CAC CAT TAG CAC CCA AA	PCR and Sequencing
R635	GAT GTG AGC CCG TCT AAA CA	PCR and Sequencing
F155	TAT TTA TCG CAC CTA CGT TC	Sequencing
R16410m	GAG GAT GGT GGT CAA GGG A	Sequencing

PCR: Polymerase chain reaction.

Table 3. Clinical and laboratory characteristics of patients with Behçet's disease

Category items	Male (n=31)		Female (n=67)		Total (n=98)		p
	n	%	n	%	n	%	
Age (year)		47.7±12.5		48.2±12.0		48±2.8	
Gender ratio (M:F)					1: 2.16		
Time since diagnosis (year)		8.9±5.9		7.8±5.9		8.1±2.8	0.386
Symptom duration (year)		13.8±9.6		12.0±7.5		12.6±11.3	0.297
Recurrent oral ulcer	31		67		98/98	100	
Deep ulcer	15	48.4	31	47	46/97	47.42	1.000
Multiple	22	71.0	55	83.3	77/97	79.38	0.257
Posterior	5	16.1	12	18.5	17/96	17.70	1.000
Genital ulcer	22	71.0	52	77.6	74/98	75.51	0.646
Erythema nodosum-like skin lesion	22	71.0	46	68.7	68/98	69.39	1.000
Pseudofolliculitis	12	38.7	22	32.8	34/98	34.69	0.734
Uveitis	12	38.7	22	32.8	34/98	34.69	0.734
Optic neuritis	0	0.0	2	3.0	2/98	2.04	0.839
Positive pathergy test	8	30.8	15	24.6	23/87	26.44	0.739
Thrombosis	7	22.6	0	0.0	7/98	7.14	<0.001
Arthralgia and/or arthritis	13	41.9	31	46.3	45/98	45.92	0.710
Fever	9	29.0	13	19.4	22/98	22.45	0.467
Vasculitis	6	19.4	7	10.6	13/97	13.27	0.390
Gastrointestinal involvement	3	9.7	7	10.9	10/94	10.20	0.765
Rebamipide use	24	77.4	51	76.1	75/98	76.53	1.000
Colchicine use	24	77.4	57	85.1	81/97	83.51	0.428
Steroid use	22	71.0	45	67.2	67/98	68.37	0.886
Azathioprine use	11	35.5	20	29.9	31/98	31.63	0.746
Cyclosporine use	7	22.6	5	7.5	12/98	12.24	0.073
Methotrexate use	4	12.9	7	10.4	11/98	11.22	0.989
Sulfasalazine use	3	9.7	3	4.5	6/97	6.19	0.599
Dapsone use	0	0.0	1	1.5	1/98	1.02	1.000
Family history of recurrent oral ulcer	3	9.7	9	13.4	12/98	12.24	0.845
Laboratory HLA-B51 positivity	8	44.4	10	55.6	18/48	37.50	0.483
Antinuclear antibody positivity	3	13.6	6	10.2	9/81	1.11	0.226
Rheumatoid factor positivity	1	4.8	4	7.4	5/75	6.67	1.000
WBC (/mm ³)		7395.5±2458.5		6795.7±2244.9		6985.4±2318.79	0.236
Hgb (g/dL)		14.4±1.5		12.5±1.16		13.1±1.53	0.061
Hct (%)		43.0±4.6		37.12±4.31		38.98±5.19	0.085
Platelet (/mm ³)		314612.9±360844.8		268955.2±68079.2		283.398±209475.2	0.490
ESR (mm/hr)		17.6±17.6		24.0±20.9		21.95±20.03	0.146
CRP (mg/dL)		0.4±0.6		0.8±1.5		0.69±1.30	0.99

SD: Standard deviation; HLA: Human leukocyte antigen; WBC: White blood count; Hgb: Hemoglobin; Hct: Hematocrit; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.

Table 4. Associations between mitochondrial deoxyribonucleic acid alterations and Behçet's disease

rCRS position	Map locus	Gene position (np)	Description	Nucleotide alteration	BD (n=98)		HC (n=196)		p	OR* (95% CI)
					n	%	n	%		
248	MT-HV2	57-372	Hypervariable segment 2	A del	12	12.2	23	11.7	1.000	1.050 (0.499-2.209)
304	MT-HV2	57-372	Hypervariable segment 2	C>A	0	0	0	0	NA	NA
709	MT-RNR1	648-1601	12S ribosomal RNA	G>A	24	24.5	35	17.9	0.236	1.492 (0.929-2.686)
3010	MT-RNR2	1671-3229	16S ribosomal RNA	G>A	19	19.4	50	25.5	0.307	0.702 (0.387-1.273)
3970	MT-ND1	3307-4262	NADH Dehydrogenase subunit 1	T>C	14	14.3	19	9.7	0.327	1.553 (0.743-3.246)
4883	MT-ND2	4470-5511	NADH Dehydrogenase subunit 2	C>T	25	25.5	59	30.1	0.494	0.795 (0.460-1.374)
5178	MT-ND2	4470-5511	NADH Dehydrogenase subunit 2	C>A	25	25.5	59	30.1	0.494	0.795 (0.460-1.374)
6392	MT-COI	5904-7445	Cytochrome c oxidase subunit I	T>C	14	14.3	19	9.7	0.327	1.553 (0.743-3.246)
6962	MT-COI	5904-7445	Cytochrome c oxidase subunit I	G>A	12	12.2	18	9.2	0.540	1.380 (0.636-2.993)
10310	MT-ND3	10059-10404	NADH Dehydrogenase subunit 3	G>A	16	16.3	24	12.2	0.434	1.398 (0.705-2.774)
10609	MT-ND4L	10470-10766	NADH Dehydrogenase subunit 4L	T>C	12	12.2	16	8.2	0.361	1.570 (0.711-3.463)
12406	MT-ND5	12337-14148	NADH Dehydrogenase subunit 5	G>A	12	12.2	17	8.7	0.447	1.469 (0.672-3.213)
12882	MT-ND5	12337-14148	NADH Dehydrogenase subunit 5	C>T	12	12.2	16	8.2	0.361	1.570 (0.711-3.463)
13928	MT-ND5	12337-14148	NADH Dehydrogenase subunit 5	G>C	14	14.3	19	9.7	0.327	1.553 (0.743-3.246)
14668	MT-ND6	14149-14673	NADH Dehydrogenase subunit 6	C>T	19	19.4	49	25.0	0.353	0.722 (0.398-1.310)
16129	MT-HV1	16024-16383	Hypervariable segment 1	G>A	23	23.5	42	21.4	0.804	1.124 (0.631-2.005)
16182	MT-HV1	16024-16383	Hypervariable segment 1	A>C	22	22.4	24	12.2	0.061	2.062 (1.089-3.906)
16183	MT-HV1	16024-16383	Hypervariable segment 1	A>C	32	32.7	42	21.4	0.092	1.766 (1.026-3.040)
16189	MT-HV1	16024-16383	Hypervariable segment 1	T>C	42	42.9	64	32.7	0.112	1.547 (0.939-2.548)
16304	MT-HV1	16024-16383	Hypervariable segment 1	T>C	15	15.3	18	9.2	0.170	1.787 (0.859-3.720)

BD: Behçet's disease; HC: Healthy control; rCRS: Revised Cambridge Reference Sequence; OR: Odds ratio; CI: Confidence interval; NA: Non-applicable; RNA: Ribonucleic acid; NADH: Nicotinamide adenine dinucleotide, reduced form; * Chi-square test.

Table 5. Associations between symptoms of Behçet's disease and mitochondrial deoxyribonucleic acid alterations

Symptom	Mitochondrial DNA SNPs	<i>p</i>
Deep oral ulcer	m.16183A>C	0.001
	m.16189T>C	0.042
Posterior oral ulcer	m.4883C>T	0.025
	m.5178C>A	0.025
Arthralgia/arthritis	m.16182A>C	0.043
Thrombosis	m.16189T>C	0.048

DNA: Deoxyribonucleic acid; SNP: Single nucleotide polymorphism.

25 µL volumes with 1 ng of genomic DNA, 2.5 µL of Gold ST*R 10X Buffer (Promega, Madison, Fitchburg, WI, USA), 0.3 µL AmpliTaq Gold® DNA Polymerase (5 U/µL, Applied Biosystems, Foster City, CA, USA) and 15 pmol of each primer, using a SimpliAmp™ Thermal Cycler (Life Technologies, Carlsbad, CA, USA). Cycling parameters were as follows: initial denaturation at 95°C for 11 minutes, followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds, and a final extension of 72°C for seven minutes. Amplified PCR products were purified using Exo-AP PCR Clean-up (MG MED, Inc., Seoul, Korea). Sanger sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with an ABI PRISM 3730 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Sequencing results were compared with the revised Cambridge Reference Sequence, and the extent of variation that occurred was determined. Chi square or Fisher's exact tests were used to analyze associations of the mitochondrial DNA SNPs with BD susceptibility and its clinical characteristics. We calculated an odds ratio (OR), a 95% confidence interval (CI), and a two-tailed *p*-value. All statistical analyses were performed using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria), based on a significance level of 0.05.

RESULTS

Clinical and laboratory characteristics of BD patients were presented in Table 3. Of the patients,

68.4% were females and 31.6% were males, yielding a female to male ratio of 2.16. The mean ages of the female and the male patients were 48.2±12.0 and 47.7±12.5 years, respectively. Symptom duration and disease duration after diagnosis did not differ between the female and male patients. Among the clinical and laboratory characteristics of BD patients, thrombosis was more frequent in male patients than female patients (7 [22.6%] vs. 0 [0%], *p*<0.001).

Analysis results of the 20 mitochondrial DNA SNPs were shown in Table 4. The frequencies of m.248A>G, m.304C>A, m.709G>A, m.3010G>A, m.3970C>T, m.4883C>T, m.5178C>A, m.6392T>C, m.6962G>A, m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.14668C>T, m.16129G>A, and m.16304T>C did not differ between the BD and HC groups. However, m.16182A>C and m.16183A>C were more common in the patient group than the HC group (22 [22.4%] vs. 24 [12.2%], *p*=0.061 and 32 [32.7%] vs. 42 [21.4%], *p*=0.092) although without statistical significance. The m.304C>A SNP was not observed in either the BD and HC group.

Thirty-four of the 98 BD patients (34.69%) had had at least one or more uveitis in their lifetimes (Table 3). The frequencies of female and male patients with histories of uveitis were 22/67 (33%) and 12/31 (39%), respectively, and there was no significant difference in mitochondrial DNA SNPs between the two groups (*p*=0.734).

m.16182A>C, m.16183A>C, m.16189T>C were not found to be associated with uveitis (*p*=0.278, 0.239 and 0.976, respectively). m.16182A>C was more frequently observed in the patient group than the HC group, and was significantly associated with methotrexate use, and arthralgia/arthritis (*p*=0.020, 0.043, respectively). m.4883C>T and m.5178C>A were associated with posterior location of oral ulcers (*p*=0.025 for each) and m.16183A>C was associated with deep oral ulcers (*p*=0.001), while m.16189T>C was associated with deep oral ulcers and thrombosis (*p*=0.042, 0.048, respectively) (Table 5).

DISCUSSION

In a previous analysis, m.248A>G, m.709G>A, m.3970C>T, m.6392T>C, m.6962G>A,

m.10310G>A, m.10609T>C, m.12406G>A, m.12882C>T, m.13928G>C, m.16129G>A, and m.16304T>C were more frequently observed in the patient group than in HCs, though the effects were without statistical significance. In addition, m.16182A>C, m.16183A>C, and m.16189T>C were associated with uveitis ($p=0.041$, 0.022 , and 0.014 , respectively). The current extended study did not confirm any mitochondrial DNA alterations that had been postulated to be candidate susceptibility mitochondrial SNPs for development of BD in the previous study. Even though m.16182A>C and m.16183A>C were more frequent in the patient group (without statistical significance), this was not consistent with the earlier observations. The difference between the two sets of findings might be explained by the fact that different subjects were involved, and certainly the current analysis involving many more subjects ought to be more reliable from a statistical perspective. Secondly, the diagnoses of BD in the patients examined were based on combinations of symptoms and signs without pathognomonic measures, which could have affected the sensitivity and specificity of the diagnoses.

m.16182A>C and m.16183A>C were more frequently observed in the patient group than the HC group (22 [22.4%] vs. 24 [12.2%], $p=0.061$ and 32 [32.7%] vs. 42 [21.4%], $p=0.092$) although the effects did not have statistical significance. They could be defined as SNPs according to the criterion that a SNP should occur in more than 1% of the population. In the results of Lee et al.⁶ for the whole mitochondrial DNA control region sequences of 593 Korean subjects, m.16182A>C and m.16183A>C were observed in 71 (12.0%) and 143 (24.1%) subjects respectively. Additionally, in an online human mitochondrial genome database, m.16182A>C and m.16183A>C were found in 7,817 (7.0%) and 16,470 (14.7%) cases, respectively, in 112,173 datasets (42,616 full-length and 69,557 control region datasets).⁷ Also, these SNPs were not among the ethnically unique alterations which Han et al.⁸ described among 100 Korean subjects. m.16182 and m.16183 are located in hypervariable region I (HV1, 16024-16383) of the control region of mitochondrial DNA. The control region is an approximately 1000-bp non-coding region that encompasses the displacement loop (D-loop) and promoter regions,

which play important roles in mitochondrial ribonucleic acid and DNA synthesis. The D-loop includes three hypervariable segments, HV-I(16024-16383), HV2(57-372), and HV3(438-574) in which mutations are more frequent than in any other mitochondrial DNA regions and result in altered mitochondrial gene replication and/or transcription and mitochondrial gene function, and are responsible for a number of diseases.⁹⁻¹² Links between mitochondria and inflammasomes have been studied to a point where the underlying mechanisms are quite well understood.¹³⁻¹⁸ When there were defects in mitochondrial respiratory enzymes which are encoded by several nuclear and mitochondrial genes, it led to excessive reactive oxygen species formation, which in turn activated inflammasomes, and resulted in secretion of proinflammatory cytokines such as interleukin (IL)-1 β . As some researchers have suggested, BD might belong to the category of autoinflammatory diseases.¹ And in line with this viewpoint, we hypothesize that certain mitochondrial genetic alterations in the control region could possibly cause defects in mitochondrial oxidative phosphorylation enzymes (complex I, III, IV and V), increase reactive oxygen species formation, activate inflammasomes, and finally provoke the episodic clinical symptoms and signs of BD.

Several of the mitochondrial SNPs were associated with clinical symptoms and signs; arthralgia with m.16182A>C ($p=0.043$), posterior location of oral ulcers with m.4883C>T and m.5178C>A ($p=0.025$ for each), deep oral ulcers with m.16183A>C ($p=0.001$) and m.16189T>C ($p=0.042$), and thrombosis with m.16189T>C ($p=0.048$). These novel findings require further study. Arslan Taş et al.¹⁹ have reported that neurologic involvement was more frequent in Turkish BD patients with c.769-3C>T in the mevalonate kinase gene ($p=0.012$) and, in another Turkish report, CD40 rs1883832 CC and rs1883832 C were found to be associated with genital ulcers (OR 2.30, 95% CI [1.07-4.94], $p<0.05$, and, OR 1.78, 95% CI [1.06-2.97], $p<0.05$, respectively), although the significance was lost after Bonferroni correction.²⁰ The AA and GA genotypes of factor V Leiden polymorphism were seen in Spanish BD patients and in patients with thrombosis (OR 2.51, 95% CI [1.68-3.74], $p<0.00001$).²¹ In the Han Chinese population, IL-10 rs1800871T was associated with BD and

genital ulcers, skin lesions, and positive pathergy tests, and haplotypes of IL-17A were associated with a risk of intestinal BD in a Korean report, which suggests that the IL-17/23 axis has a significant role in the pathogenesis of intestinal BD.^{22,23}

The more severe clinical manifestations and poorer prognoses of BD in male patients are well known. Differences between clinical manifestations according to sex were studied among the 98 BD patients in the current study; however, there were no significant differences except that thrombosis was more frequent among the male patients (7 [22.6%] vs. 0 [0%], $p < 0.001$). All seven of the BD patients with thrombosis were male, and the mean age of these patients at enrollment was 44.57 years. One 51-year-old male patient without a history of hypertension, diabetes mellitus or smoking had cerebral infarction, and the other six patients had deep vein thrombosis in the lower legs. A smoking history was present only in one 46-year-old male patient with deep vein thrombosis, indicating that there was no association of thrombosis with smoking in these patients. A Japanese and a further Turkish group have similarly reported that genital ulcers were more frequent among female patients (OR 0.29, 95% CI [0.25-0.32], $p < 0.001$ and male 971/1138 [85.6%] vs. female 995/1095 [91.0%], $p < 0.001$),^{24,25} while this was not observed in our data. Ocular manifestations showed male predominance in both data sets; on the other hand, only the Turkish group reported male predominance of papulopustular eruptions, thrombophlebitis, and neurologic, pulmonary and vascular involvement.

There are some limitations to the current study. First, there were difficulties enrolling patients because BD is rare, even though East Asia including Korea is a region with a relatively high prevalence of BD. We had to reduce patient enrollment to half of what was originally intended and even had to extend the enrollment period. Second, an important pitfall of studies recruiting patients and HCs is the possibility that HCs could be future patients, and this is a clear limitation of most genetic association studies. Third, aberrant mitochondrial function is very difficult to validate, though there is still value in descriptive mitochondrial genetic studies.

In conclusion, our findings indicate that m.16182A>C and m.16183A>C may be associated with BD susceptibility in the Korean population and also suggest some associations of mitochondrial SNPs with clinical characteristics.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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