Clinical implications of genetic polymorphisms in blepharospasm

JEONG-KYEONG JANG[1*], MIN-JUNG KWON[2*], NAM-KEUN KIM[2] and HELEN LEW[1]

[1]Department of Ophthalmology, Bundang CHA Medical Center, CHA University, Seongnam, Gyeonggi 13496, Republic of Korea; [2]Department of Biomedical Science, College of Life Science, CHA University, Seongnam, Gyeonggi 13488, Republic of Korea

Received November 23, 2023; Accepted May 14, 2024

DOI: 10.3892/etm.2024.12621

Abstract. The possible genetic variants associated with blepharospasm (BSP) and facial dystonia have been investigated. Although genetic variants associated with BSP have been extensively studied, the contribution of single-nucleotide polymorphisms towards this condition remains poorly understood. In addition, the etiology of BSP remains to be fully elucidated. Therefore, the present study aimed to assess the role of polymorphisms in the torsin 1A (TOR1A), dopamine receptor D (DRD)2 and DRD5 genes in South Korean patients with BSP. Furthermore, the role of genetic variants of these three aforementioned genes was investigated. A prospective case-control study was established, where 56 patients with BSP and 115 healthy controls were recruited at the Department of Ophthalmology of CHA Bundang Medical Center (Seongnam, South Korea) using single nucleotide polymorphisms analysis by real-time PCR. The *TOR1A* rs1182CC/*DRD5* rs6283TC genotype combination was found to be associated with decreased BSP risk [adjusted odds ratio (AOR), 0.288; P=0.013]. *DRD5* rs6283 was observed to be associated with the periocular type of BSP in the co-dominant (for the TC genotype; AOR, 0.370; P=0.029) and dominant models (AOR, 0.406; P=0.029). The recessive model of *TOR1A* rs1801968 (AOR, 0.245; P=0.030), and the recessive (AOR, 0.245; P=0.029) and over-dominant models (AOR, 2.437; P=0.019) of *DRD2* rs1800497 were found to be associated with superior responses to botulinum neurotoxin A (BoNT) treatment. By contrast, dominant (AOR, 0.205; P=0.034) and additive (AOR, 0.227; P=0.030) models of *DRD5* rs6283 were associated with poor responses to BoNT treatment. To conclude, these results suggested that *DRD2* rs1800497 can confer genetic susceptibility to BSP responses to BoNT treatment, whereas the *TOR1A* rs1182CC/*DRD5* rs6283TC genotype combination appeared to contribute to the association with BoNT efficacy in BSP.

Introduction

Blepharospasm (BSP) is the most common form of focal dystonia that involves the periocular muscles. It causes involuntary forceful eyelid closure and functional blindness (1). Unlike other forms of focal dystonia, the pathophysiology of BSP can readily spread to other body parts (2), causing dystonia in the eyes, and lower facial and masticatory muscles (3). Meige syndrome is characterized by the combined presence of BSP and oromandibular dystonia (4). Evidence suggests that both genetic and environmental factors can contribute to BSP (5). The prevalence of BSP in various populations was reported to range between 12 and 133 cases per million between 1976 and 1995. However, BSP is more common in women compared with men, with a male:female ratio of 1:2.3 in Chinese patients (6).

Regarding the epidemiology of BSP, a previous nationwide Taiwanese study reported that the mean annual incidence was 0.10%, with the peak incidence in the 50-59 years age group (0.19%) (7). In addition, the prevalence of BSP in the general population was found to be 1.2-13 in 100,000 according to another previous Korean study (8). Because the clinical features of BSP are inconsistent and the etiology of BSP is multifactorial, to the best of our knowledge, there is currently no cure for BSP. Treatments for BSP include botulinum neurotoxin A (BoNT-A) injection, oral medication such as benzodiazepine and surgical intervention like myectomy (4). Although BoNT-A injection is efficacious and has no clinically significant long-term side effects caused by the impaired neural transmission (9), treatment response varies and repeat injections are needed (9). Furthermore, it remains difficult to predict the response to BoNT-A, since patients with apraxia of eyelid opening can respond poorly to BoNT-A injections (10,11). Such patients typically require surgery to restore ocular functionality (10).

A number of previous studies have searched for genetic variants associated with BSP and dystonia (12,13). Whole-exome sequencing of 31 patients with BSP in the US from 21 independent pedigrees suggested potential roles of several genes in the pathogenesis of BSP, including calcium voltage-gated channel subunit α1A, receptor accessory protein 4 (REEP4), torsin 2A (TOR2A), sarco/endoplasmic reticulum Ca^{2+}-ATPase and other deleterious variants, including G protein subunit

Correspondence to: Dr Helen Lew, Department of Ophthalmology, Bundang CHA Medical Center, CHA University, 59 Yatap-ro, Bundang, Seongnam, Gyeonggi 13496, Republic of Korea
E-mail: eye@cha.ac.kr

*Contributed equally

Key words: blepharospasm, gene, genotype analysis, polymorphism, botulinum neurotoxin

alpha 14, HCLS1 binding protein 3 and neurofilament heavy chain (12). Genetic screening studies have also been previously performed to elucidate associations between genetic variants and BSP. Among 20 Chinese patients with BSP, screening of 151 genes associated with movement disorders revealed that spectrin repeat containing nuclear envelope protein 1 (SYNE1) and Cip1-interacting zinc finger protein (CIZ1) mutations can contribute to BSP (6). Another study of 132 mainly Caucasian patients with BSP previously suggested that all exonic variants of guanine nucleotide-binding protein G(Olf) subunit α, CIZ1 and TOR2A may be benign, whereas there may be two REEP4 non-synonymous single-nucleotide variants (SNVs) (13). In addition, a recent case-control study of 78 Chinese patients (53 with BSP and 25 with Meige syndrome) and 96 healthy individuals revealed that exonic variants of TOR1A and THAP domain-containing protein 1 (THAP1) can contribute to the etiology of BSP and Meige syndrome (14).

TOR1A mutations have previously been found to be present in patients with early-onset torsion dystonia (15) and have been associated with late-onset focal, segmental and multifocal dystonia, including BSP, oromandibular dystonia and Meige syndrome (16-18). A previous case-control study revealed an association between the rs1182 variant of *TOR1A* and the risk of spread in BSP (19). By contrast, the results of another study suggested that rs2296793, rs1182, mitochondrial DNA deletion of Dystonia 1 (DYT1) and a three-nucleotide deletion (ΔGAG) variant of *TOR1A* were not associated with BSP (16,17). Two different case-control series of allelic association study previously focused on the role of polymorphisms in dopamine receptor D (DRD) and transporter genes, which revealed that polymorphisms in the DRD5 gene were associated with 75 BSP patients (16,20). Although a screening test indicated that the DRD2 gene does not contribute to BSP (6), decreased DRD2 binding activity and expression in the striatum have previously been identified in patients with TOR1A-related dystonia (21,22). Furthermore, *DRD2* variants have been previously found to be associated with cervical dystonia, highlighting the importance of DRD2 in motor control (23,24).

Several studies have revealed genetic risk factors for dystonia and BSP (25-28). Based on the associations with dystonia, if there is existing evidence of dopaminergic involvement in dystonia, then the role of genetic variations in dopamine-related genes could be investigated in BSP (16). Certain forms of dystonia, including those affecting the face and eyes, may respond to dopaminergic medications (20). Investigating the function of genetic factors in the dopaminergic pathway may reveal the reason for variations in clinical responses to dopaminergic medications, providing insights into the possibility of individualized treatment approaches. However, such studies have not been able to produce consistently reproducible results, implying variability in the architecture of BSP genetics (28,29). To the best of our knowledge, there remains a lack of genetic research involving Asian populations with BSP, except in China (6). Therefore, the present study explored the possible association between genetic variants and BSP in a Korean population.

Although there is accumulating evidence suggesting that gene alterations may lead to BSP and extensive data concerning pathogenic variants in BSP already exist (6,12-14), the contributions of single-nucleotide polymorphisms (SNPs) in BSP remain poorly understood. Notably, results tended to differ according to ethnicity (6,16), rendering the role of genetic variants in the risk for BSP unclear. Therefore, hypotheses or clinical observations may suggest a role for dopamine dysregulation in the etiology of BSP. As there is reason to suspect dopamine involvement in BSP, exploring the genetic underpinnings of the dopaminergic pathway may prove beneficial for understanding the pathophysiology of BSP. In addition, identifying genetic factors in the dopaminergic pathway may contribute to the development of personalized treatment strategies for each patient with BSP.

In the present study, the roles of *TOR1A*, *DRD2* and *DRD5* SNPs in South Korean patients with BSP were assessed, which were selected based on major allele frequency and previous association studies (Tables SI and SII). In addition, the associations among symptoms, treatment response to BoNT-A and genetic variants were investigated.

Materials and methods

Participants and clinical evaluations. In the present prospective case-control study of South Korean individuals, peripheral blood samples of 56 patients with BSP (7 men and 49 women; mean age, 64.1±12.6 years) and 115 healthy controls (23 men and 92 women; mean age, 60.8±12.1 years) who came for check-ups at the outpatient clinic were collected from the Department of Ophthalmology at CHA Bundang Medical Center (Seongnam, South Korea) between March 2021 and May 2022. Patients with BSP were diagnosed by an ophthalmologist. The inclusion criterion was the diagnosis of primary BSP (1). The exclusion criterion was secondary BSPs, such as those caused by mass lesions, trauma or exposure to neuro-substances. The 'controls' were age-matched individuals who did not have any periocular muscle dystonia. All study protocols were reviewed and approved by the Institutional Review Board of CHA Bundang Medical Center (approval no. CHAMC 2020-10-012; Seongnam, South Korea). Genotype frequencies in the patients with BSP were analyzed based on periocular manifestations, such as involuntary forceful facial muscle contraction. BSP was classified as periocular dystonia (dystonia located only around the eyes) or wide-spread dystonia (contractions affecting the face, as well as regions around the eyes) (1).

Patients with BSP were treated with an injection of 2 U/0.1 cc BoNT-A (onabotulinumtoxin A, BOTOX®; Allergan; AbbVie, Inc.) except for 5 patients, who requested observation. The mean BoNT-A injection dose among the patients with BSP was 0.8±0.2 cc. Clinical outcomes were evaluated using the Jankovic rating scale (30) or changes in injection dose. Both frequency scores and severity scores were >3 points for all patients. Good responders were patients who improved to 0 or 1 on the rating scale and maintained the same injection dose at the next injection as that applied at the initial injection. Poor responders were patients who exhibited >2 points on the rating scale at the initial injection, along with an increase of 50% in the BoNT-A dose at the next injection (31).

Analysis of peripheral blood. To measure physiological parameters, including electrolytes, 3 ml blood was collected after an overnight fast. Plasma glucose levels were measured in duplicate using the hexokinase method on an automated

analyzer (Cobas® C 702; Roche Diagnostics). High-density lipoprotein cholesterol (HDL-C) levels were measured using an enzymatic colorimetric method with commercial reagents (TBA 200FR NEO; Canon Medical Systems Corporation), and total cholesterol and blood urea nitrogen were also measured using commercial enzymatic colorimetric tests (Cobas® C 702; Roche Diagnostics). Prothrombin and activated partial thromboplastin times were measured using an ACL TOP automated photo-optical coagulometer (LSI Medience Corporation).

Genetic analysis. Genomic DNA (gDNA) was extracted from peripheral blood leukocytes using a GDEX II gDNA Extraction kit (Intron Biotechnology, Inc.). All genetic polymorphisms were determined by PCR-restriction fragment length polymorphism (PCR-RFLP) analysis and TaqMan allele discrimination analysis, using the isolated gDNA as the template. The PCR-RFLP primers used were as follows: *TOR1A* rs1182 C>A forward, 5'-CAACAACTTAGAATCTGA GCAGTCTCTC-3' and reverse, 5'-ATGTGACAGGAATTC TCCCTGG-3'; and *DRD2* rs1800497 G>A forward, 5'-AGC ACCTTCCTGAGTGTCATCAAC-3' and reverse, 5'-TGT GCAGCTCACTCCATCCT-3' (annealing at 58˚C) by using Solgent premix (Solgent). The thermocycling conditions was based on Solgent protocol (initial denaturation at 95˚C for 5 min, 35 cycle of denaturation at 95˚C for 30 sec, annealing at 58˚C for 30 sec and extension at 72˚C for 1 min, and final extension at 72˚C for 5 min). The size of the PCR product (Fig. S1) for *TOR1A* rs1182 C>A was 278 bp, and digestion would yield CC (278 1bp), CA (278, 207 and 71 bp) or AA (207 and 71 bp) allele fragments. The size of the PCR product (Fig. S1) for *DRD2* rs1800497 G>A was 174 bp and digestion would yield GG (128 and 46 bp), GA (174, 128 and 46 bp) or AA (174 bp) fragments. Both PCR products were digested with *Taq*I at 37˚C for 16 h and examined on 3% agarose gels with eco dye (Biofact).

For allelic discrimination, *TOR1A* rs1801968 C>G and *DRD5* rs6283 T>C were genotyped using real-time PCR (RG-3000; Qiagen, Inc.). Real-time PCR was performed using the 2X Real-time Smart mix (Solgent Corporation) (initial denaturation at 95˚C for 5 min, 40 cycles of denaturation at 95˚C for 30 sec, annealing at 58˚C for 45 sec and final extension at 72˚C for 5 min). TaqMan probes were designed using Primer Express Software (v2.0) and synthesized by Bioneer (Daedeok-gu) with the FAM and JOE reporter dyes. SNPs were detected using a TaqMan probe assay kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). Genotyping was performed according to the manufacturer's protocols (sequences were not provided by the supplier; *TOR1A* rs1801968 C>G: Assay ID C_7428931_10, cat. no. 4351379; and *DRD5* rs6283 T>C: Assay ID C_226374441_10, cat. no. 4351379) (Fig. S2). To validate the analysis, ~20% of the samples were randomly selected for DNA sequencing using an ABI 3730XL DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.) (Fig. S3). The concordance of the quality control samples was 100%.

Statistical analysis. Associations of each genotype with BSP were estimated by calculating the adjusted odds ratios (adjusted by age and sex). Genotyping and statistical analysis methods were performed as previously described (26,29,32). Genotyping was done by considering most frequently occurring homozygous genotype as the dominant model while considering less frequently occurring homozygous genotype as the recessive model. Statistical analyses were conducted with GraphPad Prism 4.0 (Dotmatics) or MedCalc (ver. 12.7.1.0; MedCalc Software Ltd.). To analyze differences in clinical characteristics between the study groups, Fisher's exact test was used for categorical data. Independent-samples t-tests or one-way analysis of variance and Scheffé's post-hoc test were used for continuous data from the same samples. The genotype distributions of *TOR1A*, *DRD2* and *DRD5* SNPs were compared between patients with BSP and controls using binary logistic regression analyses. All polymorphisms were confirmed to be suitable for research if they satisfied the Hardy-Weinberg equilibrium (P>0.05). Odds ratios and 95% CIs were used to measure the strength of association between each polymorphism and BSP. Multivariate analyses were conducted with adjustments for sex and age, which are reported as risk factors for BSP (7). Allele combinations of multiple loci were analyzed using HAPSTAT (v.3.0; https://dlin.web.unc.edu/software/hapstat/). P<0.05 was considered to indicate a statistically significant difference.

Results

Baseline characteristics. The present study included 56 patients with BSP (7 men and 49 women) and 115 age- and sex-matched controls (23 men and 92 women). The mean age was 64.1±12.6 years (range, 52-78 years; median, 65 years) for the group of patients with BSP and 60.8±12.1 years (range, 49-73 years; median, 58 years) for the control group.

There were no differences in the proportions of hypertension, diabetes mellitus or dyslipidemia diagnoses between the BSP and control groups. By contrast, the lipid profile was significantly higher in the control group than in the BSP group in terms of total cholesterol levels (P=0.007) and triglyceride levels (P=0.042), but lower in HDL-C levels (P<0.001). However, no differences in low-density lipoprotein cholesterol (LDL-C) levels were observed. No significant differences were observed for the other parameters assessed (Table I).

Comparison of genotype frequencies. The genotype frequencies for each polymorphism were measured. The frequency of *DRD2* rs1800497 was found to significantly higher in the BSP than the control group in the recessive model (P<0.05). However, none of the other SNVs significantly differed between the BSP and control groups (Table II).

Genotype combination. Genotype combination analysis was next performed to investigate the combined genotype effects of each SNP. For the *TOR1A* rs1801968 C>G/*TOR1A* rs1182 C>A/*DRD2* rs1800497 G>A/*DRD5* rs6283 T>C genotype combination in Table IIIA and F, the combined *TOR1A* rs1182CC/*DRD5* rs6283TC genotype was found to be associated with a risk of BSP (P=0.013; Table III).

Genotype frequencies according to symptoms. *DRD5* rs6283 was observed to be associated with the periocular type of BSP in the co-dominant (for the TC genotype, P=0.029) and dominant (P=0.029) models (Table IV).

Table I. Baseline characteristics of BSP and control subjects.

Characteristic	Control (n=115)	BSP (n=56)	P-value[a]
Age, years	60.8±12.1	64.1±12.6	0.079
Sex (male:female)	23:92	7:49	0.319
Total cholesterol, mg/dl	196.28±39.75	172.56±41.69	0.007
High-density lipoprotein cholesterol, mg/dl	46.08±11.38	64.84±17.36	<0.001
Low-density lipoprotein cholesterol, mg/dl	111.27±52.66	98.37±39.79	0.422
Triglyceride, mg/dl	132.21±65.90	102.23±36.79	0.042
Fasting blood sugar, mg/dl	110.12±33.46	103.68±14.53	0.906
Hemoglobin A1c, %	6.43±1.06	6.13±0.70	0.405
Blood urea nitrogen, mg/dl	15.49±5.27	14.65±4.94	0.463
Na^+, mEq/l	141.69±2.17	141.43±2.1	0.621
K^+, mEq/l	4.28±0.37	4.28±0.34	0.985
Mg^{2+}, mg/dl	1.60±0.00	2.14±0.17	0.422
Ca^{2+}, mg/dl	9.50±0.28	9.34±0.44	0.076
Troponin T, ng/ml	0.01±0.00	0.01±0.00	0.726
Thyroid stimulating hormone, uIU/ml	3.49±2.59	2.34±1.62	0.105
Prothrombin time, sec	11.66±0.76	11.38±0.75	0.054
Activated partial thromboplastin time, sec	33.46±23.64	30.83±4.19	0.861
Hypertension	88 (76.5)	20 (35.7)	0.873
Diabetes mellitus	35 (30.4	6 (10.7)	0.561
Dyslipidemia	23 (20.0)	16 (28.6)	0.289

[a]P-values were calculated using a two-sided t-test for continuous variables and Fisher's exact test for categorical variables comparing between control subjects and the BSP group. Values are expressed as the mean ± standard deviation, n or n (%). BSP, blepharospasm.

Genotype frequencies according to treatment response. After BoNT-A therapy, 82.4% (42/51) of the patients were considered good responders, whereas 17.6% (9/51) were considered poor responders. Five patients refused BoNT injection and preferred to be observed with conservative management such as sunglasses and artificial eye drops. They did not present any sign of improvement or worsening during the follow-up. The recessive model of *TOR1A* rs1801968 (P=0.030), in addition to the recessive (P=0.029) and over-dominant models (P=0.019) of *DRD2* rs1800497 were associated with superior responses to BoNT treatment. By contrast, the dominant (P=0.034) and additive (P=0.030) models of *DRD5* rs6283 were associated with a poor responses to BoNT treatment (Table V).

Discussion

In the present study, the relationship between genetic polymorphisms and BSP was examined through comparisons between age- and sex-matched controls in a South Korean population. There is accumulating evidence that certain genetic variants are associated with BSP and other diseases (6,16).

Hypercholesterolemia has been documented to aggravate systemic inflammation (33). On a cellular level, a dysregulated lipid status can mediate important alterations in the innate and adaptive immune system by interrupting antigen-presenting cell and lymphocyte function (33). The effects of LDL-C, free fatty acids and cholesterol crystals on the activation and inflammatory action of macrophages have been extensively described (33,34).

In the present study, patients with BSP had lower total cholesterol levels and higher HDL-C levels but similar LDL-C levels compared with those in the control group. Hypercholesterolemia has been previously found to induce toll-like receptor signaling, which could decrease the cholesterol efflux from cells, resulting in the further accumulation of cholesterol and aggravation of the inflammatory response (34,35). Accordingly, the roles of cholesterol and cholesterol-lowering medications must be investigated in terms of BSP onset and progression.

TOR1A encodes torsion A and serves important roles in synapse formation and connectivity organization in the spinal sensorimotor circuit (36). The *TOR1A* rs1182 polymorphism has been reported to influence the spread of BSP to adjacent regions (16). However, previous studies of ΔGAG in *TOR1A* found that this mutation was not associated with BSP (17,18). To the best of our knowledge, there is limited information on the role of *TOR1A* mutations in BSP. In the present study, no significant associations between *TOR1A* SNVs and BSP were found. Since BSP was not found to be associated with *TOR1A* rs1182 or *TOR1A* rs1801968, the variants of these two polymorphisms were concluded to bear no relationship with BSP.

The generalizability of genetic findings across different populations is a complex and nuanced aspect of genetic research. Therefore, it is of importance to validate the results by comparing the findings of the present study with those for other ethnicities. No pathogenic or likely pathogenic sequence variants in the BSP-associated genes assessed, including

Table II. Comparison of genotype frequencies in patients with BSP and control subjects.

Mode	Genotypes	Controls (n=115), n (%)	BSP (n=56), n (%)	COR (95% CI)	P-value	AOR (95% CI)	P-value
A, *TOR1A* rs1801968C>G							
Co-dominant	CC	100 (86.9)	53 (94.6)	1.000 (reference)		1.000 (reference)	
	CG	14 (12.2)	3 (5.4)	0.404 (0.111-1.470)	0.169	0.429 (0.117-1.582)	0.204
	GG	1 (0.9)	0 (0.0)	N/A	-	N/A	-
Dominant	CC vs. CG + GG	N/A	N/A	0.377 (0.105-1.362)	0.137	0.404 (0.111-1.477)	0.171
Recessive	CC + CG vs. GG	N/A	N/A	N/A	-	N/A	-
Over-dominant	CC + GG vs. CG	N/A	N/A	0.408 (0.112-1.484)	0.174	0.433 (0.117-1.595)	0.208
Additive	N/A	N/A	N/A	0.38 (0.109-1.323)	0.129	0.406 (0.115-1.434)	0.161
B, *TOR1A* rs1182C>A							
Co-dominant	CC	73 (63.5)	30 (53.6)	1.000 (reference)		1.000 (reference)	
	CA	35 (30.4)	22 (39.3)	1.53 (0.773-3.025)	0.222	1.588 (0.794-3.179)	0.191
	AA	7 (6.0)	4 (7.1)	1.391 (0.379-5.102)	0.619	1.252 (0.333-4.714)	0.739
Dominant	CC vs. CA + AA	N/A	N/A	1.506 (0.788-2.880)	0.215	1.529 (0.791-2.955)	0.206
Recessive	CC + CA vs. AA	N/A	N/A	1.187 (0.333-4.236)	0.792	1.062 (0.291-3.880)	0.927
Over-dominant	CC + AA vs. CA	N/A	N/A	1.479 (0.759-2.883)	0.250	1.553 (0.786-3.068)	0.205
Additive	N/A	N/A	N/A	1.329 (0.798-2.214)	0.275	1.315 (0.784-2.207)	0.300
C, DRD2 rs1800497G>A							
Co-dominant	GG	40 (34.8)	19 (33.9)	1.000 (reference)		1.000 (reference)	
	GA	48 (441.7)	31 (55.4)	1.36 (0.669-2.762)	0.396	1.482 (0.715-3.069)	0.290
	AA	27 (223.5)	6 (10.7)	0.468 (0.165-1.323)	0.152	0.482 (0.169-1.374)	0.172
Dominant	GG vs. GA + AA	N/A	N/A	1.039 (0.530-2.036)	0.912	1.086 (0.547-2.154)	0.814
Recessive	GG + GA vs. AA	N/A	N/A	0.391 (0.151-1.012)	0.053	0.369 (0.141-0.966)	0.042
Over-dominant	GG + AA vs. GA	N/A	N/A	1.731 (0.909-3.296)	0.095	1.883 (0.973-3.645)	0.060
Additive	N/A	N/A	N/A	0.791 (0.504-1.243)	0.310	0.794 (0.504-1.253)	0.322
D, *DRD5* rs6283T>C							
Co-dominant	TT	33 (28.7)	24 (42.9)	1.000 (reference)		1.000 (reference)	
	TC	58 (50.4)	22 (39.3)	0.506 (0.246-1.041)	0.064	0.51 (0.244-1.065)	0.073
	CC	24 (20.9)	10 (17.9)	0.556 (0.224-1.377)	0.205	0.659 (0.252-1.726)	0.396

Table II. Continued.

D, *DRD5* rs6283T>C

Mode	Genotypes	Controls (n=115), n (%)	BSP (n=56), n (%)	COR (95% CI)	P-value	AOR (95% CI)	P-value
Dominant	TT vs. TC + CC	N/A	N/A	0.52 (0.267-1.015)	0.056	0.559 (0.284-1.103)	0.094
Recessive	TT + TC vs. CC	N/A	N/A	0.815 (0.36-1.849)	0.625	0.919 (0.398-2.127)	0.844
Over-dominant	TT + CC vs. TC	N/A	N/A	0.636 (0.332-1.217)	0.172	0.623 (0.322-1.206)	0.161
Additive	N/A	N/A	N/A	0.712 (0.451-1.123)	0.144	0.756 (0.475-1.205)	0.240

HWE P-value (Control, BSP): *TOR1A* rs1801968C>G (0.523, 0.837), *TOR1A* rs1182C>A (0.322, 0.990), *DRD2* rs1800497G>A (0.098, 0.203), *DRD5* rs6283T>C (0.873, 0.226). *TOR1A*, torsin 1A; *DRD*, dopamine receptor D; AOR, adjusted odds ratio (adjusted by age and sex of subjects); BSP, blepharospasm; COR, crude odds ratio; HWE, Hardy-Weinberg equilibrium; N/A, not applicable.

TOR1A, could be identified in a previous whole-exome sequencing study of a multiplex African-American pedigree (37). Clarimon *et al* (16) reported an analysis in two independent cohorts of Italian and North American patients with BSP. They revealed an association with the same risk genotype of Torsin A (Dystonia 1) as in an Icelandic population. In addition, the frequencies of *TOR1A* rs1435566780 and *THAP1* rs545930392 were previously found to be higher in patients with BSP compared with those in the control group in an analysis of Han Chinese populations (14).

The dopaminergic pathway can influence the basal ganglia damage and the abnormality of a dopaminergic pathway may be implicated in the pathogenesis of dystonic conditions, including BSP (38-40). Fayers *et al* (41) previously reported that patients with BSP had fewer nerves in the sub-basal plexus than the controls, suggesting that impaired corticosensory processing due to a defect in the sensorimotor gating mechanism resulted in the loss of blink reflex inhibition. A previous study found a number of genetic variants that may be associated with BSP, including allele 2 of *DRD5* and the D1.1 SNVs of the D1 receptor gene (20). However, the results of another study suggested that *DRD5* did not contribute to BSP risk (16). In the present study, it was found that the *DRD2* rs1800497 SNV was associated with increased susceptibility to BSP. For *DRD5* rs6283, the lack of statistical power due to the limited number of patients may explain the absence of an association between this SNV and BSP. However, *DRD5* rs6283 was found to be associated with the periocular type of BSP. Because *DRD2* rs1800497 is located in the 3'-untranslated region, which serves an important role in mRNA stability upstream of translation (23), one mechanism of dystonia (and therefore BSP) as a result of this SNV could be by altering mRNA translation. *DRD5* rs6283 was found to be associated with the periocular type of BSP in the present study, suggesting that the level of DRD5 gene expression differs according to the locations of involved neurons.

As an aspect of the clinical spread type in primary BSP, the present study confirmed that there was different genetic susceptibility in *DRD2* and *DRD5*. Under physiological conditions, the blink reflex is inhibited if conditioned by a preceding peripheral nerve stimulus. This is presumably mediated through an inhibitory brainstem reticular pathway involving the pedunculopontine nucleus, which is disrupted by damage to the pedunculopontine nucleus or the adjacent architecture (42,43). Blink reflex excitability in patients with BSP implies a degree of variation in the sensorimotor signal pathway because of impaired corticosensory processing and defect in the sensorimotor gating mechanism (41). Given these differences according to the genetic variant could influence the facial nerve by a distinct sensorimotor pathway from the cornea to the brain stem, the degree of genetic expression may vary. If genetic variants are used for BSP screening, it may be necessary to target different SNPs depending on the clinical parameters.

Comparing the two responder groups to BoNT-A injection, the previous study reported that the poor responders exhibited a different blink profile such as increased lid closure time and decreased closing speed, including apraxia of eyelid opening (31). Therefore, a more detailed classification of BSP is required, along with a revised approach to treatment selection.

Table III. Genotype combinations of polymorphisms.

Characteristics	Controls (n=115), n (%)	BSP (n=56), n (%)	AOR (95% CI)	P-value
A, *TOR1A* rs1801968 C>G/*TOR1A* rs1182 C>A				
CC/CC	62 (53.9)	27 (48.2)	1.000 (reference)	
CC/CA	31 (27.0)	22 (44.9)	1.755 (0.847-3.636)	0.130
CC/AA	7 (6.1)	4 (8.2)	1.177 (0.307-4.514)	0.812
CG/CC	10 (8.7)	3 (6.1)	0.764 (0.191-3.053)	0.703
CG/CA	4 (3.5)	0 (0.0)	N/A	-
CG/AA	0 (0.0)	0 (0.0)	N/A	-
GG/CC	1 (0.9)	0 (0.0)	N/A	-
GG/CA	0 (0.0)	0 (0.0)	N/A	-
GG/AA	0 (0.0)	0 (0.0)	N/A	-
B, *TOR1A* rs1801968 C>G/*DRD2* rs1800497 G>A				
CC/GG	33 (28.7)	18 (36.7)	1.000 (reference)	
CC/GA	44 (38.3)	29 (59.2)	1.290 (0.600-2.777)	0.514
CC/AA	23 (20.0)	6 (12.2)	0.481 (0.164-1.414)	0.183
CG/GG	7 (6.1)	1 (2.0)	0.259 (0.029-2.301)	0.226
CG/GA	4 (3.5)	2 (4.1)	1.231 (0.180-8.418)	0.832
CG/AA	3 (2.6)	0 (0.0)	N/A	-
GG/GG	0 (0.0)	0 (0.0)	N/A	-
GG/GA	0 (0.0)	0 (0.0)	N/A	-
GG/AA	1 (0.9)	0 (0.0)	N/A	-
C, *TOR1A* rs1801968 C>G/*DRD5* rs6283 T>C				
CC/TT	30 (26.1)	24 (49.0)	1.000 (reference)	
CC/TC	50 (43.5)	19 (38.8)	0.460 (0.211-1.000)	0.050
CC/CC	20 (17.4)	10 (20.4)	0.765 (0.283-2.067)	0.597
CG/TT	3 (2.6)	0 (0.0)	N/A	-
CG/TC	7 (6.1)	3 (6.1)	1.304 (0.365-4.660)	0.683
CG/CC	4 (3.5)	0 (0.0)	N/A	-
GG/TT	0 (0.0)	0 (0.0)	N/A	-
GG/TC	1 (0.9)	0 (0.0)	N/A	-
GG/CC	0 (0.0)	0 (0.0)	N/A	-
D, *TOR1A* rs1182 C>A/*DRD2* rs1800497 G>A				
CC/GG	27 (23.5)	18 (36.7)	1.000 (reference)	
CC/GA	28 (24.3)	29 (59.2)	1.451 (0.563-3.740)	0.441
CC/AA	19 (16.5)	6 (12.2)	0.398 (0.096-1.648)	0.204
CA/GG	12 (10.4)	1 (2.0)	1.125 (0.332-3.815)	0.850
CA/GA	19 (16.5)	2 (4.1)	2.075 (0.724-5.945)	0.174
CA/AA	4 (3.5)	0 (0.0)	1.671 (0.313-8.920)	0.548
AA/GG	1 (0.9)	0 (0.0)	4.567 (0.366-6.961)	0.238
AA/GA	2 (1.7)	0 (0.0)	2.671 (0.289-4.667)	0.386
AA/AA	3 (2.6)	0 (0.0)	N/A	-
E, *TOR1A* rs1182 C>A/*DRD5* rs6283 T>C				
CC/TT	19 (16.5)	17 (34.7)	1.000 (reference)	
CC/TC	38 (33.0)	10 (20.4)	0.288 (0.108-0.769)	0.013

Table III. Continued.

E, *TOR1A* rs1182 C>A/*DRD5* rs6283 T>C				
Characteristics	Controls (n=115), n (%)	BSP (n=56), n (%)	AOR (95% CI)	P-value
CC/CC	16 (13.9)	3 (6.1)	0.260 (0.062-1.095)	0.066
CA/TT	11 (9.6)	7 (14.3)	0.802 (0.236-2.726)	0.723
CA/TC	18 (15.7)	8 (16.3)	0.424 (0.140-1.287)	0.130
CA/CC	6 (5.2)	7 (14.3)	1.508 (0.396-5.744)	0.547
AA/TT	3 (2.6)	0 (0.0)	N/A	-
AA/TC	2 (1.7)	4 (8.2)	2.256 (0.350-4.535)	0.392
AA/CC	2 (1.7)	0 (0.0)	N/A	-
F, *DRD2* rs1800497 G>A/*DRD5* rs6283 T>C				
GG/TT	8 (7.0)	6 (12.2)	1.000 (reference)	
GG/TC	21 (18.3)	12 (24.5)	0.787 (0.217-2.855)	0.715
GG/CC	11 (9.6)	1 (2.0)	0.105 (0.009-1.297)	0.079
GA/TT	14 (12.2)	15 (30.6)	1.670 (0.430-6.495)	0.459
GA/TC	26 (22.6)	10 (20.4)	0.530 (0.144-1.951)	0.340
GA/CC	8 (7.0)	6 (12.2)	1.571 (0.186-3.247)	0.678
AA/TT	11 (9.6)	3 (6.1)	0.356 (0.066-1.919)	0.230
AA/TC	11 (9.6)	0 (0.0)	N/A	-
AA/CC	5 (4.3)	3 (6.1)	0.551 (0.074-4.101)	0.560

TOR1A, torsin 1A; *DRD*, dopamine receptor D; BSP, blepharospasm; AOR, adjusted odds ratio (adjusted by age and sex); N/A, not applicable.

It may be possible to establish a diagnosis by detecting genetic variants and implement appropriate treatment strategies that avoid unnecessary medical procedures and expenses, such as repeated injections. Early detection of these genetic differences may facilitate potential patient-specific gene therapy.

BoNT-A interferes with neural transmission by blocking acetylcholine release at the presynaptic motor neurons at neuromuscular junctions, which causes muscle paralysis (44). BoNT-A was approved for the treatment of BSP by the FDA in 1991, since various studies have demonstrated its efficacy without significant long-term side effects (45,46). In the present study, *TOR1A* rs1801968 and *DRD2* rs1800497 were found to be associated with superior treatment responses, whereas *DRD5* rs6283 was associated with poor responses.

Amongst the treatment responders, the recessive model of *TOR1A* rs1801968 was associated with a superior response to BoNT-A in this study. Individuals with this mutation may have altered neuronal function, potentially affecting motor control (47,48). The SNP rs1800497 on chromosome 11q23.2 is located in exon 8 of the ankyrin repeat domain containing one gene downstream of DRD2 (49). Genetic variations in this receptor have been found to influence dopamine signaling, impacting motor function (50). These genetic variations may contribute to alterations in the basal ganglia circuitry, a region implicated in motor control (50,51). Changes in DRD function may affect the balance between inhibitory and excitatory signals of blink (25). Therefore, the poor responder group could be associated with *DRD5* rs6283. The SNP rs1800497 on chromosome 4p16.1 is located in exon 1 of a G-protein coupled receptor which stimulates adenyl cyclase of downstream of DRD5. Variations in this receptor have been found to alter dopamine responsiveness in certain neural circuits such as cortico-basal ganglia-thalamo-cortical loops (50,51). Altered dopamine D5 receptor function may affect the responsiveness of postsynaptic neurons, potentially reducing the efficacy of BoNT-A, which relies on neurotransmission processes (25).

The aforementioned findings suggest that the effect of BoNT will likely differ among the genetic variants associated with BSP, such as those in TOR1A and DRD5. Several studies have explored the effects of BoNT-A on gene expression (52,53). Their results indicated that BoNT-A may affect the expression of genes in other sensorimotor pathways, suggesting that the treatment itself may influence the clinical outcomes among patients with BSP.

Numerous studies have previously examined the etiology of BSP and the contributions of genetic variants to BSP onset. Since a whole-exome sequencing analysis identified several variants among 31 patients with BSP from 21 independent pedigrees (12), another study explored potential candidate genes, CIZ1, TOR2A and REEP4, using exome sequencing in 132 patients with BSP (13). In addition, in another previous study, to understand the genetic etiology of BSP, genetic screening of 151 genes associated with movement disorders was performed in 20 patients with BSP. However, only a few genes (*SYNE1* and *CIZ1*) were found to contribute to the etiology of BSP (6). Additional case-control studies have previously demonstrated associations with polymorphisms, although conflicting and population-specific results were

Table IV. Genotype frequencies for each polymorphism according to the clinical symptoms.

Mode	Genotypes	Periocular (n=33), n (%)	AOR (95% CI)	P-value	Facial (n=23), n (%)	AOR (95% CI)	P-value
A, *TOR1A* rs1801968C>G							
Co-dominant	CC	31 (93.9)	1.000 (reference)		22 (95.7)	1.000 (reference)	
	CG	2 (6.1)	0.493 (0.104-2.339)	0.374	1 (4.3)	0.321 (0.040-2.595)	0.286
	GG	0 (0.0)	N/A	-	0 (0.0)	N/A	-
Dominant	CC vs. CG + GG	N/A	0.463 (0.099-2.175)	0.329	N/A	0.305 (0.038-2.456)	0.265
Recessive	CC + CG vs. GG	N/A	N/A	-	N/A	N/A	-
Over-dominant	CC + GG vs. CG	N/A	0.497 (0.105-2.356)	0.378	N/A	0.323 (0.040-2.612)	0.289
Additive	N/A	N/A	0.463 (0.103-2.073)	0.314	N/A	0.310 (0.040-2.419)	0.264
B, *TOR1A* rs1182C>A							
Co-dominant	CC	18 (54.5)	1.000 (reference)		12 (52.2)	1.000 (reference)	
	CA	12 (36.4)	1.412 (0.605-3.294)	0.425	10 (43.5)	1.888 (0.734-4.857)	0.187
	AA	3 (9.1)	2.019 (0.451-9.030)	0.358	1 (4.3)	0.975 (0.103-9.210)	0.982
Dominant	CC vs. CA + AA	N/A	1.503 (0.677-3.341)	0.317	N/A	1.763 (0.705-4.408)	0.225
Recessive	CC + CA vs. AA	N/A	1.800 (0.416-7.799)	0.432	N/A	0.789 (0.088-7.047)	0.832
Over-dominant	CC + AA vs. CA	N/A	1.307 (0.571-2.992)	0.527	N/A	1.906 (0.750-4.843)	0.175
Additive	N/A	N/A	1.420 (0.764-2.638)	0.268	N/A	1.405 (0.678-2.912)	0.361
C, *DRD2* rs1800497G>A							
Co-dominant	GG	10 (30.3)	1.000 (reference)		9 (39.1)	1.000 (reference)	
	GA	20 (60.6)	1.825 (0.750-4.444)	0.185	11 (47.8)	1.134 (0.418-3.075)	0.805
	AA	3 (9.1)	0.494 (0.121-2.018)	0.326	3 (13.0)	0.518 (0.126-2.122)	0.361
Dominant	GG vs. GA + AA	N/A	1.325 (0.566-3.105)	0.517	N/A	0.898 (0.352-2.290)	0.821
Recessive	GG + GA vs. AA	N/A	0.324 (0.090-1.162)	0.084	N/A	0.495 (0.135-1.811)	0.288
Over-dominant	GG + AA vs. GA	N/A	2.329 (1.035-5.245)	0.041	N/A	1.376 (0.550-3.440)	0.495
Additive	N/A	N/A	0.853 (0.493-1.478)	0.571	N/A	0.792 (0.426-1.472)	0.461
D, *DRD5* rs6283T>C							
Co-dominant	TT	17 (51.5)	1.000 (reference)		7 (30.4)	1.000 (reference)	
	TC	11 (33.3)	0.370 (0.152-0.901)	0.029	11 (47.8)	0.845 (0.296-2.411)	0.753
	CC	5 (15.2)	0.586 (0.176-1.951)	0.384	5 (21.7)	1.450 (0.360-5.832)	0.601
Dominant	TT vs. TC + CC	N/A	0.406 (0.181-0.912)	0.029	N/A	0.949 (0.353-2.554)	0.917
Recessive	TT + TC vs. CC	N/A	0.855 (0.289-2.536)	0.778	N/A	1.306 (0.424-4.023)	0.641
Over-dominant	TT + CC vs. TC	N/A	0.46 (0.202-1.050)	0.065	N/A	0.817 (0.329-2.029)	0.664
Additive	N/A	N/A	0.61 (0.336-1.108)	0.104	N/A	1.082 (0.556-2.104)	0.817

TOR1A, torsin 1A; *DRD*, dopamine receptor D; AOR, adjusted odds ratio (adjusted by age and sex); N/A, not applicable.

Table V. Genotype frequencies for each polymorphism according to the response to BoNT treatment.

Mode	Genotypes	BoNT good responder (n=42), n (%)	AOR (95% CI)	P-value	BoNT poor-responder (n=9), n (%)	AOR (95% CI)	P-value
A, *TOR1A* rs1801968C>G							
Co-dominant	CC	39 (92.9)	1.000 (reference)		9 (100.0)	1.000 (reference)	
	CG	3 (7.1)	0.578 (0.155-2.156)	0.414	0 (0.0)	N/A	-
	GG	0 (0.0)	N/A	-	0 (0.0)	N/A	-
Dominant	CC vs. CG + GG	N/A	0.546 (0.148-2.022)	0.365	N/A	N/A	-
Recessive	CC + CG vs. GG	N/A	0.245 (0.069-0.868)	0.030	N/A	N/A	-
Over-dominant	CC + GG vs. CG	N/A	0.582 (0.156-2.173)	0.421	N/A	0.419 (0.097-1.814)	0.245
Additive	N/A	N/A	0.541 (0.152-1.918)	0.341	N/A	N/A	N/A
B, *TOR1A* rs1182C>A							
Co-dominant	CC	23 (54.8)	1.000 (reference)		5 (55.6)	1.000 (reference)	
	CA	16 (38.1)	1.517 (0.704-3.268)	0.288	3 (33.3)	1.234 (0.273-5.571)	0.785
	AA	3 (7.1)	1.589 (0.359-7.031)	0.542	1 (11.1)	2.536 (0.239-6.954)	0.440
Dominant	CC vs. CA + AA	N/A	1.527 (0.735-3.174)	0.257	N/A	1.408 (0.350-5.661)	0.630
Recessive	CC + CA vs. AA	N/A	1.367 (0.318-5.878)	0.675	N/A	2.365 (0.238-3.521)	0.463
Over-dominant	CC + AA vs. CA	N/A	1.451 (0.683-3.084)	0.333	N/A	1.101 (0.254-4.771)	0.898
Additive	N/A	N/A	1.375 (0.770-2.456)	0.282	N/A	1.444 (0.499-4.182)	0.498
C, *DRD2* rs1800497G>A							
Co-dominant	GG	13 (31.0)	1.000 (reference)		5 (55.6)	1.000 (reference)	
	GA	26 (61.9)	1.780 (0.795-3.988)	0.161	3 (33.3)	0.638 (0.138-2.938)	0.564
	AA	3 (7.1)	0.358 (0.092-1.393)	0.139	1 (11.1)	0.328 (0.036-3.016)	0.325
Dominant	GG vs. GA + AA	N/A	1.255 (0.579-2.720)	0.565	N/A	0.510 (0.127-2.049)	0.343
Recessive	GG + GA vs. AA	N/A	0.245 (0.069-0.868)	0.029	N/A	0.411 (0.048-3.500)	0.416
Over-dominant	GG + AA vs. GA	N/A	2.437 (1.156-5.141)	0.019	N/A	0.827 (0.192-3.557)	0.798
Additive	N/A	N/A	0.794 (0.476-1.324)	0.376	N/A	0.590 (0.219-1.584)	0.295
D, *DRD5* rs6283T>C							
Co-dominant	TT	17 (40.5)	1.000 (reference)		6 (66.7)	1.000 (reference)	
	TC	18 (42.9)	0.594 (0.266-1.328)	0.204	3 (33.3)	0.271 (0.062-1.182)	0.082
	CC	7 (16.7)	0.827 (0.267-2.558)	0.741	0 (0.0)	N/A	-
Dominant	TT vs. TC + CC	N/A	0.638 (0.299-1.357)	0.243	N/A	0.205 (0.047-0.889)	0.034
Recessive	TT + TC vs. CC	N/A	0.951 (0.364-2.484)	0.919	N/A	N/A	-
Over-dominant	TT + CC vs. TC	N/A	0.695 (0.337-1.435)	0.325	N/A	N/A	-
Additive	N/A	N/A	0.804 (0.473-1.367)	0.421	N/A	0.227 (0.059-0.863)	0.030

TOR1A, torsin 1A; *DRD*, dopamine receptor D; BoNT, botulinum neurotoxin; AOR, adjusted odds ratio (adjusted by age and sex); N/A, not applicable.

found (16,19,26). In the present study, only *DRD2* was found to be associated with genetic susceptibility in all patients with BSP, and the *TOR1A* rs1182CC/*DRD5* rs6283TC genotype combination tended to be associated with BSP. These findings suggest that the combined genotype effect of *TOR1A* and *DRD5* contributes to BSP risk. In addition, these results suggest that multiple SNVs can affect susceptibility to BSP. However, it should be noted that the risk of BSP can be influenced by a diverse range of factors, including multiple genes, ethnicity, population and environmental factors (25).

The present study has a number of important limitations. It analyzed a relatively small number of SNPs that were potentially associated with BSP. The study population also included a small number of poor responders to BoNT-A treatment, although the proportions of good and poor responders were similar to those reported in a previous study (31). Because the present study had a small sample size, its statistical power was <75%. However, it did offer insights supporting larger studies into associations between genetic variants and BSP. Considering these limitations, future larger cohort functional studies are required to evaluate the associations between genetic variants and BSP risk.

Despite these limitations, to the best of our knowledge, the present study was the first to analyze the genetic variation among patients with BSP in a South Korean population, where the association between genetic variants and treatment response was also analyzed. The generalizability of genetic findings across different populations is a complex and nuanced aspect of genetic research. Therefore, the results of the present study must be validated in other Asian populations before these findings can be applied clinically. Similar studies in diverse Asian populations can validate whether the observed genetic associations are consistent across various demographic groups. Collaborative validation studies and a nuanced understanding of the genetic and environmental factors involved will enhance the applicability of the results across diverse populations.

In conclusion, the results of the present study suggested that *DRD2* rs1800497 could increase BSP risk, and the *TOR1A* rs1182CC/*DRD5* rs6283TC genotype combination was associated with the response to BoNT-A in BSP. Identification of genetic variants expressed in the sensorimotor signaling pathway may elucidate the mechanisms by which each of the proposed genetic factors contributes to BSP and influences the treatment response. There is potential for predicting BSP risk through analyses of genetic susceptibility, which may facilitate patient-specific treatment.

Acknowledgements

Not applicable.

Funding

The present study was supported by National Research Foundation grants of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute, funded by the National Research Foundation of Korea (grant no. NRF 2021R1A2C201052311).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

Conceptualization: JKJ, NKK and HL. Methodology: JKJ and HL. Software: JKJ, MJK. Validation: MJK and NKK. Formal analysis: JKJ, MJK. Investigation: JKJ, MJK. Resources: JKJ and MJK. Data curation: JKJ and MJK. JKJ and MJK checked and confirm the authenticity of all the raw data. Writing-original draft preparation: JKJ, MJK. Writing-review and editing: JKJ and HL. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of CHA Bundang Medical Center (Seongnam, South Korea; approval no. CHAMC 2020-10-012). Written informed consent was obtained from all individuals involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Valls-Sole J and Defazio G: Blepharospasm: Update on epidemiology, clinical aspects, and pathophysiology. Front Neurol 7: 45, 2016.
2. Weiss EM, Hershey T, Karimi M, Racette B, Tabbal SD, Mink JW, Paniello RC and Perlmutter JS: Relative risk of spread of symptoms among the focal onset primary dystonias. Mov Disord 21: 1175-1181, 2006.
3. LeDoux MS: Meige syndrome: What's in a name? Parkinsonism Relat Disord 15: 483-489, 2009.
4. Pandey S and Sharma S: Meige's syndrome: History, epidemiology, clinical features, pathogenesis and treatment. J Neurol Sci 372: 162-170, 2017.
5. Defazio G, Hallett M, Jinnah HA, Conte A and Berardelli A: Blepharospasm 40 years later. Mov Disord 32: 498-509, 2017.
6. Dong H, Luo Y, Fan S, Yin B, Weng C and Peng B: Screening gene mutations in Chinese patients with benign essential blepharospasm. Front Neurol 10: 1387, 2020.
7. Sun Y, Tsai PJ, Chu CL, Huang WC and Bee YS: Epidemiology of benign essential blepharospasm: A nationwide population-based retrospective study in Taiwan. PLoS One 13: e0209558, 2018.
8. Lee JM, Baek JS, Choi HS, Kim SJ and Jang JW: Clinical features of benign essential blepharospasm in Korean patients. Korean J Ophthalmol 32: 339-343, 2018.
9. Simpson DM, Hallett M, Ashman EJ, Comella CL, Green MW, Gronseth GS, Armstrong MJ and Gloss D: Practice guideline update summary: Botulinum neurotoxin for the treatment of blepharospasm, cervical dystonia, adult spasticity, and headache: Report of the guideline development subcommittee of the American academy of neurology. Neurology 86: 1818-1826, 2016.
10. Krack P and Marion MH: 'Apraxia of lid opening,' a focal eyelid dystonia: Clinical study of 32 patients. Mov Disord 9: 610-615, 1994.

11. Ababneh OH, Cetinkaya A and Kulwin DR: Long-term efficacy and safety of botulinum toxin A injections to treat blepharospasm and hemifacial spasm. Clin Exp Ophthalmol 42: 254-261, 2014.
12. Tian J, Vemula SR, Xiao J, Valente EM, Defazio G, Petrucci S, Gigante AF, Rudzińska-Bar M, Wszolek ZK, Kennelly KD, *et al*: Whole-exome sequencing for variant discovery in blepharospasm. Mol Genet Genomic Med 6: 601-626, 2018.
13. Hammer M, Abravanel A, Peckham E, Mahloogi A, Majounie E, Hallett M and Singleton A: Blepharospasm: A genetic screening study in 132 patients. Parkinsonism Relat Disord 64: 315-318, 2019.
14. Teng X, Qu Q, Shu Y, Gong J, Xu B and Qu J: Genetic screening in patients of Meige syndrome and blepharospasm. Neurol Sci 43: 3683-3694, 2022.
15. Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, de Leon D, Brin MF, Raymond D, Corey DP, *et al*: The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 17: 40-48, 1997.
16. Clarimon J, Brancati F, Peckham E, Valente EM, Dallapiccola B, Abruzzese G, Girlanda P, Defazio G, Berardelli A, Hallett M and Singleton AB: Assessing the role of DRD5 and DYT1 in two different case-control series with primary blepharospasm. Mov Disord 22: 162-166, 2007.
17. Xiao J, Bastian RW, Perlmutter JS, Racette BA, Tabbal SD, Karimi M, Paniello RC, Blitzer A, Batish SD, Wszolek ZK, *et al*: High-throughput mutational analysis of TOR1A in primary dystonia. BMC Med Genet 10: 24, 2009.
18. Yoshida K: Development and validation of a disease-specific oromandibular dystonia rating scale (OMDRS). Front Neurol 11: 583177, 2020.
19. Defazio G, Matarin M, Peckham EL, Martino D, Valente EM, Singleton A, Crawley A, Aniello MS, Brancati F, Abbruzzese G, *et al*: The TOR1A polymorphism rs1182 and the risk of spread in primary blepharospasm. Mov Disord 24: 613-616, 2009.
20. Misbahuddin A, Placzek MR, Chaudhuri KR, Wood NW, Bhatia KP and Warner TT: A polymorphism in the dopamine receptor DRD5 is associated with blepharospasm. Neurology 58: 124-126, 2002.
21. Augood SJ, Hollingsworth Z, Albers DS, Yang L, Leung JC, Muller B, Klein C, Breakefield XO and Standaert DG: Dopamine transmission in DYT1 dystonia: A biochemical and autoradiographical study. Neurology 59: 445-448, 2002.
22. Asanuma K, Ma Y, Okulski J, Dhawan V, Chaly T, Carbon M, Bressman SB and Eidelberg D: Decreased striatal D2 receptor binding in non-manifesting carriers of the DYT1 dystonia mutation. Neurology 64: 347-349, 2005.
23. van der Weijden MCM, Rodriguez-Contreras D, Delnooz CCS, Robinson BG, Condon AF, Kielhold ML, Stormezand GN, Ma KY, Dufke C, Williams JT, *et al*: A gain-of-function variant in dopamine D2 receptor and progressive chorea and dystonia phenotype. Mov Disord 36: 729-739, 2021.
24. Liu Y, Xing H, Yokoi F, Vaillancourt DE and Li Y: Investigating the role of striatal dopamine receptor 2 in motor coordination and balance: Insights into the pathogenesis of DYT1 dystonia. Behav Brain Res 403: 113137, 2021.
25. Siokas V, Aloizou AM, Tsouris Z, Michalopoulou A, Mentis AA and Dardiotis E: Risk factor genes in patients with dystonia: A comprehensive review. Tremor Other Hyperkinet Mov (N Y) 8: 559, 2018.
26. Siokas V, Kardaras D, Aloizou AM, Asproudis I, Boboridis KG, Papageorgiou E, Hadjigeorgiou GM, Tsironi EE and Dardiotis E: BDNF rs6265 (Val66Met) polymorphism as a risk factor for blepharospasm. Neuromolecular Med 21: 68-74, 2019.
27. Siokas V, Kardaras D, Aloizou AM, Asproudis I, Boboridis KG, Papageorgiou E, Spandidos DA, Tsatsakis A, Tsironi EE and Dardiotis E: Lack of association of the rs11655081 ARSG gene with blepharospasm. J Mol Neurosci 67: 472-476, 2019.
28. Siokas V, Kardaras D, Aloizou AM, Liampas I, Papageorgiou E, Drakoulis N, Tsatsakis A, Mitsias PD, Hadjigeorgiou GM, Tsironi EE and Dardiotis E: CYP1A2 rs762551 and ADORA2A rs5760423 polymorphisms in patients with blepharospasm. J Mol Neurosci 70: 1370-1375, 2020.
29. Siokas V, Dardiotis E, Tsironi EE, Tsivgoulis G, Rikos D, Sokratous M, Koutsias S, Paterakis K, Deretzi G and Hadjigeorgiou GM: The role of TOR1A polymorphisms in dystonia: A systematic review and meta-analysis. PLoS One 12: e0169934, 2017.
30. Jankovic J and Orman J: Botulinum A toxin for cranial-cervical dystonia: A double-blind, placebo-controlled study. Neurology 37: 616-623, 1987.
31. Jang J and Lew H: Blink index as a response predictor of blepharospasm to botulinum neurotoxin-A treatment. Brain Behav 11: e2374, 2021.
32. Park HS, Kim IJ, Kim EG, Ryu CS, Lee JY, Ko EJ, Park HW, Sung JH and Kim NK: A study of associations between CUBN, HNF1A, and LIPC gene polymorphisms and coronary artery disease. Sci Rep 10: 16294, 2020.
33. Dehnavi S, Sohrabi N, Sadeghi M, Lansberg P, Banach M, Al-Rasadi K, Johnston TP and Sahebkar A: Statins and autoimmunity: State-of-the-art. Pharmacol Ther 214: 107614, 2020.
34. Tall AR and Yvan-Charvet L: Cholesterol, inflammation and innate immunity. Nat Rev Immunol 15: 104-116, 2015.
35. Aguilar-Ballester M, Herrero-Cervera A, Vinué Á, Martínez-Hervás S and González-Navarro H: Impact of cholesterol metabolism in immune cell function and atherosclerosis. Nutrients 12: 2021, 2020.
36. Monje MHG and Sánchez-Ferro A: Tor1a gene in GABApre interneurons: The new player in the 'impaired inhibition' game of dystonia? Mov Disord 33: 1408, 2018.
37. Xiao J, Thompson MM, Vemula SR and LeDoux MS: Blepharospasm in a multiplex African-American pedigree. J Neurol Sci 362: 299-303, 2016.
38. Defazio G, Conte A, Gigante AF, Ferrazzano G, Pellicciari R, Dagostino S, Fabbrini G and Berardelli A: Clinical heterogeneity in patients with idiopathic blepharospasm: A cluster analysis. Parkinsonism Relat Disord 40: 64-68, 2017.
39. Evinger C: Benign essential blepharospasm is a disorder of neuroplasticity: Lessons from animal models. J Neuroophthalmol 35: 374-379, 2015.
40. Karimi M, Moerlein SM, Videen TO, Luedtke RR, Taylor M, Mach RH and Perlmutter JS: Decreased striatal dopamine receptor binding in primary focal dystonia: A D2 or D3 defect? Mov Disord 26: 100-106, 2011.
41. Fayers T, Shaw SR, Hau SC and Ezra DG: Changes in corneal aesthesiometry and the sub-basal nerve plexus in benign essential blepharospasm. Br J Ophthalmol 99: 1509-1513, 2015.
42. Berardelli A, Cruccu G, Kimura J, Ongerboer de Visser BW and Valls-Solé J: The orbicularis oculi reflexes. The international federation of clinical neurophysiology. Electroencephalogr Clin Neurophysiol Suppl 52: 249-253, 1999.
43. Frauscher B, Löscher WN, Ehrmann L, Gschliesser V, Brandauer E, Högl B and Kofler M: Narcolepsy-cataplexy: Deficient prepulse inhibition of blink reflex suggests pedunculopontine involvement. J Sleep Res 21: 495-501, 2012.
44. Burgen ASV, Dickens F and Zatman LJ: The action of botulinum toxin on the neuro-muscular junction. J Physiol 109: 10-24, 1949.
45. Ferrazzano G, Conte A, Fabbrini G, Bologna M, Macerollo A, Defazio G, Hallett M and Berardelli A: Botulinum toxin and blink rate in patients with blepharospasm and increased blinking. J Neurol Neurosurg Psychiatry 86: 336-340, 2015.
46. Sung Y, Nam SM and Lew H: Clinical outcomes of individualized botulinum neurotoxin type A injection techniques in patients with essential blepharospasm. Korean J Ophthalmol 29: 115-120, 2015.
47. Xiaoxue W, Xi C and Zhibo X: Effects of botulinum toxin type A on expression of genes in keloid fibroblasts. Aesthet Surg J 34: 154-159, 2014.
48. Hong B, Yao L, Ni L, Wang L and Hu X: Antinociceptive effect of botulinum toxin A involves alterations in AMPA receptor expression and glutamate release in spinal dorsal horn neurons. Neuroscience 357: 197-207, 2017.
49. Deng Y, Huang J, Zhang H, Zhu X and Gong Q: Association of expression of DRD2 rs1800497 polymorphism with migraine risk in Han Chinese individuals. J Pain Res 11: 763-769, 2018.
50. Zeuner KE, Acewicz A, Knutzen A, Dressler D, Lohmann K and Witt K: Dopamine DRD2 polymorphism (DRD2/ANNK1-Taq1A) is not a significant risk factor in writer's cramp. J Neurogenet 30: 276-279, 2016.
51. Tanabe LM, Kim CE, Alagem N and Dauer WT: Primary dystonia: Molecules and mechanisms. Nat Rev Neurol 5: 598-609, 2009.
52. Waln O and Ledoux MS: Blepharospasm plus cervical dystonia with predominant anterocollis: A distinctive subphenotype of segmental craniocervical dystonia? Tremor Other Hyperkinet Mov (N Y) 2011: tre-01-33-140-2, 2011.
53. Saeirad S and LeDoux MS: TOR2A variants in blepharospasm. Tremor Other Hyperkinet Mov (N Y) 13: 44, 2023.